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A morphologically intergrading population facilitates plastid introgression from diploid to tetraploid *Dodecatheon* (Primulaceae)

BRAD OBERLE[1]*, ROBERT A. MONTGOMERY[2], JAMES B. BECK[3,4] and
ELIZABETH J. ESSELMAN[5]

[1]*Department of Biology, University of Missouri-St Louis, St Louis, MO 63121, USA*
[2]*Department of Biology, Washington University in St Louis, St Louis, MO 63130, USA*
[3]*Department of Biology, Duke University, Durham, NC 27708, USA*
[4]*Department of Biological Sciences, University of Wisconsin, Milwaukee, Milwaukee, WI 53201, USA*
[5]*Department of Biological Sciences, Southern Illinois University at Edwardsville, Edwardsville, IL 62026, USA*

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Polyploidy may promote diversification by generating reproductive isolation between ploidy levels, but this reproductive barrier may not be absolute. Several recent analyses of diploid–tetraploid contact zones have found evidence for hybridization. In these cases, inter-cytotype gene flow is often associated with morphologically intergrading populations. In this study, we combine cytological, fitness and population genetic data to examine the evolutionary role of a morphologically intergrading population at a contact zone between species with different ploidy levels in *Dodecatheon*. Diploid *D. frenchii* and tetraploid *D. meadia* are usually distinguished by leaf-shape characters. In southern Illinois, where these taxa occur in parapatry, a morphologically intergrading population includes the first documented tetraploid with *D. frenchii* morphology. Most plants in this intergrading population are fertile, and a nearby typical population of *D. meadia* has plastid DNA haplotypes that only occur in *D. frenchii* elsewhere in southern Illinois. These results suggest that fit neo-tetraploids in this intergrading population have facilitated local introgression between ploidy levels. Similar patterns in other regions where these taxa co-occur may explain weak range-wide genetic differentiation between these species. © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2012, **168**, 91–100.

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INTRODUCTION

Polyploidy plays an important role in evolution, particularly in plants (Otto, 2007; Paun *et al*., 2009; Soltis *et al*., 2009; Parisod, Holderegger & Brochmann, 2010). Most angiosperm genomes retain duplicated genes that diverged at a similar time, suggesting that almost all flowering plant diversity is descended from a polyploid ancestor (Cui *et al*., 2006). In addition to this ancient event, comparative cytology suggests that many angiosperms have experienced polyploidy more recently in their evolutionary histories. Across all angiosperms, phylogenetic comparative methods indicate that 15% of species are polyploid relative to the lowest diploid chromosome count in their genus (Wood *et al*., 2009).

Polyploidy may promote diversification by enhancing reproductive isolation between ploidy levels. Matings between diploids and tetraploids produce cytologically unbalanced offspring that often fail to develop or exhibit severe meiotic abnormalities (Köhler, Mittelsten Scheid & Erilova, 2010). These factors contribute to strong post-mating reproductive isolation between ploidy levels (Ramsey & Schemske, 1998; Burton & Husband, 2000). For this reason, polyploidy is one of the few genetic mechanisms that

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*Corresponding author. E-mail: brad.oberle@gmail.com satisfies theoretical criteria necessary for sympatric speciation (Coyne & Orr, 2004). Reproductive isolation between ploidy levels can further promote the evolution of pre-mating isolating mechanisms via reinforcement (Petit, Bretagnolle & Felber, 1999). For instance, co-occurring diploid and tetraploid populations of *Heuchera grossularifolia* Rydb. (Saxifragaceae) are under selection for changes in floral phenology that reduce the probability of low-fitness inter-cytotype matings (Nuismer & Cunningham, 2005).

Although reproductive isolation between ploidy levels is strong, it may, however, not be absolute. A growing number of population genetic studies at diploid–polyploid contact zones find evidence for hybridization. For instance, tetraploid accessions of *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae) contain nuclear gene sequences characteristic of diploid *C. rubella* Reut. in a region where they co-occur (Slotte *et al*., 2008). Ma *et al*. (2010) found a similar result in their recent examination of the *Achillea millefolium* L. (Asteraceae) polyploid complex in Europe. Tetraploids in a morphologically variable population had AFLP profiles that were intermediate between allotetraploid *A. collina* Becker ex. Rchb. and its co-occuring diploid parent *A. setacea* Waldst. & Kit. Thórsson, Salmela & Anamthawat-Jónsson (2001) reported an even more complex scenario for *Betula* L. (Betulaceae) in Iceland, where highly variable populations include triploids that contribute to introgressive hybridization between diploid *B. nana* L and tetraploid *B. pubescens* Ehrh. These examples demonstrate that polyploidy does not always confer instant reproductive isolation. They also suggest that morphologically variable populations may indicate inter-cytotype gene flow. Populations with extreme morphological variation could include atypical cytotypes, such as autotetraploids, triploids or polyhaploids. If these cytotypes have fitness, they can promote genetic introgression between ploidy levels near highly variable populations (Petit *et al*., 1999; Parisod *et al*., 2010).

The role of genetic introgression among polyploids may be especially relevant to understanding evolutionary relationships in *Dodecatheon* L. (Primulaceae), a clade of buzz-pollinated, perennial herbs 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 & Reveal, 2007). Frequent polyploidy and hybridization contribute to the complex taxonomic history of *Dodecatheon* (Thompson, 1953), as illustrated by two eastern North American species. Widespread *D. meadia* L. tends to inhabit relatively exposed sites such as dry forests and rocky glades. *Dodecatheon frenchii* (Vasey) Rydb., which was previously considered a taxonomic variety of *D. meadia*, is endemic to moist sandstone cliffs, with the majority of occurrences concentrated in a small region of southern Illinois (Voigt & Swayne, 1955). In this region, populations of these species grow parapatrically above and below sandstone cliffs, well within the foraging range of their shared pollinators (Macior, 1964). Leaf-shape differences distinguish these taxa. *Dodecatheon meadia* is characterized by oblanceolate leaves that gradually taper to a petiole at the base, whereas *D. frenchii* exhibits subcordate leaves that abruptly taper to a petiole at the base (Fassett, 1944).

Despite > 60 years of research, the evolutionary relationship between *D. meadia* and *D. frenchii* remains unclear. Early experimental and cytological studies in southern Illinois suggested that these two taxa are evolutionarily distinct. A reciprocal transplant experiment demonstrated that the leaf-shape differences between taxa had a genetic basis (Voigt & Swayne, 1955). Later, hundreds of chromosome counts from across southern Illinois demonstrated that *D. frenchii* is diploid ($2n = 44$) relative to tetraploid *D. meadia* ($2n = 88$) (Olah & DeFilipps, 1968, 1969). The most recent revision of the genus cites these results as support for recognizing these taxa as distinct species (Reveal, 2009).

In contrast to earlier research, more recent analyses of genetic variation have found little differentiation between these taxa. A phylogenetic analysis of the genus based on plastid DNA resolved *D. meadia* and *D. frenchii* as sister species, with sequences from the representative individuals showing only 0.04% divergence (Mast *et al*., 2004). A more recent phylogeographic study based on more than 1000 AFLP loci in 302 individuals from 32 populations found no significant range-wide genetic differentiation between *D. frenchii* and *D. meadia* (Oberle & Schaal, 2011). These results suggest that the ecological, morphological and cytological differences between these species are not associated with extensive neutral genetic differentiation.

The key to reconciling the results of early experiments and observations with more recent genetic analyses may lie in a recently recognized morphologically intermediate population. Oberle & Esselman (2011) described an intergrading population that was significantly more variable for morphological characters than typical populations of either species. This intergrading population also included plants with intermediate or otherwise unusual morphologies suggesting that atypical cytotypes may be present. Oberle & Esselman (2011) also reported similar morphologically intergrading populations in several of the regions where these taxa co-occur. If these intergrading populations promote genetic introgression, they could collectively homogenize neutral allele frequencies between taxa.

In this study, we examine whether a morphologically intergrading population facilitates genetic introgression between diploid *D. frenchii* and tetraploid *D. meadia* through a series of investigations at increasingly broader spatial scales. At the finest spatial scale, we evaluate the potential for gene flow between cytotypes based on a targeted cytological survey. If an intergrading population includes atypical cytotypes, which show a mismatch between morphology and chromosome count relative to the established taxonomic concepts, then the reproductive barrier because of differing chromosome numbers could be breached at this site (Petit *et al.*, 1999). At a broader scale, we evaluate whether plants in the intergrading population are likely to contribute maternal genes to future generations by comparing female fitness to nearby typical populations. If plants in the intergrading population are relatively fit, they could be capable of facilitating genetic introgression (Burton & Husband, 2000). Finally, we examine whether patterns of genetic variation are consistent with local maternal genetic introgression by comparing plastid haplotype differentiation between sets of populations with different expected rates of interspecific gene flow across southern Illinois. If typical populations near the intergrading population have more similar plastid haplotype frequencies than allopatric populations and parapatric populations where no intergrading plants have been reported, we can infer that the intergrading population is associated with elevated maternal gene flow.

MATERIAL AND METHODS

FIELD SAMPLING AND TAXONOMIC DETERMINATIONS

In order to represent the geographical extent of the contact zone in our analysis, we used the traditional taxonomic character of leaf shape (Fassett, 1944; Voigt & Swayne, 1955) to identify six 'typical' populations of *D. frenchii* and *D. meadia* spanning the Shawnee Hills region of southern Illinois (Fig. 1). Typical populations of *D. frenchii* had leaves that tapered abruptly to a petiole at the base and occurred in flat areas at the bases of overhanging sandstone cliffs, commonly known as 'rockhouses.' Typical populations of *D. meadia* had leaves that gradually tapered at the base and occurred in forested margins of cliff-top glades. A complimentary study showed that leaf-shape differences used to characterize these populations corresponded to significant differences in

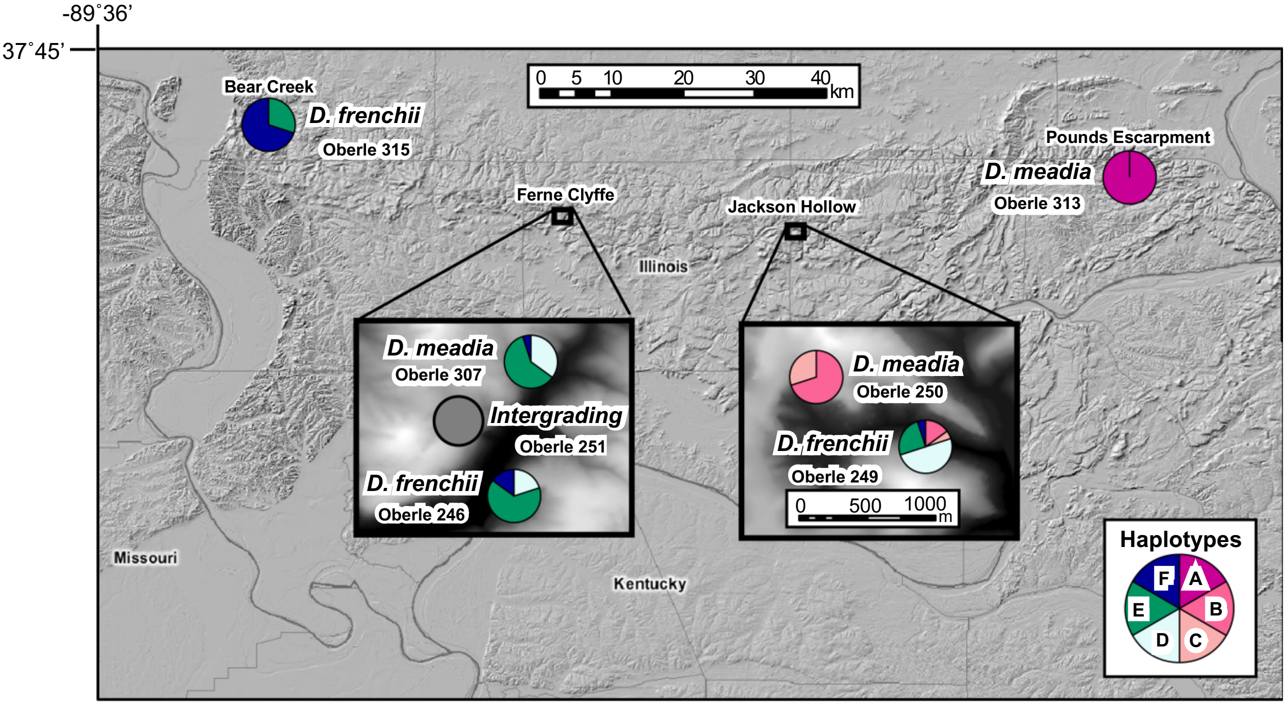

Figure 1. Map of sampling localities for *Dodecatheon* in southern Illinois. Vouchers are deposited in the Missouri Botanical Garden Herbarium (MO). Charts show the relative frequency of six different *psbA—trnH*$^{\mathrm{GUG}}$ haplotypes among 20 sequenced individuals in each population. Geographic coordinates are projected. The scales of both insets are identical.

infructescence and seed characters (Oberle & Esselman, 2011). We also identified one intergrading population of plants that spanned the range of leaf shapes between taxa (Oberle & Esselman, 2011). This population occurred in a small rockhouse, approximately 5 m long, 2 m deep and 1 m high near the top of the south-facing bluff line of Happy Hollow at Ferne Clyffe St Park, Jackson County.

CYTOLOGY

In April 2008, we collected flower buds from individuals spanning the range of leaf morphologies in the morphologically intergrading population. We used the traditional taxonomic character leaf shape to assign buds into three typological categories. Buds from plants with an angle at the base of the blade of < 15° were noted as having '*D. meadia*-like' morphology. Those from plants with thin leaves and having an angle at the base of the blade of > 25° were noted as having '*D. frenchii*-like' morphology. Buds from plants having leaves with angles at the bases of their blades between 15° and 25° were noted as having 'intermediate' morphology. Leaf-base angle correlates well with other distinguishing characters in southern Illinois and was used to make taxonomic determinations for previous chromosome counts at this locality and others (Voigt & Swayne, 1955; Olah & DeFilipps, 1968; Oberle & Esselman, 2011). We fixed buds in Farmer's fixative for 24 h and then stored them at 4 °C in 70% ethanol. Anthers were macerated, stained with 1% acetocarmine and squashed following standard methods. Stained, flattened cells were examined with brightfield microscopy at 630× to 1000× magnification using a Zeiss Axioplan 2 microscope (Carl Zeiss, Jena, Germany) and photographed at 1000× with a mounted Axiovision HR camera.

RELATIVE FITNESS

In addition to the cytological survey, we estimated the female fitness of plants in the intergrading population and compared it with that of plants in two nearby typical populations at Ferne Clyffe. In order to limit the influence of direct pollen exchange between the intergrading population and typical populations, we chose large populations (> 100 plants) growing at least 400 m away for comparison. The *D. meadia* population was growing in its standard 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 & DeFilipps, 1968). To estimate female fitness, we collected every mature fruit from up to 21 randomly selected individuals per population in June 2007 and estimated three components of fitness: fertility (ability to produce a seed), fecundity (number of seeds produced given fertility) and viability (germination rate). We determined the fertility and fecundity from simple seed counts performed under a dissecting microscope. The presence of a seed indicated fertility and the number of seeds produced indicated fecundity. We determined viability of all seeds from a randomly selected subset of fertile capsules in a greenhouse 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 (25°C) 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 (Sun-Gro, Canada). We maintained the flats on a mist bench and randomized their positions every 2 days until net daily germination rates slowed to < 5%.

To test for fitness differences among these populations, we conducted three 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 fitted models to counts for both capsules and plants using the package 'pscl' (Zeileis, Kleiber & Jackman, 2008) in R ver. 2.11.0. We also tested whether capsule-level fecundity followed a normal distribution in each population using Shapiro–Wilk *W*-tests as implemented in the package 'stats' in R ver. 2.11.0. We then tested for differences in viability using a mixed-model analysis of variance (ANOVA) on arcsine square-root transformed germination proportions with taxon treated as a fixed effect and capsule treated as a random effect nested within taxon in R ver. 2.11.0.

POPULATION GENETICS

In order to evaluate whether the morphologically intergrading population is associated with genetic introgression between taxa, we conducted a population genetic survey. We had three goals: (1) to test for genetic differentiation between *D. frenchii* and *D. meadia* across the region; (2) to assess whether the geographical configuration of populations influences relative genetic differentiation; and (3) to test whether the presence of intergrading populations affects the amount of genetic differentiation between parapatric typical populations.

In Spring 2008, we randomly sampled 20 individuals from: (1) a population of *D. frenchii* that grows in isolation (Bear Creek, IL); (2) a population of *D. meadia* that grows in isolation (Pounds Escarpement, IL); (3) parapatric populations of *D. frenchii* and *D. meadia* in which intergrading plants are not present (Jackson Hollow, IL); and (4) parapatric populations of *D. frenchii* and *D. meadia* in which intergrading plants are present (Ferne Clyffe State Park, IL) (Fig. 1). At Ferne Clyffe, we sampled from the same two typical populations included in the relative fitness analysis. At Jackson Hollow, we sampled from similarly sized populations that were also approximately 800 m apart. Our assessment that intergrading plants do not occur at Jackson Hollow was based on the results of historical surveys (Voigt & Swayne, 1955) and our own resurveys (2005–2008). Not only were no intergrading populations found during our resurveys, but we documented 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 our own and earlier surveys (Swayne, 1973).

We preserved leaf tissue in silica gel and extracted DNA using Viogene plant genomic DNA extraction kits (Viogene, Taiwan). We then PCR amplified the plastid *psbA—trnH*^{GUG} intergenic spacer using the protocol outlined in Shaw *et al*. (2005). We quantified PCR products via agarose gel electrophoresis and purified the remaining PCR product by adding 3 U exonuclease I (New England Biolabs, Ipswich, MA) and 0.015 U shrimp alkaline phosphatase (Promega, Madison, WI), followed by incubations at 37 °C for 30 min and 80 °C for 20 min. We cycle sequenced each sample using ABI BigDye chemistry (Applied Biosystems, Foster City, CA) following the manufacturer's protocol modified for 10-μL reactions and generated electropherograms using an ABI 3130xl genetic analyser.

We tested the null hypothesis of no haplotype frequency differentiation among taxa across the entire data set using a log-likelihood *G*-test with William's correction. The results of tests for population genetic differentiation based on corrected *G* values are comparable with results of more commonly used tests (Goudet *et al*., 1996; Ryman *et al*., 2006). We also tested for haplotype differentiation by calculating Chao's unbiased estimator of Jost's D ($D_{est\text{-}Chao}$). Jost's D is related to other indices of differentiation, but is mathematically independent of intra-population variation (Jost, 2008).

To test whether populations with different geographical configurations showed different levels of genetic differentiation, we calculated $D_{est\text{-}Chao}$ between three sets of populations: between allopatric typical populations (*D. frenchii* at Bear Creek and *D. meadia* at Pounds Escarpment); between parapatric typical populations in which no intergrading populations have been found (Jackson Hollow); and between parapatric typical populations in which an intergrading population does occur (Ferne Clyffe). We evaluated whether D_{est_chao} differed significantly between these comparisons by assessing the overlap of the 95% confidence intervals as calculated using the bootstrap percentile method described in Chao *et al*. (2008). In addition, we tested the null hypothesis of no difference in haplotype 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 vs. haplotypes in Ferne Clyffe vs. Jackson Hollow, differ in their degrees of association.

All estimates of $D_{est\text{-}Chao}$ were calculated using SPADE (Chao & Shen, 2010). We implemented the two-way *G*-test in R 2.11.0 (R Development Core Team, 2010) using a script written by Peter Hurd (University of Alberta). We implemented the three-way *G*-test using the VassarStats program available from Richard Lowry (Vassar College).

RESULTS

CYTOLOGY

Anthers bearing cells at countable stages of meiosis were all approximately 4 mm in length. We obtained chromosome counts from four individuals in the Ferne Clyffe intergrading population: two from '*D. meadia*-like' individuals, one from a '*D. frenchii*-like' individual, and one from an 'intermediate' individual. The counted cells counts exhibited either 44 paired bivalents at meiosis I, or two daughter cells each with 44 unpaired univalents at late meiosis I (Fig. 2). The cells exhibiting bivalents also consistently displayed both tightly associated pairs of chromosomes that are potential quadrivalents and unpaired 'B' chromosomes (Fig. 2).

RELATIVE FITNESS

We counted over 4000 seeds in 167 capsules from 59 plants from populations of *D. frenchii, D. meadia* and an intergrading population that co-occurs in Ferne Clyffe State Park. Populations differed in capsule fertility (ZINB, zero inflation model, $P < 0.001$). Capsules from the intergrading population exhibited the highest fertility rates, with three out of four capsules producing at least one seed, compared with 63% from the *D. meadia* population and 44% from the *D. frenchii* population. Plant-level fertility showed a similar pattern, but the differences among

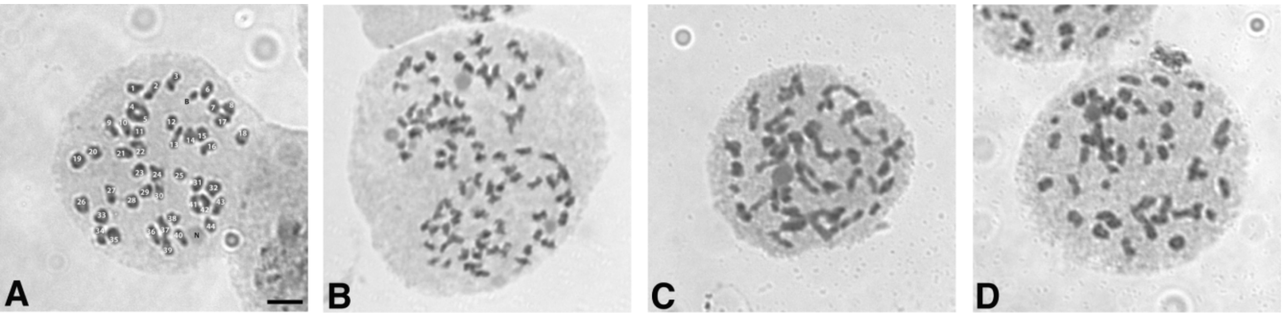

Figure 2. Chromosomes of four *Dodecatheon* individuals from the intergrading population at Ferne Clyffe State Park ($N = 44$). A, '*D. frenchii*-like' individual exhibiting 44 bivalents at meiosis I. The bivalents are numbered for interpretation. 'B' denotes a potential B chromosome and 'N' denotes the nucleolus. B, 'intermediate' individual exhibiting two daughter cells at late meiosis I, each with 44 univalents. C, '*D. meadia*-like' individual exhibiting 44 bivalents at meiosis I. D, '*D. meadia*-like' individual exhibiting 44 bivalents at meiosis I. Scale bar, 10 µm (A).

populations failed only marginally to meet the nominal significance level (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* vs. intergrading, $Z = 0.79$, $P = 0.49$). However, fecundity in these populations was significantly lower than in the *D. meadia* population (ZINB count model coefficients, *D. frenchii* vs. *D. meadia*, $Z = 4.08$, $P < 0.001$, intergrading vs. *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 (Fig. 3). The number of seeds per fertile capsule 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.

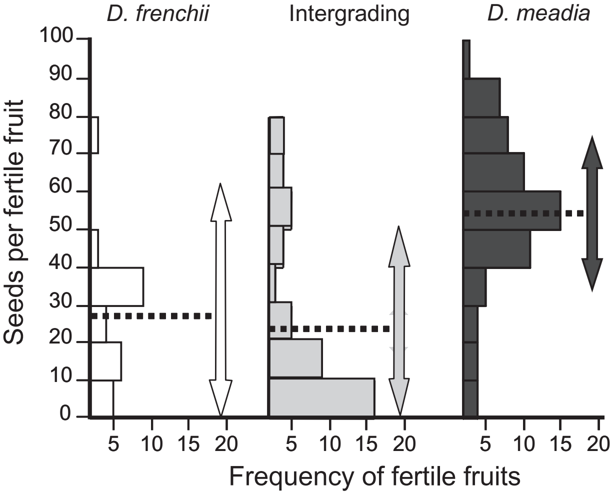

Figure 3. Fecundity of capsules from three *Dodecatheon* populations at Ferne Clyffe State Park as stacked histograms. Dashed lines represent means for each population inferred from mixed-model analysis of variance (ANOVA). Arrows alongside bars represent 95% confidence intervals bounded to be greater than zero.

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 > 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, we identified six *psbA*—*trnH*$^{\mathrm{GUG}}$ haplotypes. Sequences representing these haplotypes are deposited in GenBank under accession numbers GU066767–GU066772. These haplotypes varied at three segregating sites, including one substitution and length variation at two polynucleotide repeat sites. The number of haplotypes varied among taxa with all six occurring in *D. meadia* and five occurring in *D. frenchii*. Haplotype number also varied among populations, from one in the *D. meadia* population at Pounds Escarpment to five in the *D. frenchii* population at Jackson Hollow. Overall, haplotype frequencies differed among taxa [adjusted log-likelihood ratio statistic (G) = 58.48, χ^2 d.f. = 5, $P < 0.001$, $D_{est\_Chao} = 0.491$, 95% CI = 0.430–0.948].

Levels of genetic differentiation varied among pairs of populations with different geographical configurations (Fig. 4). Allopatric populations shared no haplotypes and were maximally differentiated. In contrast, both parapatric sets of populations shared haplotypes. Parapatric populations at Jackson Hollow still showed significant differentiation, but lower than that which occurred among allopatric populations. The parapatric populations near the intergrading population at Ferne Clyffe exhibited low genetic differentiation, with the 95% confidence interval for D_{est_Chao} including zero. The associations between haplotypes and taxa differed significantly between the two parapatric pairs of populations [adjusted log-likelihood ratio statistic (G) for three way interaction = 80.44, χ^2 d.f. = 13, $P < 0.001$]. The parapatric populations at Ferne Clyffe shared more haplotypes because the population of *D. meadia* at that locality contained haplotypes that only occur in *D. frenchii* elsewhere in southern Illinois.

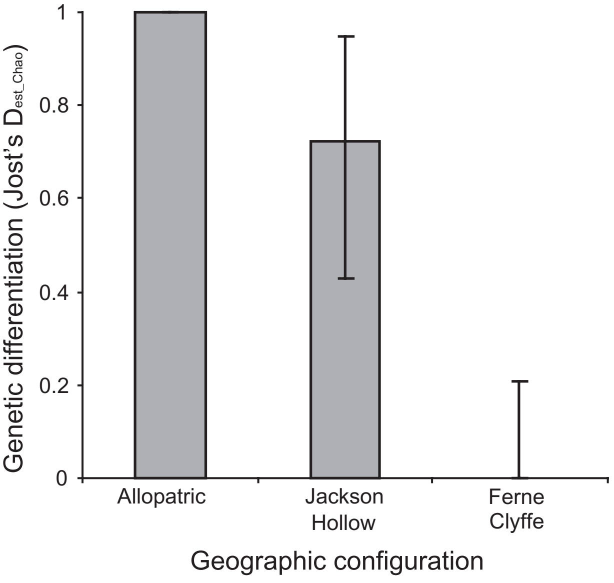

Figure 4. Genetic differentiation between southern Illinois *Dodecatheon* populations with different geographical configurations. Error bars represent 95% confidence intervals calculated using the bootstrap percentile method described in Chao *et al*. (2008).

DISCUSSION

Identifying the extent of gene flow between ploidy levels is critical for understanding the role of polyploidy in diversification. In this study, cytological, relative fitness and population genetic data provide coherent evidence that atypical cytotypes in a morphologically intergrading population have facilitated local plastid introgression from diploid *D. frenchii* into tetraploid *D. meadia* at their contact zone in southern Illinois. If similar intergrading populations also facilitate inter-cytotype gene flow in other regions, they could explain why these taxa show weak range-wide genetic differentiation despite cytological, morphological and ecological differences.

ATYPICAL CYTOTYPE

Although the cytological survey was limited, the results demonstrate that the intergrading population includes an atypical cytotype. The tetraploid individual with *D. frenchii* morphology that we report from the intergrading population at Ferne Clyffe is the first among 145 published chromosome counts from this region (Olah & DeFilipps, 1968, 1969) including only diploid counts from typical populations of *D. frenchii* at this locality. The *D. frenchii* leaf morphology and the presence of a quadrivalent during meiosis suggest that this individual may be an autopolyploid. Although autopolyploid *D. frenchii* were predicted to occur based on the frequent formation of oversized and presumably unreduced gametes (Olah & DeFilipps, 1968), firmly establishing autopolyploidy in the intergrading population will require more advanced methods, such as nuclear DNA sequencing (Schuettpelz *et al*., 2008) and/or fluorescent *in situ* hybridization (Chester *et al*., 2010). High throughput approaches, such as flow cytometry, could provide additional insights into the frequency of different cytotypes in this population and the relationships between genome content and morphology (Lihová *et al*., 2007).

RELATIVELY FIT

The hypothesis of local introgressive hybridization is supported by the relative fitness data. Female fitness varied among populations at Ferne Clyffe. *Dodecatheon meadia* ranked consistently higher than *D. frenchii*, and the relative rank of the intergrading population varied. The intergrading population exhibited a higher proportion of fertile capsules than either typical population. Critically, this result demonstrates that the intergrading population does not consist solely of sterile plants. With respect to fecundity, the average capsule from the intergrading population produced as many seeds as the average capsule from the *D. frenchii* population. The number of seeds per capsule was normally distributed in both typical populations such that differences among capsules in these populations could be attributable to random error. In contrast, the number of seeds per capsule was highly skewed in the intergrading population. The skewed distribution could result from random pollinator movements and stigmatic occlusion in an admixed population of plants from different ploidy levels (Husband & Schemske, 2000). It could also reflect the prevalence of new cytotypes with different fitness from their parents. Overall, these data suggest that plants in the intergrading population are at least as likely to contribute maternal genes to the next generation as those in a nearby population of *D. frenchii*.

Genetic introgression

Across the contact zone between *D. meadia* and *D. frenchii* in southern Illinois, these two taxa have different plastid haplotype frequencies. This differentiation is consistent with their observed ecological, morphological and cytological differences. Although they are genetically differentiated, they are not completely distinct. Sampled populations from southern Illinois share all but one plastid haplotype. Comparisons of relative genetic differentiation among sets of populations with different geographical configurations suggest site-specific differences in the rates of genetic exchange. Allopatric populations of *D. meadia* and *D. frenchii*, which should have the lowest pairwise migration rates, shared no haplotypes. In contrast, both sets of parapatric populations shared haplotypes. The pair of populations at Jackson Hollow, where no intergrading plants have been recorded, shared haplotypes but still exhibited significant genetic differentiation. Differentiation between populations near the intergrading population at Ferne Clyffe was not significantly different from zero. Overall, the association between genetic differentiation and expected effective gene flow strongly suggests that local introgression contributes to haplotype sharing between these taxa.

The maternal inheritance of plastid DNA further indicates the direction and mechanism of genetic introgression at this contact zone. Parapatric populations at Ferne Clyffe are genetically similar because the population of the tetraploid *D. meadia* includes plastid haplotypes that only occur in diploid *D. frenchii* elsewhere in southern Illinois. This population of *D. meadia* occurs near a relatively fit population of morphologically intergrading plants, including a putative autotetraploid *D. frenchii*. This pattern implies a scenario of hybridization between the autotetraploid *D. frenchii* in the intergrading population and tetraploid *D. meadia* in the nearby typical population, with subsequent backcrossing to the typical *D. meadia*. More detailed genetic characterization of the intergrading population using biparentally inherited markers could reinforce its role in local hybridization.

Range-wide implications

The association between morphological intergradation and local interspecific gene flow could explain why these taxa exhibit weak range-wide genetic differentiation. The morphologically intergrading population investigated here is not unique. Similar populations have been reported in all areas where these two taxa co-occur (Oberle & Esselman, 2011). Each could facilitate local introgression in a similar way. Because relatively little gene flow is necessary to counteract the differentiating effects of genetic drift, if the net rate of gene flow between taxa within regions is similar to the rate of gene flow between regions (Wright, 1931), then neutral variation could evolve as if the whole group were a genetically cohesive unit (Templeton, 1989). A model-based analysis of more populations and loci could provide additional insights into the generality of this pattern (Hey & Nielsen, 2007; Joly, Mclenachan & Lockhart, 2009).

CONCLUSION

Although polyploidy plays an indisputably important role in plant evolution, it may not always promote diversification by producing new reproductively isolated lineages. Rather, recurrent formation of novel cytotypes may facilitate hybridization (Parisod *et al*., 2010). This appears to be the case in *Dodecatheon*. Morphologically intergrading populations can include neotetraploids that facilitate introgression across ploidy levels, maintaining genetic connectivity despite ecological differentiation.

This conclusion is consistent with some classic early work on the biosystematics of polyploids (see, for example, Buggs, Soltis & Soltis, 2011). Raven *et al*. (1968) examined the associations between ecology, morphology, phenology and ploidy level in the North American desert shrub *Ambrosia dumosa* A.Gray ex Torr. (Asteraceae). They found local differentiation between ploidy levels, but they noted prevalent intergradation and apparent hybrid cytotypes across the species range. These observations prompted them to reject the 'unwarranted assumption that gene flow between all diploid individuals and between all polyploid individuals was negligible' and conclude that 'polyploidy may provide a measure of isolation for differential adaptation, but at the same time the species as a whole may retain a great deal of flexibility in terms of gene flow between the partly reproductively isolated derivatives.' The example presented here in *Dodecatheon* suggests that population genetic and relative fitness data may confirm the suspicions of Raven *et al*. (1968) and provide insights into the evolution of many other polyploid complexes.

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