American Journal of Botany

American Journal of Botany 100(3): 496–508. 2013.

Polyploidization in *Heuchera cylindrica* (Saxifragaceae) did not result in a shift in climatic requirements[1]

William Godsoe[2,3], Megan A. Larson[4,5], Kelsey L. Glennon[4], and Kari A. Segraves[4,6]

[2] Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand; [3] National Institute for Mathematical and Biological Synthesis, University of Tennessee, 1534 White Avenue, Knoxville, Tennessee 37996 USA; [4] Department of Biology, Syracuse University, 107 College Place, Syracuse, New York 13244 USA; and [5] Biological Sciences Department, Binghamton University, Vestal Parkway East, Binghamton, New York 13902 USA

- *Premise of the study:* Polyploidization is a key factor involved in the diversification of plants. Although polyploids are commonly found, there remains controversy on the mechanisms that lead to their successful establishment. One major problem that has been identified is that newly formed polyploids lack mates of the appropriate ploidy level and may experience severely reduced fertility due to nonproductive intercytotype crosses. Niche differentiation has been proposed as a primary mechanism that can alleviate this reproductive disadvantage and facilitate polyploid establishment. Here we test whether the establishment of tetraploid cytotypes of *Heuchera cylindrica* (Saxifragaceae) is consistent with climatic niche differentiation.
- *Methods:* We use a combination of field surveys, flow cytometry and species distribution models to: (1) examine the distribution of diploid and tetraploid cytotypes; and (2) determine whether tetraploid *Heuchera cylindrica* occupy climates that differ from those of its diploid progenitors.
- *Key results:* The geographic distributions of diploid and tetraploid cytotypes are largely allopatric as an extensive survey of 636 plants from 43 locations failed to detect any populations with both cytotypes. Although diploids and tetraploids occur in different geographic areas, polyploid *Heuchera cylindrica* occur almost exclusively in environments that are predicted to be suitable to diploid populations.
- *Conclusions:* Climatic niche differentiation does not explain the geographic distribution of tetraploid *Heuchera cylindrica*. We propose instead that tetraploid lineages were able to establish by taking advantage of glacial retreat and expanding into previously unoccupied sites.

Key words: habitat differentiation; *Heuchera;* minority cytotype exclusion; niche; plant polyploidy; Saxifragaceae; species distribution modeling

Polyploidy, or whole genome duplication, is a common mechanism of plant speciation that may account for approximately 15% of speciation events in angiosperms alone (Wood et al., 2009). In addition to its role in speciation, whole genome duplication can result in changes such as enhanced genetic diversity, genomic rearrangements, heterosis, and shifts in phenotype that place polyploid lineages on new evolutionary trajectories (e.g., Levin, 1983; Song et al., 1995; Levin, 2002; Ramsey and Schemske, 2002; Osborn et al., 2003; Adams and Wendel, 2005; Leitch and Leitch, 2008). These immediate changes combined with selection following polyploidization provide ample opportunity for further differentiation.

Although polyploid lineages are a ubiquitous feature of many plant taxa, recent efforts have shown that polyploids have higher extinction rates than their diploid counterparts, suggesting an evolutionary dead end for many newly formed polyploids (Mayrose et al., 2011). A likely explanation for this pattern is that many polyploid lineages may become extinct at the establishment phase (Thompson and Lumaret, 1992; Arrigo and Barker, 2012). Newly formed polyploids are at a reproductive disadvantage due to pollen swamping by diploids (Levin, 1975), and interbreeding between common diploids and rare polyploids can block the establishment of the minority cytotype via the production of infertile intercytotype hybrids (Kay, 1969; Van Dijk and Bakx-Schotman, 1997; Baack, 2005). The degree of the minority cytotype disadvantage will depend on the strength of selection against intercytotype hybrids, selfing rates or other mechanisms of assortative mating, the frequency of the minority cytotype, and the rate of formation of *2n* gametes by the diploid population (e.g., Felber, 1991; Rodriguez, 1996; Felber and Bever, 1997; Yamauchi et al., 2004). Consequently, mechanisms that limit intercytotype

[1] Manuscript received 7 June 2012; revision accepted 10 December 2012.

D. Althoff, C. Klunder, A. Moe, and three anonymous reviewers provided comments on an earlier draft of the manuscript. R. Ruppel helped collect samples in the field and E. Carr provided assistance with ArcGIS. M. Sklaney potted plants and provided plant care during the field season. The authors also thank N. Gonchoroff, B. Husband, and P. Kron for technical assistance with flow cytometry, A. Johncox helped with chromosome counts, and D. Althoff, R. Ruppel, M. Salinas, N. Schwarting, and M. Sklaney assisted with sample preparation. R. Hicks kindly provided the FSCPress software, P. Brunsfeld and E. Poor provided a database of plant records from the University of Idaho herbarium, and P. Wheeler provided trout blood. The Idaho, Montana, Oregon, and Washington park services provided access to field sites. The study was funded by a Ruth Meyer Summer Scholarship (to M.A.L.), a Renée Crown Honors Wise-Marcus Award (to M.A.L.), and National Science Foundation grant DEB 0743101 (to K.A.S.). W.G.'s contribution to this work was conducted while a Postdoctoral Fellow at the National Institute for Mathematical and Biological Synthesis, an Institute sponsored by the National Science Foundation, the U.S. Department of Homeland Security, and the U.S. Department of Agriculture through NSF Award #EF-0832858, with additional support from The University of Tennessee, Knoxville. K.L.G. was supported by a National Science Foundation Bioinformatics Postdoctoral Fellowship.

[6] Author for correspondence (e-mail: ksegrave@syr.edu)

doi:10.3732/ajb.1200275

matings have frequently been sought to explain the establishment of polyploids. For instance, shifts in flowering phenology or changes in floral traits that impact pollinator attractiveness may prevent cross-fertilization between cytotypes (e.g., Van Dijk and Bijlsma, 1994; Bretagnolle and Thompson, 1996; Husband and Schemske, 1998; Segraves and Thompson, 1999; Husband and Schemske, 2000; Husband and Sabara, 2003; Thompson and Merg, 2008), resulting in at least partial reproductive isolation in mixed cytotype populations.

Spatial segregation of cytotypes via niche differentiation is thought to be one of the primary mechanisms involved in alleviating the minority cytotype disadvantage (Fowler and Levin, 1984; Thompson and Lumaret, 1992), and in fact, it has been suggested to be a 'prerequisite' for polyploid speciation (Levin, 2003). Polyploids and their diploid progenitors often have different ecological requirements (e.g., Levin, 1983; Lumaret et al., 1987; Jay et al., 1991; Felber-Girard et al., 1996; Petit et al., 1999; Johnson et al., 2003; Raabová et al., 2008), and sometimes polyploids occupy new or broader niches (e.g., Thompson and Lumaret, 1992; Otto and Whitton, 2000; Brochmann et al., 2004; Treier et al., 2009). Contrastingly, broad surveys of polyploid taxa suggest that ecological breadth may not necessarily increase with polyploidy (Stebbins and Dawe, 1987; Petit and Thompson, 1999; Martin and Husband, 2009), and a number of studies of individual species have failed to reject the null hypothesis of no differentiation (e.g., Baack and Stanton, 2005; Buggs and Pannell, 2007; Sampoux and Huyghe, 2009). Indeed, since polyploids are derived from their diploid progenitors, the null prediction is one of niche conservatism (sensu Wiens and Graham, 2005) where diploids and polyploids have similar ecological requirements simply because they are close relatives. This pattern might be expected in young polyploid lineages where genome duplication does not directly impact ecological requirements. Polyploids, on the other hand, may diverge via selection favoring polyploid lineages that can survive in environments less favorable for diploids. Thus, niche divergence may be a direct result of genome duplication (Ramsey, 2011), selection, or a combination of these factors.

Given the diverse mechanisms that contribute to polyploid establishment, it is not surprising that the geographical distribution of cytotypes varies broadly, from completely nonoverlapping ranges to various degrees of admixture (reviewed by Levin, 2002). Both adaptive and nonadaptive processes have been used to explain these geographical patterns. For example, nonoverlapping distributions may occur when diploids and polyploids have different ecological niches; however, such a distribution may also be explained independently of niche differentiation. Established populations may be difficult to invade by the alternative cytotype; therefore, migration and stochastic processes may lead to geographic separation of diploids and polyploids where the first cytotype to establish in an area is the one that persists (Lewis, 1967). Although spatial segregation of cytotypes has long been recognized (e.g., Kay, 1969; Lewis, 1980; Van Dijk et al., 1992; Van Dijk and Bakx-Schotman, 1997), the underlying mechanisms creating these patterns remain poorly understood despite repeated calls for study (Stebbins, 1980; Soltis and Soltis, 2009). Consequently, there is a strong need to evaluate the relative contributions of ecological divergence, minority cytotype exclusion, and migration to the geographical distribution of naturally formed polyploids.

Here we use flow cytometry and species' distribution modeling (SDM) to determine whether ecological divergence has played a role in structuring the geographic distribution of cytotypes in the rhizomatous perennial *Heuchera cylindrica* Dougl. ex Hook. (Saxifragaceae). This species is a member of the Heucherina, a well-supported clade that includes the genera *Tolmeia*, *Elmera*, *Heuchera*, and *Tellima* (Soltis et al., 2001). Many of the phylogenetic relationships within *Heuchera* are poorly resolved (e.g., Soltis and Kuzoff, 1995; Okuyama et al., 2008), and the sister species of *H. cylindrica* remains to be determined (Okuyama et al., 2008). *H. cylindrica* grows on rocky outcrops in the Pacific Northwest and both diploid (2n = 14) and tetraploid (2n = 28) forms were previously identified via chromosome counts on a few samples (Soltis, 1984). The geographic distribution of cytotypes is largely unknown in *H. cylindrica*. Thus, the goals of this study were to: (i) determine the geographic distribution of cytotypes across the range of the species; and (ii) test whether polyploids show evidence of climatic niche divergence from their diploid ancestors. Given that we are using extant polyploid populations, any observed niche differentiation could be attributed to a direct phenotypic effect of polyploidy and/or selection on polyploids following establishment. Therefore, the current study tests whether the establishment of the geographic distribution of cytotypes is consistent with niche differentiation.

We accomplished the first goal by assessing plant nuclear DNA content using flow cytometry, a quick and accurate method to determine ploidy level (Kron et al., 2007). The second goal was addressed using SDM to test whether the two cytotypes have diverged in their climatic niche requirements. If this hypothesis is supported, we expected that tetraploid populations should occupy climatic environments that diploids do not occupy. We tested this hypothesis by fitting models to the distribution of each cytotype independently and then used these models to predict the distribution of the alternate cytotype. If diploid and tetraploid populations have similar climatic requirements, we expected the model constructed with diploid climates to accurately predict the range of tetraploids and vice versa. Together, the results demonstrate that polyploid populations occur almost exclusively in climatic environments that are predicted to be suitable to diploid populations.

MATERIALS AND METHODS

Study Species—*Heuchera cylindrica* is a common perennial found in rocky soils at low to mid elevations in the Pacific Northwest of North America (Fig. 1; Hitchcock and Cronquist, 1973). The geographic range includes the Cascade Mountains of British Columbia and Washington, Idaho, western Montana, northwestern Wyoming, Oregon, and as far south as northern California. Some *H. cylindrica* populations occur near those of the congener *H. grossulariifolia* Rydb., making an allopolyploid origin possible; however, sequencing analysis suggested that there was no hybridization between these species (Segraves et al., 1999). Additionally, preliminary analyses of controlled crosses suggested a pattern of tetrasomic inheritance, consistent with an autopolyploid origin (Ruppel and Segraves, Syracuse University, unpublished data). An autopolyploid origin would be similar to that of a number of other members of the Saxifragaceae, including two other *Heuchera* species (Soltis and Bohm, 1986; Soltis and Rieseberg, 1986; Ness et al., 1989; Wolf et al., 1989; Judd et al., 2007).

Inflorescences are scapose with 50-200 self-incompatible flowers. The flowers are generally pollinated by bumblebees, solitary bees, and the prodoxid moth *Greya enchrysa* Davis & Pellmyr, a close relative of the yucca moths (Pellmyr et al., 1996). Mid- to late summer, the small (< 1 mm), echinulate seeds are dispersed by dehiscent capsules and once the scapes have dried, they abscise, releasing any remaining seeds. Although the plants outcross via seed production, they can also reproduce asexually via rhizomes, forming dense clumps up to one meter in diameter. There is considerable variation among

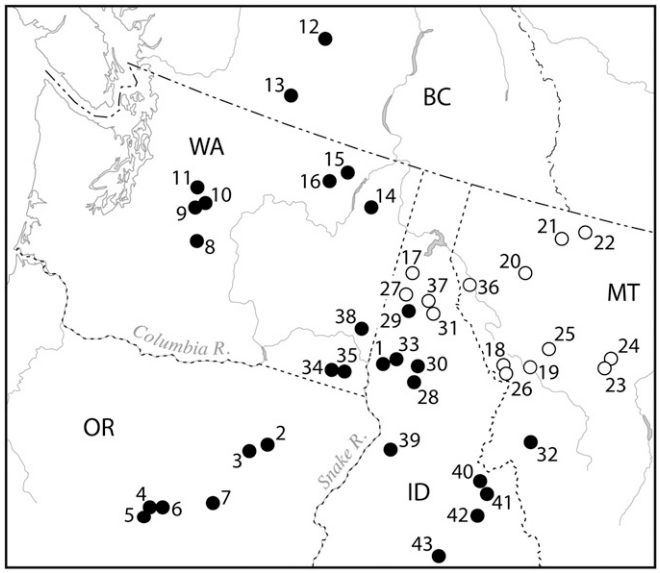

Fig. 1. Distribution of cytotypes among populations of *Heuchera cylindrica.* Closed circles indicate diploid populations and open circles indicate tetraploid populations. The numbers corespond to site information in Table 1. (BC = British Columbia, Canada; ID = Idaho, USA; MT = Montana, USA; OR = Oregon, USA; and WA = Washington, USA.)

populations in leaf shape and pubescence (Hitchcock and Cronquist, 1973; Althoff and Thompson, 2001).

***Sample collection*—**We collected 662 plants from 43 field sites (mean = 14.8 plants per site; range of 2-23 plants per site) across the range of *Heuchera cylindrica*, including Idaho, Washington, Oregon, Montana, and British Columbia (Table 1). Samples were taken from plants separated by at least one meter to avoid collecting clones. Each sample consisted of a 3 cm segment of rhizome that was wrapped in a damp paper towel and shipped overnight to Syracuse University. Rhizomes were potted in sterile Metro-Mix 360 soil in 335 cm^3 Panterra pots (ITML Horticultural Products, Middlefield, Ohio, USA) and grown in a common garden until leaf buds were available to use for flow cytometry, an average of 28 d. Plants were fertilized weekly. Of the 662 plants collected, 638 individuals survived for cytological analysis.

***Determining ploidy level*—**Flow cytometry was used to determine ploidy level by measuring the DNA content of nuclei (Kron et al., 2007). Five leaf buds were finely chopped with razor blades (Galbraith et al., 1983) for 20 s or less in a magnesium sulfate buffer (Arumuganathan and Earle, 1991), consisting of 10 mM magnesium sulfate heptahydrate (Mallinckrodt, Hazelwood, Missouri, USA), 50 mM potassium chloride (EMD Chemicals, Gibbstown, New Jersey, USA), 5 mM hepes (EMD Chemicals), 6.8 mM dithiothreitol (MP Biomedicals, Solon, Ohio, USA), 1 mM polyvinyl pyrrolidone (PVP-40; MP Biomedicals), and a final concentration of 10% w/v Triton X-100 (Alfa Aesar, Ward Hill, Massachusetts, USA). New razor blades (American Line Single Edge, American Safety Razor Company, Verona, Virginia, USA) were used for each plant. Chopped tissue was filtered using 30 μm nylon filter membranes (Cole-Parmer Instrument Co, Nile, Illinois, USA). Filtered samples were centrifuged at 13 200 rpm for 30 s and the supernatant was discarded. The nuclei were resuspended in a solution of 5 mg/ml propidium iodide stain, 10 mg/ml RNase (Sigma, St. Louis, Missouri, USA), and fresh rainbow trout blood diluted 1:11 with Alsever's solution (MP Biomedicals). The samples were mixed by vortexing and immediately analyzed on a LSR II flow cytometer (Becton Dickinson, San Jose, California, USA). The parameters for the flow cytometer required slight adjustments among runs to obtain the best results (average voltage settings: Forward Scatter Fluorescence: 604, Side Scatter Fluorescence: 418, and Propidium Iodide Fluorescence: 638). We analyzed a minimum of 10 000 nuclei per sample and ensured that both sample and standard peaks had a coefficient of variation (CV) ≤ 10%. In cases where the CV exceeded 10%, the sample was reanalyzed from fresh starting material. The average fluorescence and CV of plant and trout peaks were determined using the FCSPress 1.4 software (Ray Hicks, www.FCSPress.com). DNA content was calculated by comparing the mean peak fluorescence for plant nuclei with the mean peak fluorescence for the trout blood internal standard. Rainbow trout nuclei contain 5.05 pg per diploid genome (Vindeløv et al., 1983); thus, the mean plant peak/mean trout blood peak * 5.05 pg yields the plant DNA content. This provides the 2C value, where C is the total amount of DNA per nucleus (e.g., Harbaugh, 2008). A Welch ANOVA correcting for heteroscedasticity was used to determine whether the average 2C values differed between cytotypes. Statistical analyses were implemented using JMP 5.0.1.2 (SAS Institute, 2003).

The results from the flow cytometry analysis were confirmed with chromosome counts on two samples of each ploidy level. We used a modified method of Fukui (1996). Root tips were fixed in Farmer's fixative (3:1 absolute ethanol:glacial acetic acid) for 48 h at 4°C and then stored in 70% ethanol. Samples were transferred into a hydrochloric acid-acetocarmine solution (10% 1M HCl, 1% acetocarmine) and heated for 10 min at 60°C. The root cap and any remaining debris were removed prior to slide preparation. A drop of acetocarmine was used during preparation of the slide mounts. Mitotically active cells were observed under 1000× magnification.

***Species distribution models*—**We modeled the distribution of *Heuchera cylindrica* using climate data from the Pacific Northwest, here defined as the region between 42 to 53 degrees N latitude, 108 to 126.5 degrees W longitude (Fig. 2). This region encompasses most of the range of *H. cylindrica*. We used 17 of the bioclimatic variables that are hypothesized to represent features of climate most relevant for predicting a species' distribution at a spatial resolution of 30 arc seconds, or approximately one kilometer (Hijmans et al., 2005). Although 19 bioclimatic variables are typically used, exploratory analyses revealed an abrupt boundary for Bioclim 8 and 9 in our study area that may be an artifact of the way these variables were calculated (R. J. Hijmans, University of California-Davis, personal communication; Appendix S1: see Supplemental Data with the online version of this article). As a consequence, we eliminated these two variables from subsequent analyses.

We modeled the distribution of each ploidy level using two algorithms, maximum entropy (Maxent version 3.2.19; Phillips et al., 2006) and generalized linear models (GLMs). Maximum entropy is a machine-learning algorithm that seeks to use available information on the environment to predict presences for species of interest. Maxent fits a relatively complex model by minimizing the unexplained information in the dataset (i.e., maximizes the entropy of the residuals). Recent, extensive comparisons of available methods indicate that this algorithm is a superior choice for modeling the probability that an organism is present, and it is particularly robust to relatively small sample sizes such as those available in the current study (Elith et al., 2006; Guisan et al., 2007a; Guisan et al., 2007b). Results from previous studies indicate that algorithms such as Maxent can produce reasonably strong models with as few as five presences (Hernandez et al., 2006). We fit Maxent using default settings, i.e., a logscale output which uses a scale similar to the output of logistic regression, regularization multiplier of 1 which tunes the complexity of the model output, and a maximum of 500 iterations to find an optimal solution. We deemed the model to have reached an optimal solution using a convergence threshold of 0.00001.

The validity of the Maxent results was confirmed with GLMs. This is particularly important, as Maxent can be prone to produce poor extrapolations in novel biogeographic regions (Elith and Graham, 2009; Godsoe, 2010b). GLMs require the user to specify how the explanatory variables (climate data) shape the probability that a species will be present. We elected to include linear and squared terms in our model, implying that a species may do well at high, low, or intermediate values for a given climate variable. The squared term allows us to examine nonlinear relationships where a species might perform best at an intermediate climate (e.g., performance is best at intermediate temperatures and lower at both high and low temperatures). We tested three alternative parameterizations of GLMs per cytotype. These models included linear and squared terms for either the top three, top two, or top climate variable(s) with the highest percent contribution in the corresponding Maxent model. We then used the Akaike Information Criterion (AIC) to select the most appropriate model from this set of candidates. AIC scores provide a ranking of the relative ability of each model to explain the observed data and include a penalty term for model complexity (Burnham and Anderson, 2002). In this framework, a model with the lowest AIC score is retained as it provides the optimal balance between the simplicity of the model and the model that best fits the data. Conversely, models that differ from this best model by ≥ 2 units (hereafter Δ AIC) provide an inadequate explanation of the data and are rejected.

Reliable absence data were unavailable for the Pacific Northwest and so we fit each model using randomly selected points from the study area of interest that are interpreted as locations in which *Heuchera cylindrica* is unlikely to be

TABLE 1. Site information for *Heuchera cylindrica* samples. Site numbers correspond to the numbers in Fig. 1.

Site No.	Population	N	N Latitude	W Longitude	Elevation (m)
1	Spalding, ID	20	46°27.467’	116°47.005’	304.5
2	Hilgard, OR	16	45°20.492’	118°14.205’	1039.4
3	Red Bridge, OR	13	45°16.069’	118°21.937’	1000.7
4	Little Hay Creek, OR	16	44°24.606’	120°29.014’	1282.6
5	Ochoco, OR	17	44°24.178’	120°30.294’	1232.6
6	Moss Hill, OR	20	44°25.697’	120°21.618’	1421.6
7	Kimberly, OR	20	44°31.389’	119°37.662’	816.6
8	Durst Creek Road, WA	10	47°19.403’	120°40.621’	924.8
9	Alpine Lake, WA	17	47°34.687’	120°47.795’	709.3
10	Josephine Crag, WA	16	47°39.260’	120°43.773’	534.0
11	Powerline, WA	12	47°47.090’	120°53.564’	847.9
12	Lake Kalamalka, BC	17	50°09.628’	119°22.251’	536.4
13	Marrow Road, BC	19	49°22.125’	119°40.816’	667.8
14	Blue Creek Road, WA	19	48°17.944’	117°54.246’	643.7
15	Sherman, WA	10	48°36.217’	118°30.304’	1598.7
16	Thirteen Mile, WA	18	48°30.732’	118°44.204’	830.6
17	Coeur d’Alene Lake, ID	19	47°37.236’	116°40.727’	653.8
18	Petty Creek, MT	20	46°55.833’	114°26.720’	1021.4
19	Blackfoot River, MT	17	46°52.447’	113°51.695’	1008.0
20	Ashley Lake, MT	21	48°06.646’	114°34.788’	1068.3
21	West Glacier, MT	9	48°32.300’	113°54.672’	972.6
22	East Glacier, MT	5	48°45.145’	113°26.763’	1369.2
23	Gould Creek, MT	21	46°53.174’	112°23.031’	1496.3
24	Flesher Pass, MT	20	46°58.403’	112°20.389’	1756.0
25	Salmon Lake, MT	20	47°04.308’	113°23.058’	1229.6
26	Lolo Creek, MT	20	46°46.855’	114°24.689’	1164.6
27	Benewah Lake, ID	20	47°20.843’	116°41.882’	653.8
28	Eleven, ID	20	46°23.054’	116°10.178’	491.6
29	Santa Creek, ID	12	47°09.169’	116°31.387’	842.2
30	Dent Bridge, ID	17	46°35.956’	116°10.053’	514.2
31	Calder (Big Creek), ID	14	47°17.712’	116°07.383’	707.4
32	Sula, MT	19	45°94.000’	113°73.000’	1659.6
33	Orofino, ID	15	46°30.626’	116°33.537’	279.2
34	Blue Mountains, WA	9	46°13.000’	117°46.000’	1241.0
35	Teal Springs, Blue Mountains, WA	12	46°12.223’	117°34.363’	683.4
36	Beaver Creek, MT	12	47°42.060’	115°33.513’	817.5
37	Calder, ID	6	47°16.455’	116°13.786’	696.2
38	Albion, WA	3	46°47.244’	117°15.147’	770.5
39	Lake Creek Road, Salmon River, ID	4	45°23.967’	116°12.908’	543.2
40	Main Fork Salmon River, ID	2	45°18.973’	114°23.917’	987.6
41	Panther Creek, ID	3	45°05.041’	114°14.995’	1606.6
42	Morgan Creek, ID	13	44°42.526’	114°16.210’	1770.6
43	Stanley, ID	23	44°15.561’	114°47.808’	1885.5

found. These points are known as pseudoabsences in GLM or background points in Maxent. Models including pseudoabsences work best when a species is rare but well sampled. Thus, we designed our background sampling in a way to maximize our ability to make inferences about the niches of the two cytotypes. Recent mathematical work indicates that a distribution model can serve as an estimate for the probability that an environment is suitable if presences and absences are sampled from environments to which the study organism can disperse (Anderson and Raza, 2010; Godsoe, 2010a; Barve et al., 2011; Godsoe, 2012). As a result, we divided the Pacific Northwest into two subregions, corresponding roughly to locations closer to diploid populations and locations closer to tetraploid populations, hereafter referred to as the range of diploid and tetraploid populations, respectively (Fig. 2). We then fit each SDM using pseudoabsence or background data from the appropriate portion of the Pacific Northwest. For GLM, we fit models using all the presence data for a particular ploidy level and an equal number of pseudoabsences selected at random from the subregion associated with that cytotype. Assuming that these locations represent a good approximation of the range of each cytotype, this procedure should minimize the chance of confusing unsuitable environments with locations that are unavailable to one cytotype because of dispersal limitations or the presence of the other cytotype. In Maxent, we selected 1000 background points from the subregion associated with a particular ploidy level. Models fitted with pseudoabsence data have a slightly different interpretation from models fitted with absence data (Phillips et al., 2009). They do not represent an estimate of the probability that a species is present, and as a consequence, they are not an estimate of the probability that an environment is suitable. Rather, these models represent a surrogate for these quantities, a function that increases monotonically with the probability that a species will be present (Phillips et al., 2009).

Since each subregion contains different environments, two taxa with nonoverlapping distributions may occur in a different set of environments even if their niches are identical (Godsoe, 2010b, 2012). For this reason, we determined if differences in the environments occupied by each ploidy were a consequence of differences in the environments available in each subregion (Broennimann et al., 2007; Godsoe et al., 2009; Godsoe, 2010a). We did this using two diagnostics designed to identify regions where one cytotype has access to climates that are unavailable to the other. First, we computed the entire range of climatic conditions (minimum value and maximum value) for each environmental variable present in the range of each cytotype. We then identified locations in the range of the other cytotype where the value of at least one climatic variable was less than the minimum value or greater than the maximum, which we scored as locations with nonanalogous climates (Fig. 2). We treat extrapolation of SDMs in these locations with caution, and in one of our analyses we exclude these sites and compute model accuracy including only sites with analogous climates. Second, we generated pairwise scatterplots of the three variables that contributed the most to predictions of diploid presence and the three variables that contributed the most to predictions of tetraploid presences. These plots include presences for each cytotype and a minimum convex polygon around the climates available to each cytotype. With this information, we visually delineated examples of presences for one cytotype that occur in environments

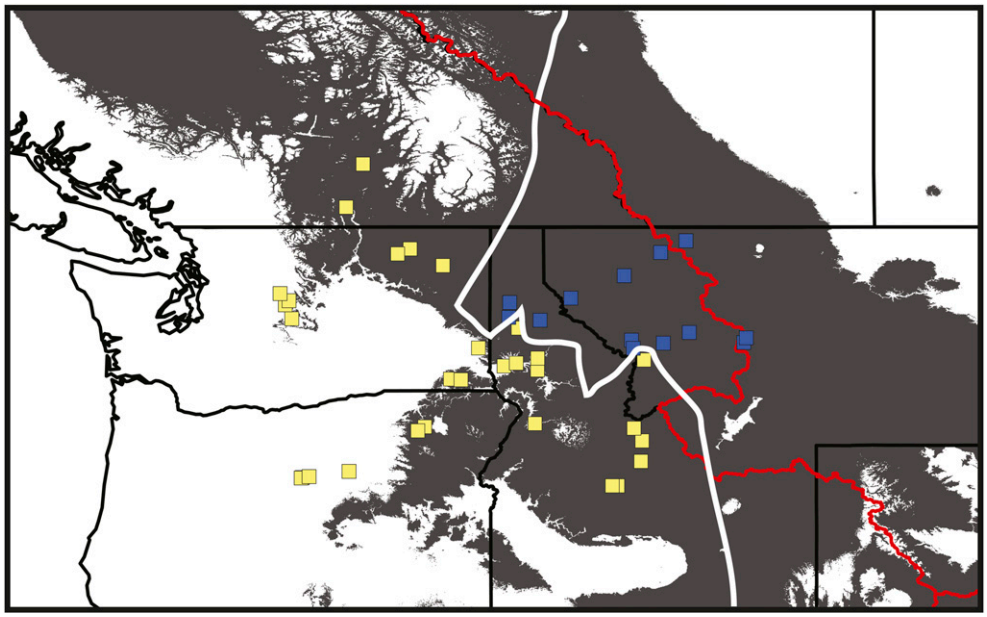

Fig. 2. An overlay of the distribution of *Heuchera cylindrica* samples with the climates available to each cytotype. Diploid populations are yellow, tetraploid populations are blue. The white line indicates the boundary between environments closer to diploid populations (to the left) and environments closer to tetraploid populations (to the right). The red line shows the continental divide. Gray shading indicates areas with climates available to both cytotypes. White portions of the map indicate areas with a climate not available to one cytotype. For example, the westernmost part of the diploid subregion has a maritime climate moderated by the Pacific Ocean. No portion of the tetraploid region has an analogous maritime climate and so this portion of the diploid subregion is white.

unavailable to the other cytotype. These scatterplots also allow visual assessment of the similarity of the distributions of the two cytotypes and the similarities of the environments available to them. We have not assessed statistical significance of these relationships using classical uni- or multivariate approaches (e.g., principal components analysis), as previous work has indicated that the *P*-values generated by these methods are suspect (Godsoe, 2010b; Broennimann et al., 2012).

We tested the accuracy of our distribution models using area under curve (AUC) scores, a measure of a model's ability to correctly distinguish presences from absences. This statistic ranges from zero to one with a score of one representing a perfect ability to distinguish presences from absences and a score of 0.5 representing a model that makes predictions that are no better than chance. We calculated AUC scores using two sets of distribution data: (1) using the locations of known ploidy; and (2) using an independent dataset of herbarium localities of unknown ploidy. We present comparable statistics across our two modeling algorithms by reporting the AUC scores calculated in the PresenceAbsence package in R (R Development Core Team, 2006; Freeman, 2007). We compared our sampling of the distribution of *Heuchera cylindrica* to the known distribution of this taxon using a database of herbarium collections (Appendix S2; see Supplemental Data with the online version of this article). This database consisted of observations from 139 locations from the University of Washington herbarium, Seattle, Washington, USA (http://biology.burke.washington.edu/herbarium/collections/vascular/search.php), 190 records from the University of Idaho herbarium, Moscow, Idaho, USA (obtained from P. Brunsfeld) and seven from the herbarium at Washington State University, Pullman, Washington, USA (http://public.wsu.edu/~wsherb/dbhome.html). We also obtained records from the University of Montana herbarium, Missoula, Montana, USA (http://herbarium.dbs.umt.edu/database/DisplayItem.aspx?id=Heuchera%20cylindrica&locality=); however, location information was only available at the county level so we did not include these data in the final analysis.

***Evaluating model similarity*—**We tested whether the distribution models were equivalent by measuring the ability of models developed for one cytotype to extrapolate the distribution of the other cytotype. To do this in one subregion (e.g., the tetraploid subregion Fig. 3D), we calculated an AUC score for an SDM generated by the cytotype occurring in that subregion (in this example, the AUC score of a model constructed from tetraploids). We then used an SDM generated for the other cytotype (diploids) to predict where we should expect to see that cytotype in the focal subregion (Fig. 3B). If the two cytotypes have similar environmental requirements, then we should expect these two models to produce similar AUC scores within a subregion. We calculated the standard error for AUC using the PresenceAbsence package, but note that this standard error is conservative (Freeman, 2007) and therefore may miss subtle shifts in environmental requirements. In a recent comparison of available methods, this procedure correctly inferred similar environmental requirements for species with identical niches across moderate environmental gradients, but was biased toward inferring different environmental requirements across strong gradients (Godsoe, 2010b), whereas several simulation studies indicate that classical methods such as Multivariate Analysis of Variance and Linear Discriminant Analysis are prone to erroneously infer ecological differences when analyzing species distributions (Godsoe, 2010b; Broennimann et al., 2012).

RESULTS

***Distribution of cytotypes*—**Chromosome counts of two diploids and two tetraploids confirmed the presence of 14 chromosomes in diploid samples and 28 chromosomes in tetraploid samples. The flow cytometry results for these four plants showed that the diploids had 0.96 and 1.01 pg of DNA whereas the tetraploids had 1.99 and 2.08 pg DNA per nucleus. Using this information, we defined diploids as those plants containing approximately one pg DNA per nucleus and tetraploids as those plants with about 2 pg DNA. We were able to confidently assess ploidy level in 636 of the 638 samples examined for DNA content. Flow cytometry indicated that the *Heuchera cylindrica* included in this survey were either diploid or tetraploid. Comparison with the trout DNA standard showed that diploid plant cells averaged 1.07 ± 0.006 pg DNA (mean ± SD) and tetraploids had approximately 2.04 ± 0.008 pg of DNA. Furthermore, diploid and tetraploid plants differed significantly in DNA content ($t = 85.99$, $df = 312$, $P < 0.0001$) and the frequency distributions did not overlap.

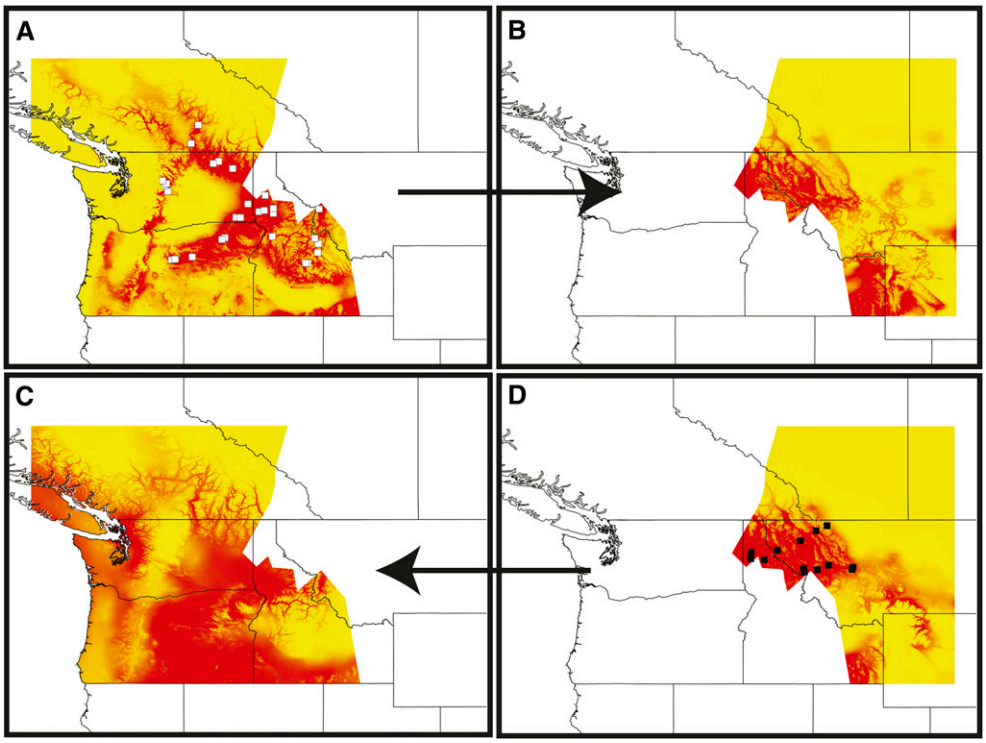

Fig. 3. Comparison of the Maxent distribution models for diploid and tetraploid samples of *Heuchera cylindrica*. Red colors indicate areas where the model predicts a high probability of occurrence, while yellow indicates environments where a particular ploidy is unlikely to be present. Model statistics are presented in Table 2. (A) A model generated from diploid samples (white squares). (B) The predictions of a model generated from diploid samples in the range of tetraploid *H. cylindrica*. (C) The predictions of a model generated from tetraploids in the range of diploids. (D) A model generated from tetraploid samples (black squares).

A total of 43 sites were examined; 30 populations were exclusively diploid and 13 were exclusively tetraploid (Fig. 1). Only single cytotype populations were found, and no triploids were observed. Tetraploids were primarily restricted to the northeastern portion of the range, and the cytotypes came into close proximity in northern Idaho and northwestern Montana.

***Species Distribution Models*—**Our SDMs produced strong predictions for the distribution of either diploid or tetraploid populations of *Heuchera cylindrica* (Table 2). The best predictors for the distribution of diploid localities were Bioclim 17 (precipitation for the driest quarter) followed by Bioclim 5 (max temperature warmest month) and Bioclim 2 (mean diurnal range, mean of [monthly max temp-monthly min temp]). The best predictors for tetraploids were Bioclim 6 (mean temperature coldest month) followed by Bioclim 15 (precipitation seasonality) and then Bioclim 4 (temperature seasonality). We note that it is often difficult to identify the climatic variables mechanistically linked to a species' distribution. Thus, we do not directly compare the variables identified as most important for each cytotype. Instead, following ideas from machine learning (Breiman, 2001), we focus on model predictions. We retained the GLM model for diploid populations that used Bioclims 17 and 5, as this model had the lowest AIC score. A model including Bioclims

TABLE 2. Summary of area under curve (AUC) scores ±SE (measures of model accuracy) for species' distribution models (SDMs) fit to the distributions of diploid and tetraploid populations of *Heuchera cylindrica*. Comparable models are presented in the same row with the model generated from the subregion of interest to the left of the model from the other subregion. For these comparisons, similar AUC scores indicate that the SDMs for each cytotype perform similarly. Conversely, lower AUC scores for the cytotype not found within the subregion (far right column) indicates that the climates used by one ploidy are a poor guide to the climates occupied by the other ploidy. (GLM = generalized linear model.)

Tetraploid subregion	SDM generated using tetraploid presences	SDM generated using diploid presences
Maxent entire subregion	0.929 ± 0.061[a]	0.928 ± 0.0609[b]
Maxent in analogous climates	0.953 ± 0.038	0.923 ± 0.050
Maxent on herbarium specimens	0.841 ± 0.0363	0.78 ± 0.043
GLM	0.84 ± 0.008	0.91 ± 0.077
Diploid subregion	SDM generated using diploid presences	SDM generated using tetraploid presences
Maxent entire subregion	0.93 ± 0.032[c]	0.64 ± 0.0723[d]
Maxent in analogous climates	0.96 ± 0.0302	0.75 ± 0.0976
Maxent on herbarium specimens	0.698 ± 0.033	0.697 ± 0.033
GLM	0.84 ± 0.0455	0.62 ± 0.0738

[a]Model shown in Fig. 3D; [b]Model shown in Fig. 3B; [c]Model shown in Fig. 3A; [d]Model shown in Fig. 3C.

17, 5, and 2 was slightly worse (ΔAIC = 2.2) and one including only Bioclim 17 was markedly worse (ΔAIC = 7.4). A tetraploid model including Bioclims 6, 15, and 4 had the lowest AIC score, though it was only trivially better than a model using Bioclims 6 and 15 (ΔAIC = 0.09). This reduced model was better than one that included only Bioclim 6 (ΔAIC = 2.49).

Comparisons of climates between subregions—The climate of the Pacific Northwest is quite complex, and as a consequence, the subregion associated with each cytotype contains environments with no equivalent in the subregion associated with the other cytotype. This is supported by nonoverlapping sections of polygons in individual scatterplots of presences and available climates for six of the most important environmental variables (Fig. 4). In most plots, a large portion of the climates available to diploids (pink polygon) is outside of the range of climates available to tetraploids (blue polygon). Conversely, for some environmental variables, the range of climates available to tetraploid populations exceeds the range of climates available to diploids. For example, the tetraploid subregion contains more seasonal climates that are outside the range of variation available in the diploid subregion (e.g., temperature seasonality, second row of Fig. 4). Likewise, diploid populations often occupy climates that are unavailable to tetraploids. For example, in the plot of precipitation seasonality vs. precipitation driest quarter (bottom right plot Fig. 4), there are four outlier diploid occurrences (red points) from environments with particularly seasonal precipitation. These sites represent some of the westernmost occurrences for diploid *Heuchera cylindrica* (sites 8-11, Fig. 1). Nonetheless, in a portion of the Pacific Northwest, comparable climates are available to both cytotypes. All tetraploid presences fall within this category, as do many of the westernmost diploid presences.

Comparisons of models between subregions—Nearly all of the Maxent models in the tetraploid subregion produced strong predictions for the range of tetraploid populations. For example, a model fit with all the tetraploid data accurately predicted the distribution of tetraploids in their own subregion (AUC 0.929 ± 0.061). A similar model generated from diploid data has nearly

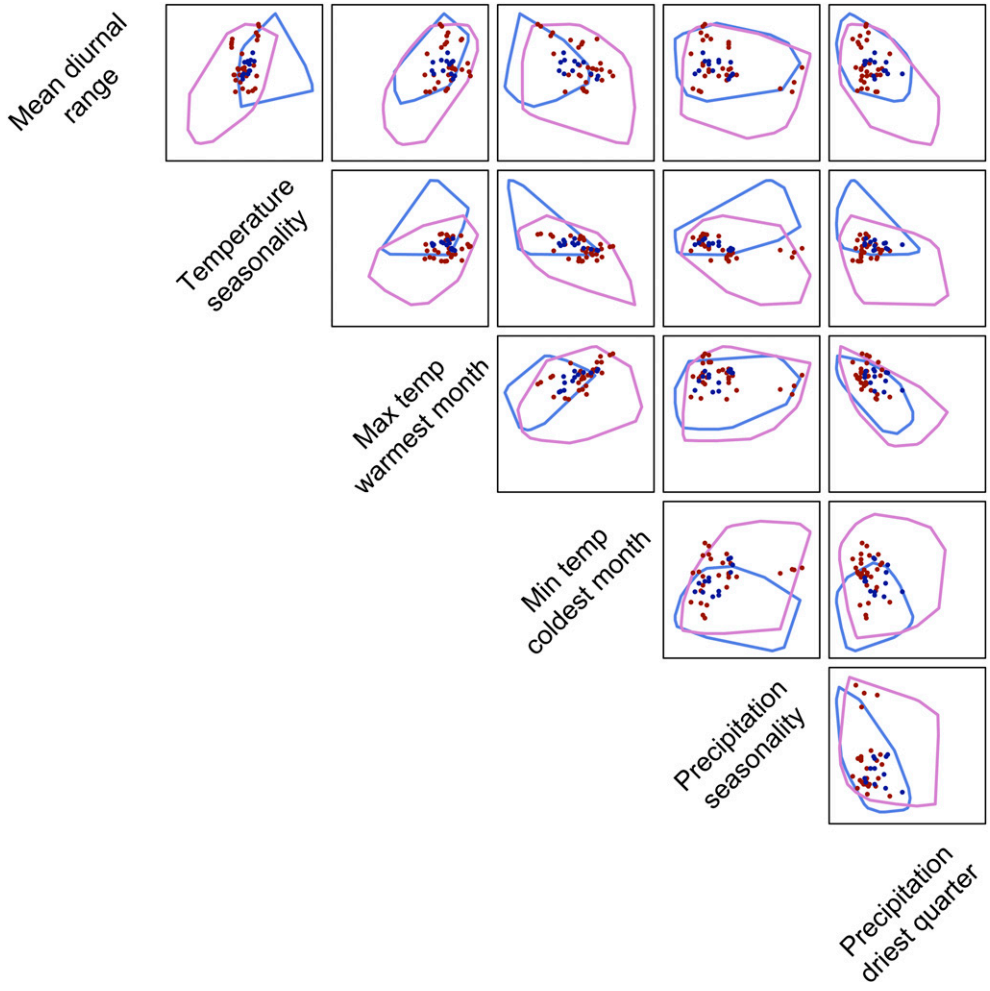

Fig. 4. Pairwise comparisons of the distribution of each cytotype in climate space. Each panel represents a plot of the two environmental variables labeled in the adjacent row and column. For example, the upper leftmost panel is a plot of mean diurnal range vs. temperature seasonality. Each plot contains a minimum convex polygon around a sample of 1000 locations with climates nearer to diploid (pink line) and tetraploid (blue line) populations. Diploid presences are identified by red dots and tetraploid presences are dark blue. These scatterplots may be used to visually assess similarity of the distributions of the two cytotypes and the similarities of the environments available to them. There is considerable overlap in the presences of the two cytotypes, with the exception of a few outlier diploid presences.

identical score in the tetraploid subregion (AUC 0.928 ± 0.0609). We obtained similar conclusions when we restricted our analyses to analogous climates. Using this restricted approach, a model generated from tetraploid data in the tetraploid subregion had an AUC score of 0.953 ± 0.038, and a model generated from diploid data in the tetraploid subregion had a similar AUC score of 0.923 ± 0.050. Likewise, both tetraploid and diploid models produced reasonably strong predictions of herbarium collection localities in the tetraploid subregion (tetraploid AUC: 0.84197 ± 0.0363; diploid AUC: 0.778 ± 0.043). Similarly, GLMs generated from both cytotypes produced strong predictions in the tetraploid region.

Diploid models generally did a better job of predicting the distribution of populations in the diploid subregion. A Maxent model generated from diploid data had an AUC score of 0.938 ± 0.032 in the diploid subregion but a model generated from tetraploid data produced comparatively poor predictions in the range of diploid populations (AUC 0.646 ± 0.0723). When we restricted our comparison to locations with analogous climates, the diploid model had an AUC score of 0.96 ± 0.0302 in the diploid subregion whereas a model generated from tetraploid data had a score of 0.75 ± 0.0976 in this subregion, slightly better than the score for a tetraploid model in the entire diploid region. We obtained similar conclusions using GLMs (Table 2). A model generated from the distribution of diploid populations produced good predictions for the range of diploids (AUC 0.8482 ± 0.0455) but a model generated from tetraploid populations produced markedly inferior predictions (AUC 0.6167 ± 0.07386) for the presence of diploids. Models for both cytotypes did a poor job at predicting the distribution of herbarium specimens in the diploid subregion. A diploid model had an AUC score of 0.698 ± 0.033 in the diploid subregion, and a tetraploid model had a score of 0.697 ± 0.033 in this subregion. This result was largely obtained because we have herbarium records but no cytotype information from portions of the diploid subregion such as southeastern Idaho near the Owyhee Mountains and the Great Basin Desert habitat in central Washington State (Appendix S2; see Supplemental Data with the online version of this article). Thus, SDMs fit using diploid data erroneously predict absences in these locations (Fig. 3A). Given that herbarium specimens were collected using a different protocol than our cytotype data, it is perhaps not surprising that our SDMs produced imperfect predictions of herbarium specimens. Nevertheless, we believe that this result highlights a case where inferences from SDMs need to be carefully integrated into existing botanical knowledge.

DISCUSSION

Although polyploidy is often cited as an important mechanism of plant diversification, we have yet to determine the processes that facilitate the establishment of new polyploid lineages, a critical first step in polyploid speciation. Niche differentiation is a commonly evoked mechanism for the successful establishment of polyploids that would reduce the effects of the minority cytotype disadvantage (Fowler and Levin, 1984; Thompson and Lumaret, 1992). Here we examined the geographic distribution of *Heuchera cylindrica* diploids and polyploids to test whether polyploids show evidence of climatic niche divergence. Specifically, we asked two questions: (1) What is the geographic distribution of cytotypes? and (2) Given this distribution, is polyploidy linked with ecological divergence? That is, do tetraploid populations occupy climates that are unsuitable to diploids? Our work provides strong answers to both of these questions; each cytotype occupies a distinct geographic range, but tetraploid populations occur in precisely the climates in which we should expect to find diploids, suggesting that polyploidy in *H. cylindrica* is not linked to specialization on distinct climates. Below, we discuss the implications of this work for the establishment of polyploids and the remaining sources of uncertainty due to the correlative nature of our analysis.

***Geographic distribution*—**Consistent with previous descriptions of the distribution of polyploids in *Heuchera cylindrica*, we find that the locations occupied by the two cytotypes are nonoverlapping. We cannot, however, completely rule out the possibility of some sympatric locations due to the topographic complexity of the Pacific Northwest and the remoteness of some portions of the boundary between the distributions of the two cytotypes. Notably, Soltis (1984) identified a single tetraploid individual collected near Stanley, Idaho in the Sawtooth Mountains Wilderness area, a locality where we collected only diploids from several populations within 12 km of this town. Although we sampled from three sites in this area and were unable to find tetraploid individuals or mixed cytotype populations, our search was far from exhaustive and did not include more remote areas. Nonetheless, given our extensive sampling throughout the Pacific Northwest and focus on sites near the boundary between the two taxa, we can be confident that the distributions of the two cytotypes are largely nonoverlapping. These results are in contrast with those for the closely related species *Heuchera grossulariifolia* where the distribution of cytotypes included both single cytotype as well as mixed populations. In this species, mixed populations had diploids and tetraploids growing side by side and were interspersed with rare triploids (Thompson et al., 1997). Presently, there are no data to explain the differences observed between *H. cylindrica* and *H. grossulariifolia*, but below we offer several hypotheses that might explain these patterns.

One possibility is that mechanisms of assortative mating may be absent in *Heuchera cylindrica*. Although *H. cylindrica* and *H. grossulariifolia* have similar pollination systems, in mixed cytotype populations, there may be differences in pollinator specificity between these plant species. If pollinators are able to recognize and selectively visit the cytotypes of *H. grossulariifolia* but not *H. cylindrica*, we might expect this disparity in geographic distributions. Indeed, field observations of mixed cytotype *H. grossulariifolia* populations indicate that some pollinators preferentially visit one cytotype (Thompson and Merg, 2008); however, we currently lack comparable data for *H. cylindrica*. Both species are pollinated by bumblebees, solitary bees, and *Greya* moths, suggesting at least the potential for differential visitation of *H. cylindrica* cytotypes. Another possibility is that phenological shifts have promoted assortative mating in *H. grossulariifolia* but not *H. cylindrica*. Differences in flowering phenology between diploids and tetraploids have been observed in mixed populations of *H. grossulariifolia* (Segraves and Thompson, 1999), but flowering phenology has yet to be examined in *H. cylindrica*. Alternatively, mixed populations may arise in *H. grossulariifolia* if the rate of polyploid formation is greater and/or ongoing. Clearly, the mechanisms underlying the disparity in geographic distribution of these species await further analysis, and their comparison may reveal interesting information about polyploid establishment.

***Ecological divergence of cytotypes*—**Contradictory to expectations, the results show that tetraploid *Heuchera cylindrica* occupy the climates in which we would expect to find diploid populations, indicating that polyploidy has not led to climatic niche divergence in *H. cylindrica*. We are reasonably confident of this result, as three of four SDM comparisons showed that a model generated from the distribution of diploid populations offered predictions for the tetraploid subregion that were as good, if not better, than the predictions generated by a model from the tetraploid dataset. The only exception was that a model fit with diploid data did a poor job of predicting the distribution of herbarium specimens in the tetraploid subregion. For this comparison, the tetraploid model was slightly better than the diploid model (AUC tetraploid: 0.84, diploid: 0.78). Although this exception offers some ambiguity, it is not surprising, given that this dataset represents a haphazard collection of observations amalgamated from several herbaria. Finally, we note that comparisons of SDMs still represent a relatively coarse way to compare the climates used by two taxa. As such, we recognize the broad similarity of the climates used by tetraploids and the climates in which we would expect to find diploids while acknowledging that we may have lacked the power to detect subtler differences in the climates occupied by the cytotypes.

Niche conservatism, or the propensity of a species to have similar ecological requirements as their ancestors, is perhaps an expected outcome of whole genome duplication as polyploids derive from diploids. We would expect this to be particularly the case in newly formed polyploid lineages that have not had time to diverge due to selection or drift. Since *Heuchera cylindrica* polyploids are well-established populations that have had the opportunity to evolve differences in ecological requirements but yet have not, the observed pattern of climatic niche requirements is most likely caused by niche conservatism. Although we know that genome duplication can create new phenotypes and may immediately confer polyploid lineages with a fitness advantage in novel environments (Ramsey, 2011), the prevalence of these shifts in ecological requirements remains unknown. It appears that, at least in *H. cylindrica*, there are no instantaneous effects of whole genome duplication on climatic niche requirements; however, the best test of this idea would be to examine early generation polyploids in the field.

The similarity of niches observed between cytotypes suggests that polyploidy is not linked to ecological divergence in *Heuchera cylindrica*. We cannot, however, entirely rule out the possibility that niche differentiation was involved in polyploid establishment. For example, fine-scale partitioning of sites has been shown in several other polyploid species (e.g., Lumaret et al., 1987; Johnson et al., 2003; Richardson and Hanks, 2011), and it is possible that early generation *H. cylindrica* polyploids may have similarly segregated during the initial stages of establishment. The SDMs used in our analysis would be unable to detect differences in microclimate as current climate data have a threshold of approximately one kilometer. However, since we were unable to find mixed cytotype populations, it seems likely that the resolution of our models is appropriate for this species and that fine-scale partitioning was unlikely involved in the initial stages of polyploid formation in *H. cylindrica*.

The finding that the ecological niche of tetraploids has not diverged from diploids is in contrast to a number of studies using SDMs that show cytotypes can specialize on distinct climates. For example, studies have found support for subtle climatic niche shifts between diploid and polyploid *Centaurea maculosa* L. (Treier et al., 2009), and niche differentiation has been shown in *Hordeum marinum* Huds. (Jakob et al., 2007), *Brachypodium distachyon* (L.) Beauv. (Manzaneda et al., 2012), the *Claytonia perfoliata* Donn ex Willd. species complex (McIntyre, 2012), and *Hyla* frogs (Otto et al., 2007). In contrast to studies employing SDM, reciprocal transplant experiments, which provide a more direct method of assessing niche divergence, show results similar to *Heuchera cylindrica*. Reciprocal transplants generally indicate little to no ecological divergence between cytotypes (Baack and Stanton, 2005; Buggs and Pannell, 2007; Raabová et al., 2008). The one exception is based on comparisons of only two populations (Flegrová and Krahulec, 1999), thus possibly confounding the effects of polyploidy with site specific local adaptation. Furthermore, our results are also consistent with broad surveys of diploid and polyploid taxa that indicate that polyploids are similar in ecological breadth to diploids (Stebbins and Dawe, 1987; Petit and Thompson, 1999; Martin and Husband, 2009). We might expect to observe disparate results among species given differences in natural history, geographic distribution, the age of the polyploid lineages, and whether interspecific hybridization was involved in polyploid formation. Together, these examples underscore that niche differentiation is one possible outcome, although it may not be an absolute requirement for the successful establishment of polyploids.

***Similarity of the environmental requirements of* Heuchera cylindrica *cytotypes*—**This work provides a valuable description of the distributions of the two cytotypes of *H. cylindrica*. We believe that such a description is useful in and of itself, but also wish to articulate the conditions under which comparisons of the distributions of two taxa can be used to make inferences about the similarity of their environmental requirements. In this section, we discuss the evidence for interpreting our results in this manner, focusing on whether our SDMs more strongly reflect environmental requirements or dispersal.

We focus on using comparisons of SDMs for formal hypothesis testing, complemented by direct comparisons of the climates occupied by each cytotype (Fig. 4). Here we used this combination of approaches since direct statistical comparisons of presences using classical multivariate methods are prone to erroneously infer ecological differences. In contrast, comparisons of SDMs have been tested over more than a decade (Peterson et al., 1999) and now have reasonably strong theoretical underpinnings (Godsoe, 2012).

Comparisons of SDMs are more likely to reflect a comparison of environmental requirements when each taxon can disperse to a similar set of climates (Godsoe, 2010b; Barve et al., 2011; Godsoe, 2012). We accounted for this by including a comparison of our SDMs in climates that are available to both taxa (Maxent in analogous climates, Table 2). All tetraploid presences in the tetraploid subregion are in climates available to diploids. Not surprisingly then, an evaluation using analogous climates produced qualitatively identical results to our Maxent comparison using the entire subregion with all models producing excellent predictions of the distribution of tetraploids (Fig. 3B vs. Fig. 3D). In contrast, it is more difficult to make comparisons in the diploid subregion as many diploid presences are in climates that are unavailable to tetraploids. Omitting locations with nonanalogous climates improved the ability of the Maxent model generated from tetraploids to predict the presence of diploids (AUC for tetraploid model in entire diploid subregion 0.64 vs. AUC limited to analogous climates 0.75; Fig. 3C). However, the score of this model is still dramatically lower than the AUC

score of SDMs generated from diploid models (0.96; Fig. 3A). We know that there is substantial overlap between the environments occupied by diploids and the environments predicted to be suitable to tetraploids, but we are less confident that all environments occupied by diploids are suitable to tetraploids. We are, however, reasonably confident that tetraploids occupy environments suitable to diploids.

A critical component of making inferences about cytotype-specific niches is to ensure that each cytotype has had an opportunity to reach the entire subregion used to model its distribution. This is equivalent to ensuring that diploids have historically had access to all of the locations in the diploid subregion while tetraploids have historically had access to all of the tetraploid subregion. Using evidence from other taxa, we have strong a priori reasons to believe that the diploid and tetraploid subregions used in this study are good approximations of the set of environments to which these plants have had an opportunity to disperse. This is because we know that the biogeographic history of organisms in the Pacific Northwest has been shaped by recent distribution shifts during the last ice age (e.g., Soltis et al., 1997; Galbreath et al., 2010; Shafer et al., 2011). Thus, even seemingly isolated populations show strong signatures of previous connectivity (Carstens et al., 2005; Brunsfeld et al., 2007), suggesting that historically, organisms such as *Heuchera cylindrica* have had substantial opportunities to reach locations throughout the Pacific Northwest.

Another important consideration is that a species' distribution is most likely to reflect its niche when the effects of environmental variation are strong relative to the effect of contemporaneous dispersal (Abrams and Wilson, 2004; Godsoe, 2010a). High migration rates can swamp the effects of local environmental conditions in situations where immigration maintains populations in suboptimal locations where they would not typically persist. We presently lack clear guidelines to determine when dispersal is sufficiently strong to bias inferences generated from SDMs. We can, however, note that environmental variability is substantial in our study region, with climates in the Pacific Northwest ranging from desert to temperate rainforest to alpine habitats. Further, *Heuchera cylindrica* has relatively weak dispersal abilities as the plants have small seeds with no obvious adaptation to assist dispersal. Collectively, these observations suggest that climate should have a significant direct influence on the distribution of *H. cylindrica* in the Pacific Northwest, and we expect our SDMs to strongly reflect the species' niche.

Conclusions—Together the results provide little support for the hypothesis that differentiation of climatic requirements alleviated the minority cytotype disadvantage of polyploid *Heuchera cylindrica*. Our observations are based on well-established polyploids that have experienced selection; thus, it is possible that selection has subsequently obscured niche differentiation that was present in newly formed polyploid populations. This scenario seems less likely as it requires multiple steps, i.e., the formation of niche divergence followed by its breakdown. Furthermore, given that a vast majority of polyploids form recurrently within species and these lineages can follow independent evolutionary trajectories (Soltis et al., 2010), it seems unlikely that all polyploid lineages would converge on the same niche. Instead, a more probable explanation is that *H. cylindrica* polyploids have avoided minority cytotype exclusion by other means than niche differentiation. Alternative mechanisms include changes in floral traits and flowering phenology that reduce intercytotype pollen transfer, but these remain to be tested.

Certainly, climatic niche differentiation is one such mechanism that can prevent minority cytotype exclusion, although that does not seem to be the case for *Heuchera cylindrica*. We currently lack additional information about the ecology of this species that would help us to resolve how polyploids became established in the northeastern portion of the range. Interestingly, the current range of tetraploid *H. cylindrica* abuts or overlaps the hypothesized extent of glaciation in Idaho and Montana. The Cordilleran ice sheet covered large parts of northern Montana, Idaho, and Washington reaching its maximum around 18 000 yr ago (Orr and Orr, 2002). During glacial retreat, the area surrounding Lake Couer d'Alene was repeatedly inundated by floods caused by the outlet of glacial Lake Missoula (Clague et al., 2003). The resulting cataclysmic flooding scoured the region downstream, creating the channeled scablands of eastern Washington. The eastern and southern shores of Lake Coeur d'Alene mark the boundary in the Idaho panhandle where diploid and tetraploid distributions abut; thus, one possible explanation for the geographic distribution is that newly formed tetraploids took advantage of glacial retreat or sites scoured clean by flooding. If tetraploids could initially become established as the majority cytotype, they would be able to locally exclude diploids. Several authors have suggested that polyploids are more successful at colonizing recently deglaciated areas (Stebbins, 1984; Brochmann et al., 2004), although this pattern is not universal (e.g., Brochmann et al., 2004; Müller et al., 2012). Even in the absence of biased colonization abilities, however, deglaciation certainly presents an opportunity for new plant populations to form. Although experimental and population genetic approaches may help to determine how the geographic distribution of *H. cylindrica* was created, answering the broader question of whether niche differentiation is a common mechanism facilitating polyploid establishment will require surveys of niche differentiation across many taxa.

LITERATURE CITED

ABRAMS, P. A., AND W. G. WILSON. 2004. Coexistence of competitors in metacommunities due to spatial variation in resource growth rates; does R* predict the outcome of competition? *Ecology Letters* 7: 929–940.

ADAMS, K. L., AND J. F. WENDEL. 2005. Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology* 8: 135–141.

ALTHOFF, D. M., AND J. N. THOMPSON. 2001. Geographic structure in the searching behaviour of a specialist parasitoid: Combining molecular and behavioural approaches. *Journal of Evolutionary Biology* 14: 406–417.

ANDERSON, R. P., AND A. RAZA. 2010. The effect of the extent of the study region on GIS models of species geographic distributions and estimates of niche evolution: Preliminary tests with montane rodents (genus *Nephelomys*) in Venezuela. *Journal of Biogeography* 37: 1378–1393.

ARRIGO, N., AND M. S. BARKER. 2012. Rarely successful polyploids and their legacy in plant genomes. *Current Opinion in Plant Biology* 15: 140–146.

ARUMUGANATHAN, K., AND E. D. EARLE. 1991. Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology Reporter* 9: 229–233.

BAACK, E. J. 2005. Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): Minority cytotype exclusion and barriers to triploid formation. *American Journal of Botany* 92: 1827–1835.

BAACK, E. J., AND M. L. STANTON. 2005. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): Niche

differentiation and tetraploid establishment. *Evolution; International Journal of Organic Evolution* 59: 1936–1944.

BARVE, N., V. BARVE, A. JIMÉNEZ-VALVERDE, A. LIRA-NORIEGA, S. P. MAHER, A. T. PETERSON, J. SOBERÓN, ET AL. 2011. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling* 222: 1810–1819.

BREIMAN, L. 2001. Statistical modeling: The two cultures. *Statistical Science* 16: 199–231.

BRETAGNOLLE, F., AND J. D. THOMPSON. 1996. An experimental study of ecological differences in winter growth between sympatric diploid and autotetraploid *Dactylis glomerata. Journal of Ecology* 84: 343–351.

BROCHMANN, C., A. K. BRYSTING, I. G. ALSOS, L. BORGEN, H. H. GRUNDT, A.-C. SCHEEN, AND R. ELVEN. 2004. Polyploidy in arctic plants. *Biological Journal of the Linnean Society. Linnean Society of London* 82: 521–536.

BROENNIMANN, O., M. C. FITZPATRICK, P. B. PEARMAN, B. PETITPIERRE, L. PELLISSIER, N. G. YOCCOZ, W. THUILLER, ET AL. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology and Biogeography* 21: 481–497.

BROENNIMANN, O., U. A. TREIER, H. MULLER-SCHARER, W. THUILLER, A. T. PETERSON, AND A. GUISAN. 2007. Evidence of climatic niche shift during biological invasion. *Ecology Letters* 10: 701–709.

BRUNSFELD, S. J., T. R. MILLER, AND B. C. CARSTENS. 2007. Insights into the biogeography of the Pacific Northwest of North America: Evidence from the phylogeography of *Salix melanopsis. Systematic Botany* 32: 129–139.

BUGGS, R. J., AND J. R. PANNELL. 2007. Ecological differentiation and diploid superiority across a moving ploidy contact zone. *Evolution; International Journal of Organic Evolution* 61: 125–140.

BURNHAM, K. P., AND R. D. ANDERSON. 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag, New York, New York, USA.

CARSTENS, B. C., S. J. BRUNSFELD, J. R. DEMBOSKI, J. M. GOOD, AND J. SULLIVAN. 2005. Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: Hypothesis testing within a comparative phylogeographic framework. *Evolution; International Journal of Organic Evolution* 59: 1639–1652.

CLAGUE, J. J., R. BARENDREGT, R. J. ENKIN, AND F. F. FOIT. 2003. Paleomagnetic and tephra evidence for tens of Missoula floods in southern Washington. *Geology* 31: 247–250.

ELITH, J., AND C. H. GRAHAM. 2009. Do they? How do they? WHY do they differ? On finding reasons for differing performances of species distribution models. *Ecography* 32: 66–77.

ELITH, J., C. H. GRAHAM, R. P. ANDERSON, M. DUDÍK, S. FERRIER, A. GUISAN, R. J. HIJMANS, ET AL. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29: 129–151.

FELBER, F. 1991. Establishment of a tetraploid cytotype in a diploid population: Effect of relative fitness of the cytotypes. *Journal of Evolutionary Biology* 4: 195–207.

FELBER, F., AND J. D. BEVER. 1997. Effect of triploid fitness on the coexistence of diploids and tetraploids. *Biological Journal of the Linnean Society. Linnean Society of London* 60: 95–106.

FELBER-GIRARD, M., F. FELBER, AND A. BUTTLER. 1996. Habitat differentiation in a narrow hybrid zone between diploid and tetraploid *Anthoxanthum alpinum. New Phytologist* 133: 531–540.

FLEGROVÁ, M., AND F. KRAHULEC. 1999. *Anthoxanthum odoratum* and *A. alpinum*: Life history parameters at two different altitudes. *Folia Geobotanica* 34: 19–31.

FOWLER, N. L., AND D. A. LEVIN. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *American Naturalist* 124: 703–711.

FREEMAN, E. A. 2007. PresenceAbsence: An R package for presence-absence model evaluation. USDA Forest Service, Rocky Mountain Research Station, Ogden, Utah, USA. Website: http://CRAN.R-project.org [accessed 05 May 2007].

FUKUI, K. 1996. Plant chromosomes at mitosis. *In* K. Fukui and S. Nakayama [eds.], Plant Chromosomes: Laboratory Methods, 1-17. CRC Press, Inc., Boca Raton, Florida, USA.

GALBRAITH, D. W., K. R. HARKINS, J. M. MADDOX, N. M. AYRES, D. P. SHARMA, AND E. FIROOZABADY. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science* 220: 1049–1051.

GALBREATH, K. E., D. J. HAFNER, K. R. ZAMUDIO, AND K. AGNEW. 2010. Isolation and introgression in the Intermountain West: Contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). *Journal of Biogeography* 37: 344–362.

GODSOE, W. 2010a. Regional variation exaggerates ecological divergence in niche models. *Systematic Biology* 59: 298–306.

GODSOE, W. 2010b. I can't define the niche but I know it when I see it: A formal link between statistical theory and the ecological niche. *Oikos* 119: 53–60.

GODSOE, W. 2012. Are comparisons of species distribution models biased? Are they biologically meaningful? *Ecography* 35: 769–779.

GODSOE, W., E. STRAND, C. I. SMITH, J. B. YODER, T. C. ESQUE, AND O. PELLMYR. 2009. Divergence in an obligate mutualism is not explained by divergent climatic factors. *New Phytologist* 183: 589–599.

GUISAN, A., C. H. GRAHAM, J. ELITH, AND F. HUETTMANN. 2007a. Sensitivity of predictive species distribution models to change in grain size. *Diversity & Distributions* 13: 332–340.

GUISAN, A., N. E. ZIMMERMANN, J. ELITH, C. H. GRAHAM, S. PHILLIPS, AND A. T. PETERSON. 2007b. What matters for predicting the occurrences of trees: Techniques, data, or species' characteristics. *Ecological Monographs* 77: 615–630.

HARBAUGH, D. T. 2008. Polyploid and hybrid origins of Pacific island sandalwoods (*Santalum*, Santalaceae) inferred from low-copy nuclear and flow cytometry data. *International Journal of Plant Sciences* 169: 677–685.

HERNANDEZ, P., C. GRAHAM, L. MASTER, AND D. ALBERT. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* 29: 773–785.

HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

HITCHCOCK, C. L., AND A. CRONQUIST. 1973. Flora of the Pacific Northwest. University of Washington Press, Seattle, Washington, USA.

HUSBAND, B.C., AND H.A. SABARA. 2003. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytologist* 161: 703–713.

HUSBAND, B. C., AND D. W. SCHEMSKE. 1998. Cytotype distribution at a diploid-tetraploid contact zone in *Chamerion* (*Epilobium*) *angustifolium* (Onagraceae). *American Journal of Botany* 85: 1688–1694.

HUSBAND, B. C., AND D. W. SCHEMSKE. 2000. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium. Journal of Ecology* 88: 689–701.

JAKOB, S. S., A. IHLOW, AND F. R. BLATTNER. 2007. Combined ecological niche modelling and molecular phylogeography revealed the evolutionary history of *Hordeum marinum* (Poaceae)–niche differentiation, loss of genetic diversity, and speciation in Mediterranean Quaternary refugia. *Molecular Ecology* 16: 1713–1727.

JAY, M., J. REYNAUD, S. BLAISE, AND D. CARTIER. 1991. Evolution and differentiation of *Lotus corniculatus/Lotus alpinus* populations from French south-western alps. III. Conclusions. *Evolutionary Trends in Plants* 5: 157–160.

JOHNSON, M. T. J., B. C. HUSBAND, AND T. L. BURTON. 2003. Habitat differentiation between diploid and tetraploid *Galax urceolata* (Diapensiaceae). *International Journal of Plant Sciences* 164: 703–710.

JUDD, W. S., D. E. SOLTIS, P. S. SOLTIS, AND G. IONTA. 2007. *Tolmiea diplomenziesii*: A new species from the Pacific Northwest and the diploid sister taxon of the autotetraploid *T. menziesii* (Saxifragaceae). *Brittonia* 59: 217–225.

KAY, Q. O. N. 1969. The origin and distribution of diploid and tetraploid *Tripleurospermum inodorum* (L.) Schultz Bip. *Watsonia* 7: 130–141.

KRON, P., J. SUDA, AND B. C. HUSBAND. 2007. Applications of flow cytometry to evolutionary and population biology. *Annual Review of Ecology Evolution and Systematics* 38: 847–876.

LEITCH, A. R., AND I. J. LEITCH. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* 320: 481–483.

LEVIN, D. A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43.

LEVIN, D. A. 1983. Polyploidy and novelty in flowering plants. *American Naturalist* 122: 1–25.

LEVIN, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, New York, New York, USA.

LEVIN, D. A. 2003. The ecological transition in speciation. *New Phytologist* 161: 91–96.

LEWIS, H. 1967. The taxonomic significance of autopolyploidy. *Taxon* 16: 267–271.

LEWIS, W. H. 1980. Polyploidy in species populations. *In* W. H. Lewis [ed.], Polyploidy: Biological Relevance, 103-144. Plenum Press, New York and London.

LUMARET, R., J.-L. GUILLERM, J. DELAY, A. AIT LHAJ LOUTFI, J. IZCO, AND M. JAY. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* 73: 436–446.

MANZANEDA, A. J., P. J. REY, J. M. BASTIDA, C. WEISS-LEHMAN, E. RASKIN, AND T. MITCHELL-OLDS. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytologist* 193: 797–805.

MARTIN, S. L., AND B. C. HUSBAND. 2009. Influence of phylogeny and ploidy on species ranges of North American angiosperms. *Journal of Ecology* 97: 913–922.

MAYROSE, I., S. H. ZHAN, C. J. ROTHFELS, K. MAGNUSON-FORD, M. S. BARKER, AND L. H. RIESEBERG. 2011. Recently formed polyploid plants diversify at lower rates. *Science* 333: 1257.

MCINTYRE, P. J. 2012. Polyploidy associated with altered and broader ecological niches in the *Claytonia perfoliata* (Portulacaceae) species complex. *American Journal of Botany* 99: 655–662.

MÜLLER, E., P. B. EIDESEN, D. EHRICH, AND I. G. ALSOS. 2012. Frequency of local, regional, and long-distance dispersal of diploid and tetraploid *Saxifraga oppositifolia* (Saxifragaceae) to Arctic glacier forelands. *American Journal of Botany* 99: 459–471.

NESS, B. D., D. E. SOLTIS, AND P. E. SOLTIS. 1989. Autopolyploidy in *Heuchera micrantha* (Saxifragaceae). *American Journal of Botany* 76: 614–626.

OKUYAMA, Y., O. PELLMYR, AND M. KATO. 2008. Parallel floral adaptations to pollination by fungus gnats within the genus *Mitella* (Saxifragaceae). *Molecular Phylogenetics and Evolution* 46: 560–575.

ORR, W. N., AND E. L. ORR. 2002. Geology of the Pacific Northwest. McGraw-Hill, New York, New York, USA.

OSBORN, T. C., J. C. PIRES, J. A. BIRCHLER, D. L. AUGER, Z. J. CHEN, H.-S. LEE, L. COMAI, ET AL. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics* 19: 141–147.

OTTO, C. R., J. W. SNODGRASS, D. C. FORESTER, J. C. MITCHELL, AND R. W. MILLER. 2007. Climatic variation and the distribution of an amphibian polyploid complex. *Journal of Animal Ecology* 76: 1053–1061.

OTTO, S., AND J. WHITTON. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* 34: 401–437.

PELLMYR, O., J. N. THOMPSON, J. M. BROWN, AND R. G. HARRISON. 1996. Evolution of pollination and mutualism in the yucca moth lineage. *American Naturalist* 148: 827–847.

PETERSON, A. T., J. SOBERON, AND V. SANCHEZ-CORDERO. 1999. Conservatism of ecological niches in evolutionary time. *Science* 285: 1265–1267.

PETIT, C., AND J. D. THOMPSON. 1999. Species diversity and ecological range in relation to ploidy level in the flora of the Pyrenees. *Evolutionary Ecology* 13: 45–66.

PETIT, C., F. BRETAGNOLLE, AND F. FELBER. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends in Ecology & Evolution* 14: 306–311.

PHILLIPS, S. J., R. P. ANDERSON, AND R. E. SCHAPIRE. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190: 231–259.

PHILLIPS, S. J., M. DUDÍK, J. ELITH, C. H. GRAHAM, A. LEHMANN, J. LEATHWICK, AND S. FERRIER. 2009. Sample selection bias and presence-only distribution models: Implications for background and pseudo-absence data. *Ecological Applications* 19: 181–197.

R DEVELOPMENT CORE TEAM. 2006. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: http://www.R-project.org [accessed 23 June 2009]

RAABOVÁ, J., M. FISCHER, AND Z. MÜNZBERGOVÁ. 2008. Niche differentiation between diploid and hexaploid *Aster amellus. Oecologia* 158: 463–472.

RAMSEY, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences, USA* 108: 7096–7101.

RAMSEY, J., AND D. W. SCHEMSKE. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* 33: 589–639.

RICHARDSON, M. L., AND L. M. HANKS. 2011. Differences in spatial distribution, morphology, and communities of herbivorous insects among three cytotypes of *Solidago altissima* (Asteraceae). *American Journal of Botany* 98: 1595–1601.

RODRIGUEZ, D. J. 1996. A model for the establishment of polyploidy in plants. *American Naturalist* 147: 33–46.

SAMPOUX, J.-P., AND C. HUYGHE. 2009. Contribution of ploidy-level variation and adaptive trait diversity to the environmental distribution of taxa in the 'fine-leaved fescue' lineage (genus *Festuca* subg. *Festuca*). *Journal of Biogeography* 36: 1978–1993.

SAS INSTITUTE. 2003. SAS JMP5.0.1.2 for Mac OS X. SAS Institute, Cary, North Carolina, USA.

SEGRAVES, K. A., AND J. N. THOMPSON. 1999. Plant polyploidy and pollination: Floral traits and insect visits to diploid and autotetraploid *Heuchera grossulariifolia. Evolution; International Journal of Organic Evolution* 53: 1114–1127.

SEGRAVES, K. A., J. N. THOMPSON, P. S. SOLTIS, AND D. E. SOLTIS. 1999. Multiple origins of polyploidy and the geographic structure of *Heuchera grossulariifolia. Molecular Ecology* 8: 253–262.

SHAFER, A. B. A., S. D. COTE, AND D. W. COLTMAN. 2011. Hot spots of genetic diversity descended from multiple Pleistocene refugia in an alpine ungulate. *Evolution; International Journal of Organic Evolution* 65: 125–138.

SOLTIS, D. E. 1984. Karyotypic relationships among *Elmera, Heuchera*, and *Tellima* (Saxifragaceae). *Systematic Botany* 9: 6–11.

SOLTIS, D. E., AND B. A. BOHM. 1986. Flavonoid chemistry of diploid and tetraploid cytotypes of *Tolmiea menziesii* (Saxifragaceae). *Systematic Botany* 11: 20–25.

SOLTIS, D. E., R. J. A. BUGGS, J. J. DOYLE, AND P. S. SOLTIS. 2010. What we still don't know about polyploidy. *Taxon* 59: 1387–1403.

SOLTIS, D. E., M. A. GITZENDANNER, D. D. STRENGE, AND P. S. SOLTIS. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution* 206: 353–373.

SOLTIS, D. E., AND R. K. KUZOFF. 1995. Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). *Evolution; International Journal of Organic Evolution* 49: 727–742.

SOLTIS, D. E., R. K. KUZOFF, M. MORT, M. ZANIS, M. FISHBEIN, L. HUFFORD, J. KOONTZ, ET AL. 2001. Elucidating deep-level phylogenetic relationships in Saxifragaceae using sequences for six chloroplastic and nuclear DNA regions. *Annals of the Missouri Botanical Garden* 88: 669–693.

SOLTIS, D. E., AND L. H. RIESEBERG. 1986. Autopolyploidy in *Tolmiea menziesii* (Saxifragaceae): Genetic insights from enzyme electrophoresis. *American Journal of Botany* 73: 310–318.

SOLTIS, D. E., AND P. S. SOLTIS. 2009. The role of hybridization in plant speciation. *Annual Review of Plant Biology* 60: 561–588.

SONG, K., P. LU, K. TANG, AND T. C. OSBORN. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proceedings of the National Academy of Sciences, USA* 92: 7719–7723.

STEBBINS, G. L. 1980. Polyploidy in plants: Unsolved problems and prospects. *In* W. H. Lewis [ed.], Polyploidy: Biological Relevance, 495-520. Plenum Press, New York, New York, USA.

STEBBINS, G. L. 1984. Polyploidy and the distribution of the arctic–alpine flora: New evidence and a new approach. *Botanica Helvetica* 94: 1–13.

STEBBINS, G. L., AND J. C. DAWE. 1987. Polyploidy and distribution in the European flora: A reappraisal. *Botany Jahrb. Systematics* 108: 343–354.

THOMPSON, J. D., AND R. LUMARET. 1992. The evolutionary dynamics of polyploid plants: Origins, establishment and persistence. *Trends in Ecology & Evolution* 7: 302–307.

THOMPSON, J. N., B. M. CUNNINGHAM, K. A. SEGRAVES, D. M. ALTHOFF, AND D. WAGNER. 1997. Plant polyploidy and insect/plant interactions. *American Naturalist* 150: 730–743.

THOMPSON, J. N., AND K. F. MERG. 2008. Evolution of polyploidy and the diversification of plant-pollinator interactions. *Ecology* 89: 2197–2206.

TREIER, U. A., O. BROENNIMANN, S. NORMAND, A. GUISAN, U. SCHAFFNER, T. STEINGER, AND H. MÜLLER-SCHÄRER. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa. Ecology* 90: 1366–1377.

VAN DIJK, P., AND T. BAKX-SCHOTMAN. 1997. Chloroplast DNA phylogeography and cytotype geography in autopolyploid *Plantago media. Molecular Ecology* 6: 345–352.

VAN DIJK, P., AND R. BIJLSMA. 1994. Simulations of flowering time displacement between two cytotypes that form inviable hybrids. *Heredity* 72: 522–535.

VAN DIJK, P., M. HARTOG, AND W. VAN DELDEN. 1992. Single cytotype areas in autopolyploid *Plantago media* L. *Biological Journal of the Linnean Society. Linnean Society of London* 46: 315–331.

VINDELØV, L. L., I. J. CHRISTENSEN, AND N. I. NISSEN. 1983. Standardization of high-resolution flow cytometric DNA analysis by the simultaneous use of chicken and trout red blood cells as internal reference standards. *Cytometry* 3: 328–331.

WIENS, J. J., AND C. H. GRAHAM. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics* 36: 519–539.

WOLF, P. G., P. S. SOLTIS, AND D. E. SOLTIS. 1989. Tetrasomic inheritance and chromosome pairing behaviour in the naturally occurring autotetraploid *Heuchera grossulariifolia* (Saxifragaceae). *Genome* 32: 655–659.

WOOD, T. E., N. TAKEBAYASHI, M. S. BARKER, I. MAYROSE, P. B. GREENSPOON, AND L. H. RIESEBERG. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences, USA* 106: 13875–13879.

YAMAUCHI, A., A. HOSOKAWA, H. NAGATA, AND M. SHIMODA. 2004. Triploid bridge and role of parthenogenesis in the evolution of autopolyploidy. *American Naturalist* 164: 101–112.