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Climatic niche differences between diploid and tetraploid cytotypes of *Chamerion angustifolium* (Onagraceae)[1]

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- *Premise of the study:* Polyploidy—the possession of more than two copies of each chromosome in the nucleus—is common in flowering plants. Polyploid plants can occupy different geographic ranges than their diploid progenitors, but the factors responsible for maintaining these range differences are poorly understood. Polyploidy can have significant physiological consequences, and the present study aims to determine whether previously described physiological differences between cytotypes are correlated with climatic niches and geographic distributions.
- *Methods:* Prior research indicates that tetraploid plants of *Chamerion angustifolium* (Onagraceae) are more tolerant of drought and less tolerant of freezing than diploids, which suggests that they should occupy a niche that is warmer and drier than that of diploids. We extracted climate data for 134 populations of *C. angustifolium* classified as pure diploid, pure tetraploid, or mixed-ploidy. We compared climatic conditions between these population categories and generated ecological niche models to compare their geographic distribution with prior qualitative estimates.
- *Key results:* Pure tetraploid populations occupy habitats that are warmer and drier than those of pure diploid populations. Mixed-ploidy populations occur in habitats that are not strictly intermediate between pure diploid and pure tetraploid populations, but are as cold as pure diploid populations and have intermediate soil moisture deficits. Our niche models were similar to previous qualitative estimates of cytotype geographic distribution.
- *Conclusions:* The correspondence between the physiological tolerances of cytotypes, their climatic niches, and their geographic distributions suggests that physiological traits are at least partially responsible for differences in the realized climatic niches of diploid and tetraploid *C. angustifolium*.

Key words: cytogeography; drought tolerance; ecological niche model; fireweed; MaxEnt; niche differentiation; polyploidy.

Polyploidy—the possession of more than two copies of each chromosome in the nucleus after a genome duplication event—has arisen recurrently throughout the evolutionary history of plants (Soltis and Soltis, 1999; Cui et al., 2006) and may be responsible for the rapid diversification of all flowering plants (*Amborella* Genome Project, 2013). In addition, there is typically strong reproductive isolation between diploid and polyploid cytotypes (e.g., Husband and Schemske, 2000; Husband and Sabara, 2003), and polyploidy is recognized as a mechanism of instantaneous sympatric speciation (Otto and Whitton, 2000; Wood et al., 2009). Polyploidy has immediate phenotypic consequences, such as increased cell size (Masterson, 1994), and can increase the potential for plants to adapt to novel environments (e.g., Ramsey, 2011). Although polyploidy does not necessarily have consistent effects on geographic range attributes (Martin and Husband, 2009), spatial differences between diploids and polyploids are frequently observed (Lewis, 1980). The magnitude and direction of physiological and ecological differences between polyploids and their diploid progenitors, however, are highly species-specific (reviewed by Levin, 2002). Many comparative studies have identified physiological and ecological differences between diploids and polyploids (reviewed by Levin, 2002; also see te Beest et al., 2012), but little is known about how these differences influence the geographic distribution of diploid and polyploid cytotypes.

The factors responsible for causing differences in the geographic ranges of diploid and polyploid cytotypes may be historical, ecological, or a combination of the two. If historical factors—such as the expansion of one cytotype into glacial refugia—are primarily responsible for distributional differences between diploids and polyploids, then their geographic ranges will be characterized by similar environmental conditions (e.g., Godsoe et al., 2013). If ecological differences between diploid and polyploid cytotypes are primarily responsible for distributional differences, then their geographic ranges will be characterized by different environmental conditions that correspond with the functional characteristics of each cytotype (e.g., Manzaneda et al., 2012). Historical and ecological factors are not necessarily mutually exclusive; differences in colonization history as well as ecological divergence between cytotypes can jointly influence cytotype distribution (e.g., Laport et al., 2013).

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The functional differences that contribute to ecological segregation between cytotypes may arise directly from the physiological consequences of genome duplication (Levin, 1983; Li et al., 1996, 2009; Maherali et al., 2009) and their effects on climatic tolerance (Gaston, 2003; Manzaneda et al., 2012). Few studies, however, have determined whether physiological differences between diploids and polyploids contribute to differentiation in their realized climatic niches (Soltis et al., 2010; Manzaneda et al., 2012). A close correspondence between physiological traits and the realized climatic niche of cytotypes would suggest that cytotype distributions are the result of sorting along environmental gradients. Comparative studies of polyploid complexes with well-understood and ecologically relevant physiological traits are needed to understand the importance of physiological traits for determining the realized climatic niches of diploids and polyploids (Soltis et al., 2010).

To examine whether physiological differences between cytotypes are correlated with their respective climatic niches and—by association—their geographic distributions, we used the autopolyploid *Chamerion angustifolium* L. Holub (Onagraceae). Previous studies of physiological traits in *C. angustifolium* have shown that tetraploid plants have xylem conduits that are 21% wider and have 87% higher hydraulic conductivity than those of diploids, but do not differ in their vulnerability to water-stress-induced xylem cavitation (Maherali et al., 2009). Diploid and tetraploid cytotypes also do not differ in their photosynthetic and stomatal responses to decreasing leaf water potential (Maherali et al., 2009). However, higher hydraulic conductance associated with wider xylem conduits permits tetraploids to extract more water from drying soils than diploids without a severe reduction in leaf water potential, facilitating the avoidance of water stress under drought (Maherali et al., 2009). Specifically, tetraploids take 30% longer to wilt and die than diploids after watering ceases (Maherali et al., 2009). Although differences in cytotype freezing tolerances have not been investigated directly in *C. angustifolium*, the narrower xylem conduits of diploid plants compared with tetraploid plants suggest that their xylem conduits are more resistant to freezing-induced cavitation than those of tetraploids (see Davis et al., 1999). Martin and Husband (2013) found that diploid and tetraploid populations of *C. angustifolium* in the Rocky Mountains were adapted to elevation and not local environment, indicating that the physiological differences between cytotypes are conserved among populations at a regional scale.

We characterized the climatic niches of diploid and tetraploid *C. angustifolium* to determine whether they are correlated with differences in cytotype physiology. We hypothesized that diploid and tetraploid cytotypes of *C. angustifolium* would occupy different climatic niches because of their different physiological tolerances to drought and freezing. Because tetraploids are able to maintain their physiological functions at greater water deficits than diploids (Maherali et al., 2009), we predicted that their niche would be drier than the diploid niche. Because diploids may be more resistant than tetraploids to freezing-induced cavitation, we predicted that their niche would have a higher incidence of freezing temperatures than the tetraploid niche. Previously, no hypotheses or empirical studies have been published on the climatic conditions that favor coexistence between cytotypes at a regional scale. We predicted that mixed-ploidy populations would occur in a climatic niche intermediate between those of pure diploid and pure tetraploid populations. To test our predictions, we used climate data to compare the environmental variables that characterize pure diploid, pure tetraploid, and mixed-ploidy populations. We generated ecological niche models to qualitatively compare the modeled climatic niches of cytotypes with estimates of their geographic distributions.

MATERIALS AND METHODS

Study system—*Chamerion angustifolium* (Onagraceae), fireweed, is a well-studied (Levin, 2002; Soltis et al., 2007), herbaceous, perennial plant with diploid ($2n = 2x = 36$), triploid, and autotetraploid ($2n = 4x = 72$) cytotypes (Mosquin, 1966, 1967; Husband and Schemske, 1998). In North America, *C. angustifolium* tetraploids tend to occupy lower latitudes, whereas diploids tend to occupy higher latitudes. The cytotypes co-occur along the southern border of the boreal forest and in the Rocky Mountains (Mosquin, 1967; Husband and Schemske, 1998). There are maps that depict the estimated geographic distribution of both cytotypes (Mosquin and Small, 1971), but the climatic envelopes of cytotypes have not yet been evaluated.

Population data—We compiled a dataset of 134 georeferenced *C. angustifolium* populations located in North America ($n = 130$), Iceland ($n = 3$), and Greenland ($n = 1$). These populations were located and sampled for ploidy over the last 15 yr, and cytotype frequencies for each population were estimated using flow cytometry (for detailed methods, see Sabara et al., 2013). The three mixed-ploidy populations in the Rocky Mountains used by Maherali et al. (2009) to study comparative cytotype physiology were included in the present study. A mean of 26.1 ± 4.0 plants were screened in each population. Some ($n = 16$) populations had only 3 to 10 plants screened. We included these populations in our analyses to increase the geographic extent of our sampled populations, but their omission would not have altered our conclusions. Of the 134 sampled populations, 14 were pure diploid, 77 were pure tetraploid, and 43 were mixed-ploidy, containing both diploid and tetraploid plants. Cytotype frequency estimates for 73 of these populations have been published previously by Husband and Schemske (1998), Roy (2008), Martin and Husband (2013), and Sabara et al. (2013); all others were previously unpublished (see Dryad data package [doi:10.5061/dryad.g791n]).

Climate data—To test our predictions about drought and temperature conditions, we retrieved data for specific climate variables that were relevant to cytotype physiology. Specifically, we retrieved data for mean annual temperature (BIO1), maximum temperature of the warmest month (BIO5), mean annual precipitation (BIO12), as well as mean monthly temperature and precipitation data, from WorldClim (http://www.worldclim.org; Hijmans et al., 2005). To investigate soil moisture conditions in more detail than WorldClim data allow, we retrieved monthly potential evapotranspiration (PET) data from the CGIAR Consortium for Spatial Information (http://www.cgiar-csi.org; Trabucco et al., 2008); PET characterizes the atmospheric demand for water in a habitat. All climate data were at the highest available resolution (30 arc second ≈ 1 km^2) and were extracted for all populations using QGIS version 2.0.1 (QGIS Core Development Team, 2014).

Using climate data from WorldClim and CGIAR CSI, we calculated a subset of climate variables that characterized the growing season, which we defined as May to September inclusively. We investigated climate conditions during the growing season because of an a priori assumption that perennial plants are most actively responding to climatic conditions when they are photosynthetic and growing, rather than when they are dormant during winter. To test our prediction that tetraploid *C. angustifolium* would occupy a drier climatic niche than diploids, we calculated the total growing season soil moisture deficit as the difference between precipitation and PET, for which more negative values indicate drier conditions. We also calculated the total soil moisture deficit for both the early and late growing season by taking the sum of monthly soil-moisture-deficit values for May and June and for July and August, respectively. Another metric of habitat aridity—the Global Aridity Index (GRI)—is available but was not used, because it is calculated as a ratio of (rather than a difference between) PET and precipitation. In situations where both precipitation and PET are low, a ratio can artificially inflate small differences between them. To test our prediction that diploid plants of *C. angustifolium* would occupy a colder climatic niche than tetraploids, we calculated the mean minimum growing season temperature as the mean of all monthly minimum temperature values from May to September.

Statistical analysis—To test our predictions that cytotypes would occupy different climatic niches, we made univariate comparisons of climate variables across the three population categories (pure diploid, pure tetraploid, and

mixed-ploidy) and compared population categories in multivariate climate space. All statistical analyses were conducted in R version 3.0.2 (R Development Core Team, 2014). We used one-way analysis of variance and Tukey's HSD post hoc tests to determine whether particular climate variables were significantly different between population categories. To visualize the populations in multivariate climate space and determine which climate factors were most important for characterizing the environmental conditions experienced by population categories, we conducted a principal component analysis (PCA) with varimax rotation ('psych' package; Revelle, 2014). The loading factors were BIO1 (mean annual temperature), BIO5 (maximum temperature of the warmest month), BIO12 (mean annual precipitation), minimum May temperature, and mean annual PET. Minimum May temperature was used rather than BIO6 (minimum temperature of the coldest month) because it characterizes the temperature of the early growing season. We adhered to the Kaiser criterion (eigenvalues ≥1) when deciding which factors to consider in the PCA.

***Ecological niche models*—**To visualize the climatic niche of each cytotype, we generated ecological niche models in MaxEnt version 3.3.3k (Phillips and Dudík, 2008). We note that the use of niche models here is to illustrate the distribution of each cytotype, and that the models are not meant to test hypotheses about differences in the climatic niches of cytotypes. We allocated 75% of occurrences for training the models and 25% of occurrences for testing the models (McIntyre, 2012). Training occurrences are used to build the niche models, whereas testing occurrences are used to determine the ability of the trained models to "predict" real occurrences.

An assumption of niche modeling is that occurrence data represent a complete and random sampling effort throughout the range of model construction. Although our occurrence data spanned the extent of our models, they were typically clustered within 300-km radii. To reduce the effect of data clustering on model quality, we randomly excluded populations within clusters until all remaining populations were separated by ≥300 km. We included mixed-ploidy populations in both of our models. After accounting for clustering, our diploid model was produced using 13 occurrences and our tetraploid model was produced using 26 occurrences. These sample sizes were likely suitable for our purposes, given that the MaxEnt algorithm has been shown to produce strong fundamental niche predictions for species with just 5 occurrences (Pearson et al., 2007; Wisz et al., 2008).

We produced estimates of the climatic niche by generating 10 models for each cytotype using subsampling replication, and taking the mean model generated from the 10 replicates. Model replication ensures that the final model does not rely on a single training–testing split of the data, but rather considers all available occurrence data. With subsampling replication, the specific occurrences used for training and testing a model are repartitioned randomly without replacement before each model is generated, such that all replicates are statistically independent (Phillips, 2008). We generated logistic models, which estimate the probability (from 0 to 1) that the cytotype being modeled will occur at a particular locality. We set the maximum number of iterations—the number of times the model training algorithm is repeated to find the model parameters—to 1000; all other settings were default. The same climate variables used in the PCA were used to construct the niche models. We assessed the predictive ability of our models using the "area under the receiver operating characteristic curve" (AUC; Fielding and Bell, 1997) and rates of omission on test data points. Strong models have AUC scores >0.5 and omit few test occurrences. We used the mean models to calculate Schoener's *D*—a niche overlap metric that ranges from 0 (no overlap) to 1 (identical niches) (Schoener, 1968)—in ENMTools version 1.4.3 (Warren et al., 2008).

RESULTS

Soil moisture deficit differs across population categories, and these differences vary over the course of the growing season (Table 1). Mean annual PET is highest in pure tetraploid populations, followed by mixed-ploidy populations, which have intermediate mean annual PET (Table 1). Pure tetraploid populations experience a total growing-season soil moisture deficit that is 91 mm more negative than that in pure diploid populations ($F_{2, 131} = 91.2$; $P = 0.005$), but there is no difference in total growing-season soil moisture deficit between pure tetraploid and mixed-ploidy populations ($F_{2, 131} = 37.3$; $P = 0.125$) or between pure diploid and mixed-ploidy populations ($F_{2, 131} = 53.8$; $P = 0.190$). When considering only the early growing season, pure tetraploid populations experience soil moisture deficits that are 31 mm and 35 mm more negative than those in pure diploid populations ($F_{2, 131} = 31.3$; $P = 0.022$) and mixed-ploidy populations ($F_{2, 131} = 34.9$; $P < 0.001$), respectively, but there is no difference between pure diploid and mixed-ploidy populations ($F_{2, 131} = 3.60$; $P = 0.954$). When considering only the late growing season, pure tetraploid populations experience soil moisture deficits that are 45 mm more negative than those in pure diploid populations ($F_{2, 131} = 45.5$; $P = 0.009$). During the late growing season, there is no difference in soil moisture deficit between pure tetraploid and mixed-ploidy populations ($F_{2, 131} = 5.10$; $P = 0.865$), and mixed-ploidy populations experience a mean soil moisture deficit that is 40 mm more negative than that in pure diploid populations ($F_{2, 131} = 40.4$; $P = 0.034$). Across population categories, all months have negative soil-moisture-deficit values during the growing season (Fig. 1A).

Pure tetraploid populations of *C. angustifolium* occupy habitats with temperature conditions that differ from those of pure diploid and mixed-ploidy populations (Table 1). Mean annual temperature in pure tetraploid populations is 2.9°C higher than in pure diploid populations ($F_{2, 131} = 28.8$; $P = 0.002$) and 3.0°C higher than in mixed-ploidy populations ($F_{2, 131} = 30.4$; $P < 0.001$), but does not differ between pure diploid and mixed-ploidy populations ($F_{2, 131} = 1.64$; $P = 0.981$). Similarly, the mean minimum growing-season temperature is 3.5°C higher in pure tetraploid populations than in pure diploid populations ($F_{2, 131} = 3.45$; $P < 0.001$) and 3.8°C higher in pure tetraploid populations than in

TABLE 1. Mean values of five climate variable for pure diploid ($n = 14$), mixed-ploidy ($n = 43$), and pure tetraploid ($n = 77$) *Chamerion angustifolium* populations in North America, Greenland, and Iceland. Significant differences ($P < 0.05$) between population categories are indicated by different letters within rows. Statistical differences were assessed with analysis of variance (ANOVA) and Tukey's HSD post hoc tests. *F*-test statistics for the full ANOVA are shown.

Environmental variable	Pure diploid	Mixed-ploidy	Pure tetraploid	$F_{2, 131}$
Mean annual temperature (BIO1) (°C)	−0.628 a	−0.793 a	2.251 b	17.59**
Mean minimum growing season temperature (°C)	1.967 a	1.585 a	5.422 b	31.33**
Minimum May temperature (°C)	−1.764 a	−2.046 a	1.344 b	27.84**
Mean annual potential evapotranspiration (mm)	520.3 a	625.7 b	717.3 c	25.31**
Total soil moisture deficit (mm)				
Entire growing season (May–September)	−132.71 a	−186.6 ab	−223.9 b	5.732**
Early growing season (May–June)	−63.14 a	−59.53 a	−94.40 b	11.78**
Late growing season (July–August)	−79.35 a	−119.8 b	−124.9 b	4.532*

* $P < 0.05$.
** $P < 0.01$.

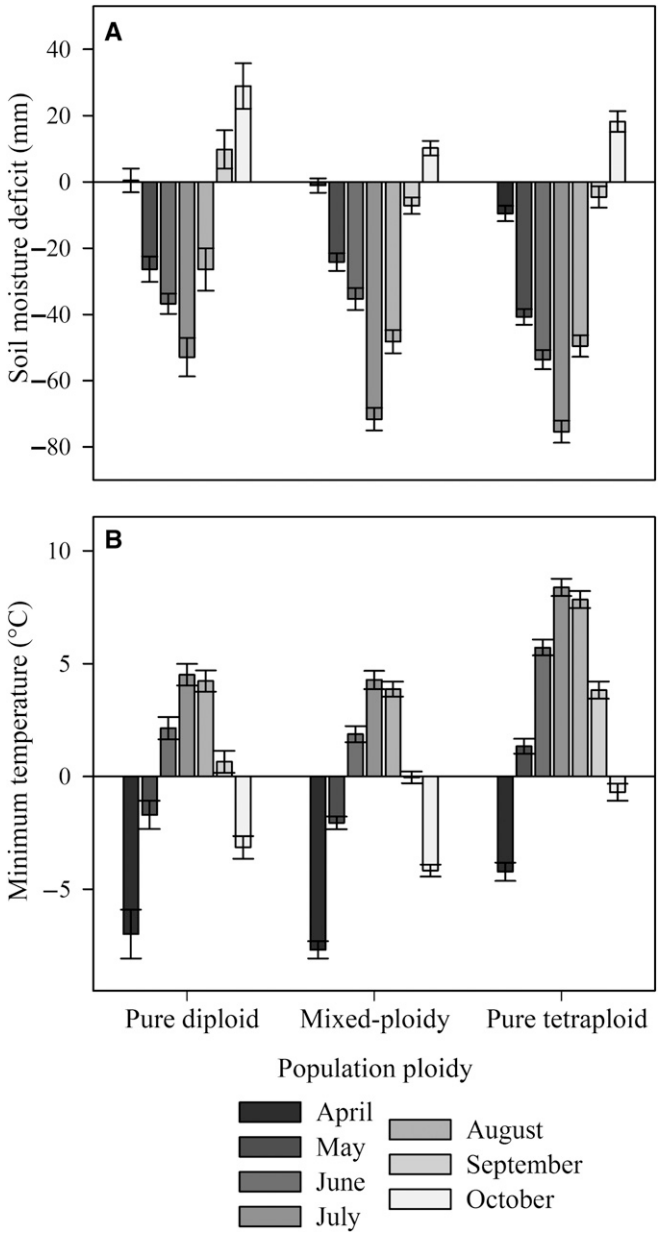

Fig. 1. Mean monthly (± SE) (A) soil moisture deficit and (B) minimum temperature for each *Chamerion angustifolium* cytotype population category in North America, Greenland, and Iceland for April through October. Soil moisture deficit was calculated as the difference between monthly potential evapotranspiration and precipitation; more negative values indicate that habitats are drier.

mixed-ploidy populations ($F_{2, 131} = 3.84$; $P < 0.001$), but does not differ between pure diploid and mixed-ploidy populations ($F_{2, 131} = 0.38$; $P = 0.890$). If the boundaries of the growing season are limited by freezing temperatures, then pure tetraploid populations occupy habitats with longer growing seasons than pure diploid and mixed-ploidy populations (Fig. 1B). Specifically, pure tetraploid populations experience a minimum of 5 mo each year without freezing temperatures, whereas pure diploid and mixed-ploidy habitats experience only 3 or 4 mo each year without freezing temperatures.

TABLE 2. Summary of principal component analysis.

	PC1	PC2
Factor loadings		
Mean annual PET	0.902	0.129
Maximum temperature (BIO5)	0.900	0.414
Minimum May temperature	0.312	0.889
Mean annual precipitation	–	0.199
Mean annual temperature	0.393	0.517
Eigenvalue	3.247	1.091
Proportion of variance explained	0.375	0.257

The PCA revealed two principal components that cumulatively explained 63.2% of variation in the data (Table 2 and Fig. 2). The first component was highly positively correlated with maximum temperature of the warmest month (BIO5) and with mean annual PET. The second component was highly correlated with minimum May temperature. Pure tetraploid populations tended not to occur in the lower-left quadrat of the PCA ordination (Fig. 2), which was characterized by low temperatures and low PET and contained most mixed-ploidy and pure diploid populations. The PCA ordination indicates that mixed-ploidy and pure diploid populations occupy a similar climatic niche, which is different from that of pure tetraploid populations.

The ecological niche models produced predictions of *C. angustifolium* cytotype niches that were well supported by the model-evaluation procedures. The models predicted that diploid plants occur at higher latitudes than tetraploid plants and

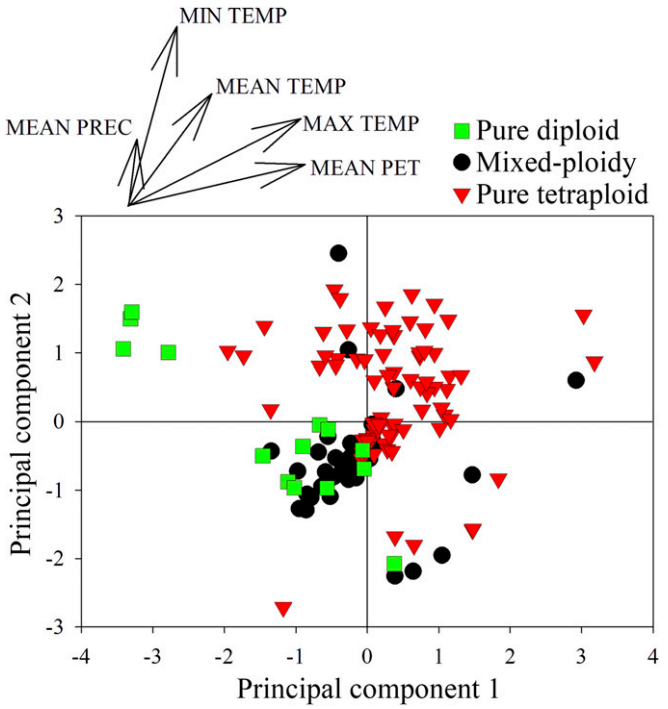

Fig. 2. Scatterplot of principal component analysis axis scores for the two principal components grouped by *Chamerion angustifolium* cytotype population category. A biplot depicting the relative importance and direction of the environmental variables is included above the scatterplot (MEAN PET = mean annual PET; MAX TEMP = maximum temperature of the warmest month [BIO5]; MEAN TEMP = mean annual temperature [BIO1]; MIN TEMP = minimum May temperature; MEAN PREC = mean annual precipitation [BIO12]).

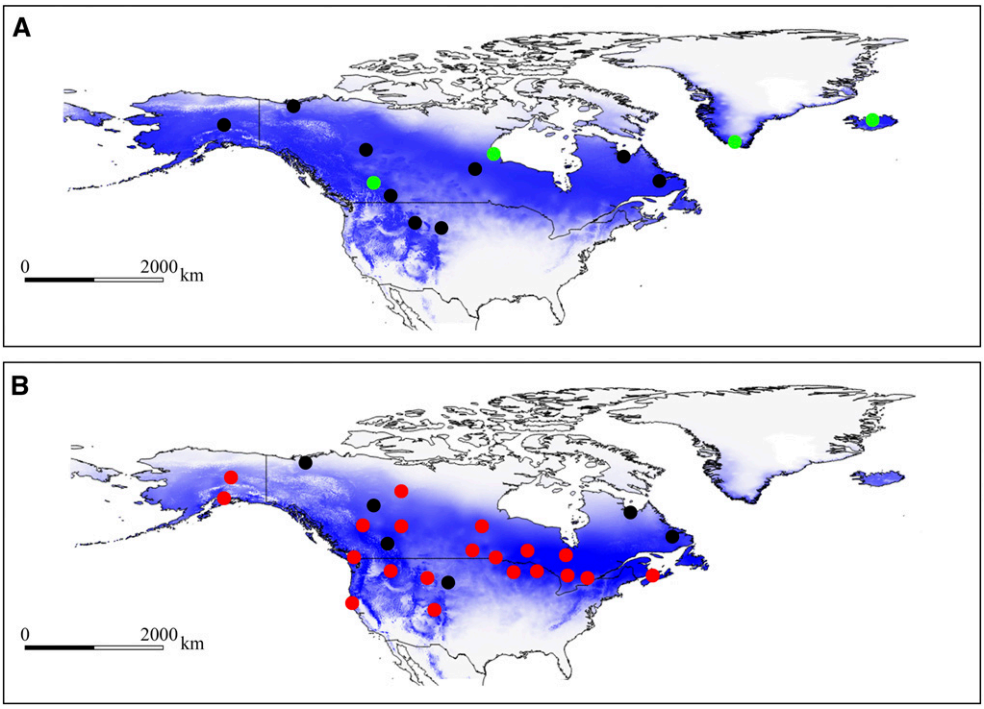

Fig. 3. Average ecological niche models generated in MaxEnt after 10 rounds of subsampling for (A) diploids (n = 13) and (B) tetraploids (n = 26) of *Chamerion angustifolium* across North America, Greenland, and Iceland. Occurrences used for model generation were separated by ≥300 km. White indicates unsuitable habitats, and dark shades of blue indicate highly suitable habitats. Pure diploid populations are indicated by green dots, mixed-ploidy populations are indicated by black dots, and pure tetraploid populations are indicated by red dots. Both models are depicted on the same color–habitat-suitability scale. The models indicate that diploids occupy more northern latitudes than tetraploids, and that tetraploids occupy more southern latitudes. However, the models also indicate a higher degree of niche overlap between cytotypes (Schoener's D = 0.780) than would be extrapolated from previous estimates of cytotype distribution in *C. angustifolium* and our univariate analyses.

that the climatic niche of both cytotypes is continuous across the longitudinal extent of the North American continent (Fig. 3). High AUC scores (diploid model: 0.887 ± 0.030; tetraploid model: 0.885 ± 0.030) and low omission rates of test occurrences (diploid model: 0.066 ± 0.140; tetraploid model: 0.033 ± 0.140) demonstrate that the models predicted test occurrences with high accuracy. Schoener's D metric of niche overlap was 0.780 between the diploid and tetraploid niche models.

DISCUSSION

Our results support the prediction that tetraploid plants of *C. angustifolium* occupy a drier climatic niche than diploids. Specifically, we found that pure tetraploid populations experience a drier growing season than pure diploid populations (Fig. 1A). Although differences in adaptation to water stress were characterized for plants obtained from three mixed-ploidy populations sampled at a single time (Maherali et al., 2009), the predictions derived from these physiological differences were supported using populations sampled over a much larger spatial scale and accumulated over a 15-yr period. These findings suggest that physiological differences observed by Maherali et al. (2009) likely characterize the cytotypes throughout their respective ranges in North America. Nevertheless, it is possible that populations of *C. angustifolium* are locally adapted throughout their range, and physiological differences between cytotypes are likely more variable than characterized previously. Our findings are generally consistent with those of previous studies that have identified a positive correlation between aridity and ploidy level in populations sampled in the field (Hunter et al., 2001; Manzaneda et al., 2012), and with those that have demonstrated that polyploids are more tolerant of drought than their diploid progenitors (Bouharmont and Mace, 1972; Pustovoitova and Borodina, 1981; Garbutt and Bazzaz, 1983; Li et al., 1996, 2009; Maherali et al., 2009; Van Laere et al., 2011; Hao et al., 2013), as well as with the majority of research that has compared diploid and polyploid water relations (reviewed by Levin, 2002; also see te Beest et al., 2012).

Pure diploid populations occur in habitats that are colder than those of pure tetraploid populations, in agreement with our prediction. Previous research demonstrated that diploid plants of *C. angustifolium* have xylem conduits that are narrower than those of tetraploids (Maherali et al., 2009), and narrow conduits are more resistant to freezing-induced cavitation than wide conduits (Davis et al., 1999). Therefore, our finding that diploids occupy colder habitats than tetraploids is consistent with the hypothesis that diploid plants of *C. angustifolium* experience lower levels of freezing-induced cavitation in their range than tetraploids. However, estimates of the degree of freezing-induced cavitation in situ are required to test this prediction. Several field studies that examined the fine-scale distribution of diploids and polyploids found that diploids occur at higher elevations (Husband and Sabara, 2003; Schönswetter et al., 2007) and lower minimum temperatures (Pockman and Sperry, 1997) than polyploids, which suggests that ecological sorting based on cold tolerance can occur between cytotypes. Although the experimental induction of polyploidy has been shown to decrease cold tolerance

(Dvorak and Fowler, 1978), presumably through an increase in cell size (Stebbins, 1950; Davis et al., 1999; Maherali et al., 2009), relative cold tolerances between diploids and polyploids are inconsistent across polyploid taxa (reviewed by Levin, 2002; also see Martin and Husband, 2009). For example, Bowden (1940) planted 100 species of related diploids and polyploids and found no consistent patterns of cold hardiness between them. This inconsistency may be because of selection for increased cold tolerance after polyploidization in some taxa (Dvorak and Fowler, 1978), or because of physiological trade-offs between growth rate and freezing tolerance that maintain variation in cold tolerance between populations (e.g., Medeiros et al., 2012).

In addition to influencing climatic tolerance, physiological differences between cytotypes could contribute to the observed climatic-niche differences by affecting intercytotype competition. For example, tetraploid plants are able to deplete soil moisture to a greater degree than diploids before wilting (Maherali et al., 2009) and could therefore maintain photosynthetic function for longer periods than diploids in water-limited habitats. If this photosynthetic advantage positively affects growth and reproduction, tetraploids could competitively exclude diploids in dry habitats. By contrast, if diploids are more resistant to freezing than tetraploids, they could be at a competitive advantage in relation to tetraploids in cold habitats.

Our results suggest that mixed-ploidy populations occupy a unique climatic niche, rather than one that is strictly intermediate between those of pure diploid and pure tetraploid populations. Although mixed-ploidy populations experience temperatures and early-growing-season soil moisture deficits that are similar to those in pure diploid populations, they experience soil moisture deficits during the late growing season that are similar to those in pure tetraploid populations (Table 1 and Fig. 1A). The transition from low soil moisture deficit early in the growing season to high soil moisture deficit late in the growing season may promote cytotype coexistence, because such dry conditions are likely advantageous to tetraploids and disadvantageous to diploids. These findings are supported by reciprocal transplant experiments with *C. angustifolium* populations across an elevation gradient in the Rocky Mountains, which have shown that mixed-ploidy populations occur in habitats with environmental conditions that are not ideal for either cytotype (Martin and Husband, 2013). Specifically, while diploids outperformed tetraploids at high elevations and tetraploids outperformed diploids at low elevations, neither cytotype had an advantage at the intermediate elevations where mixed-ploidy populations tend to occur (Martin and Husband, 2013). One limitation of our study is that most (72%) of our mixed-ploidy populations were located in the Rocky Mountains. Because of this, our conclusions about environmental conditions promoting coexistence between cytotypes require further study and should be treated as tentative.

It is unlikely that the observed differences in climatic niches and geographic ranges between cytotypes are caused by other factors, such as spatial variation in interactions with pollinators. Kennedy et al. (2006) surveyed three disparate mixed-ploidy populations of *C. angustifolium* in the Rocky Mountains and found that pollinator composition did not differ between cytotypes. In addition, the three most abundant pollinators of *C. angustifolium*—all bees—were very widespread and occurred at a range of elevations and latitudes (Kennedy et al., 2006). In another study, Routley and Husband (2006) examined pollination efficiency on flowers of both diploids and tetraploids from the Rocky Mountains. The authors found no difference between cytotypes in either pollen deposition or pollen removal per visit for any of the dominant bumblebee visitors (Routley and Husband, 2006). Collectively, these observations suggest that pollinator abundances and distributions are unlikely to be responsible for observed differences in geographic range between cytotypes, at least in the Rocky Mountains, where segregation occurs between cytotypes along altitudinal gradients (Martin and Husband, 2013).

The high degree of correspondence between the niche models for *C. angustifolium* and the previously reported geographic distribution of cytotypes provides further evidence that cytotypes are nonrandomly distributed with respect to prevailing climatic conditions. Mosquin and Small (1971) described the geographic distribution of *C. angustifolium* in North America (redrawn by Soltis et al., 2007) and reported cytotype range limits that are similar to those captured by the niche model. One discrepancy between our models and the distribution estimates of Mosquin and Small (1971) is the diploid southern range limit, which extends farther south in our model. Our population data support the description of Mosquin and Small (1971) rather than our model predictions—no diploids were found in any populations located in northern Ontario ($n = 22$), despite our model predicting moderate diploid habitat suitability there. Habitat suitability estimates were higher for tetraploids than for diploids in northern Ontario (Fig. 3), and an ecological explanation for the absence of diploids may be that they are outcompeted by tetraploids in that particular habitat (Grime, 1973; also see Laport et al., 2013). The inability of ecological niche models to account for biotic interactions, such as competition, may be why our estimate of niche overlap (Schoener's $D = 0.78$) is higher than would be extrapolated from the geographic overlap estimate of Mosquin and Small (1971).

The high incidence of niche overlap between our niche models contrasts with the results of our univariate analyses and raises questions about the utility of ecological niche modeling for comparisons of cytotype distribution. On the basis of our niche models alone and the associated *D*-metric, it appears as if there is broad-scale niche conservatism between *C. angustifolium* cytotypes. Our univariate analyses, however, suggest that this is not likely the case in nature. For example, total growing-season soil moisture deficit was 69% higher in pure tetraploid populations than in pure diploid populations (Table 1), which indicates that climatic conditions differ considerably between these population categories. This discrepancy may be due to the MaxEnt algorithm itself, which has been criticized recently for underpredicting species range sizes compared with conventional likelihood methods (see Royle et al., 2012). Our results contrast with a recent study by Glennon et al. (2014), who calculated climatic niche overlap for 20 diploid–polyploid pairs and found that the broad-scale climatic niches were largely conserved between cytotypes. Similarly, Martin and Husband (2009) found that diploid and polyploid congeners tended to occupy similar climate envelopes. Given the discrepancy between our univariate analyses and our niche models, we argue that broad niche-modeling approaches may not be sufficiently sensitive to detect biologically meaningful differences in the climatic niches of diploid and polyploid cytotypes. We suggest that a more targeted approach, testing species-specific predictions that are rooted in an understanding of the species' biology, may be more effective for uncovering ecologically relevant climatic differences between related diploids and polyploids.

***Conclusion*—**Our findings are consistent with the hypothesis that the geographic distributions of North American diploid

and tetraploid *C. angustifolium* cytotypes can be influenced by physiological and ecological responses to climate. Specifically, we observed that differences in the climatic niches of cytotypes were consistent with their respective physiological tolerances. Moreover, the observed climatic niche differentiation between cytotypes makes it unlikely that historical factors are responsible for differences in cytotype ranges (Godsoe et al., 2013). Although climatic and geographic differences between cytotypes vary among species, our observation that tetraploid *C. angustifolium* occupy a drier niche than diploids is consistent with previous findings that polyploids are generally more tolerant of drought than diploids. To further test hypotheses about the correspondence between cytotype functioning and distribution, future research could examine whether polyploids consistently tolerate drought better than congeneric diploids in the field, and whether physiological differences between cytotypes can predict the outcome of competition across relevant resource gradients.

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