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The role of genome duplication in big sagebrush growth and fecundity

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Abstract

Premise: Adaptive traits can be dramatically altered by genome duplication. The study of interactions among traits, ploidy, and the environment are necessary to develop an understanding of how polyploidy affects niche differentiation and to develop restoration strategies for resilient native ecosystems.

Methods: Growth and fecundity were measured in common gardens for 39 populations of big sagebrush (*Artemisia tridentata*) containing two subspecies and two ploidy levels. General linear mixed-effect models assessed how much of the trait variation could be attributed to genetics (i.e., ploidy and climatic adaptation), environment, and gene–environment interactions.

Results: Growth and fecundity variation were explained well by the mixed models (80% and 91%, respectively). Much of the trait variation was attributed to environment, and 15% of variation in growth and 34% of variation in seed yield were attributed to genetics. Genetic trait variation was mostly attributable to ploidy, with much higher growth and seed production in diploids, even in a warm-dry environment typically dominated by tetraploids. Population-level genetic variation was also evident and was related to the climate of each population's origin.

Conclusions: Ploidy is a strong predictor growth and seed yield, regardless of common-garden environment. The superior growth and fecundity of diploids across environments raises the question as to how tetraploids can be more prevalent than diploids, especially in warm-dry environments. Two hypotheses that may explain the abundance of tetraploids on the landscape include selection for drought resistance at the seedling stage, and greater competitive ability in water uptake in the upper soil horizon.

KEYWORDS

Artemisia tridentata, common garden, C–S–R strategy, cytotype, genetic, gene–environment interaction, genetic adaptation, mixed-effect model, polyploidy

Genome duplication, polyploidy, is believed to be a potent force in plant adaptation and evolution. From a cytogeographic and phylogenetic context, numerous examples suggest that geographic patterns of cytotypes (i.e., ploidy level) reflect niche differentiation (Petit and Thompson, 1999; Treier et al., 2009; Sanderson, 2011; Laport et al., 2012). Others have classified different types of niche differentiation, suggesting that polyploids often occupy intermediate rather than novel niches and that these differences are typically explained by microclimatic factors (Marchant et al., 2016). Fewer studies have explored the genetic processes underlying these geographic patterns, connecting polyploid-related trait modifications and fitness to a

particular niche (Raabová et al., 2008; Maherali and Walden, 2009; Ramsey, 2011; Hao et al., 2013; Wei et al., 2018). To obtain a more comprehensive understanding of the role between ploidy variation on niche differentiation, focus is needed on the connection among ploidal variation, trait modification, and niche differentiation (Ramsey and Ramsey, 2014; Soltis et al., 2016).

Big sagebrush (*Artemisia tridentata*, subgenus *Tridentatae*; Asteraceae) is a native dominant shrub species found over much of the vast cold desert in North America that is referred to as sagebrush steppe. Three widespread subspecies have been described in big sagebrush with a large environmental breadth,

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together ranging from low elevation basins to dry montane, 100 to 3000 m a.s.l. Two subspecies, *A. tridentata* subsp. *tridentata* (basin big sagebrush; hereafter, *tridentata*) and *A. tridentata* subsp. *wyomingensis* (Wyoming big sagebrush; hereafter, *wyomingensis*) are the focus of this study. Previous research has suggested that the low-elevation subspecies of *A. tridentata* segregate into different microsites distinguished by topographic and edaphic conditions (Barker and McKell, 1983; McArthur and Sanderson, 1999b). Subspecies *tridentata* seedlings grow faster than *wyomingensis* (McArthur and Welch, 1978) and are highly responsive to conditions that favor soil water storage for growth in summer, such as greater winter precipitation and deeper soils or areas where landscape drainage is collected (Germino and Reinhart, 2014). Subspecies *wyomingensis* is more common in uplands and plateaus with shallow soils (McArthur and Plummer, 1978; Barker and McKell, 1983; McArthur and Sanderson, 1999a). However, examination of belowground traits shows a more nuanced adaptive strategy. Studies have shown that *wyomingensis* seedling root growth is relatively greater than *tridentata* (Welch and Jacobson, 1988), and adult plants have greater water uptake in shallow lateral roots compared to other variants (Zaiats et al., 2020). These belowground adaptive traits may explain higher juvenile survival and growth among *wyomingensis* populations under drier environments of the sagebrush ecosystem (Brabec et al., 2017). The differences in traits between *tridentata* and *wyomingensis* suggest two differing adaptive strategies have evolved to accommodate these environmental differences.

Taxonomy and ploidy are currently confounded among big sagebrush subspecies. Subspecies *tridentata* has been reported to be predominately diploid ($2n = 2x = 18$), with tetraploidy ($4n = 4x = 36$) being less frequent. Subspecies *wyomingensis* is considered to be tetraploid ($4n = 4x = 36$) or rarely pentaploid ($5n = 5x = 54$, McArthur et al., 1981; McArthur and Sanderson, 1999a). Characteristics that distinguish these subspecies (growth habit and leaf morphology) are often intermediate, making determination based purely on morphology difficult (Shultz, 2009). Mixed taxonomic characters likely stem from multiple polyploidization events from different diploid lineages (Richardson et al., 2012), introgression with the higher elevation subspecies *A. tridentata* subsp. *vaseyana* in ecotones, and the species strong preference for outcrossing (McArthur et al., 1988). Ploidy in big sagebrush appears to be the key diagnostic and biologically relevant characteristic between these two subspecies. However, it remains unclear whether these polyploidization events stem from autopolyploidy (within subspecies) or allopolyploidy (among subspecies or other species). Given the diverse morphological forms of polyploids in this taxon, both auto- and allopolyploidy could be at play.

Our understanding of the connection among ploidy, trait variation, and niche differentiation remain limited, especially among woody plants and in natural systems. However, this information is important in developing concepts between polyploidy and ecological function (Soltis

et al., 2016). For sagebrush ecosystem restoration, tetraploid sagebrush occupies the warm-dry spectrum of the sagebrush ecosystem, which is more vulnerable to disturbance, conversion to exotic annual grasses (Chambers et al., 2013), and climate change (Still and Richardson, 2015; Schlaepfer et al., 2015). Trait variation between ploidy levels in big sagebrush can be distinct enough to suggest differences in physiological stress-response strategies, such as freezing avoidance in tetraploids compared to freezing tolerance in diploids of low-elevation big sagebrush (Brabec et al., 2017). Improving our understanding of how genome duplication affects the modification of adaptive traits has important implications for restoration strategies, especially in how it relates to niche differentiation. Knowledge of how polyploidy and environment affect fitness traits is key to developing restoration guidelines that use environmental features in determining the appropriate subspecies and cytotype.

Previous work from common garden has shown phenology and cold hardiness variation are largely governed by phenotypic plasticity and climate-associated genetic trait variation with little to no subspecies and cytotype effects on these traits (Chaney et al., 2017; Richardson et al., 2017; Lazarus et al., 2019). However, as mentioned above, subspecies and cytotypes have been shown to be influential in growth (McArthur and Welch, 1978) and seed mass (Richardson et al., 2015). These traits are likely critical to maintaining fitness and resilient sagebrush ecosystems. The aim of this study was to characterize variation in growth and fecundity into genetic (i.e., subspecies, ploidy, and climatic adaptation), environment (i.e., phenotypic plasticity) and gene–environment interaction (GxE). Using common gardens that represent climates that support different compositions of subspecies, we addressed how growth and fecundity trait variation is attributed to ploidy with the following questions: (1) To what extent do subspecies and ploidy explain growth and fecundity variation in comparison to other sources of genetic and environmental variance? (2) Do growth and fecundity interact in different environments?

MATERIALS AND METHODS

Seed collection and common gardens

Big sagebrush seed was collected from 39 sites in the fall of 2010. Collection sites are hereafter referred to as populations. Seed collections were sampled from wild stands in an approximately 2-ha area. Flow cytometry was conducted on at least three wild-collected maternal plants and each offspring growing in common gardens in Orchard, Idaho and Majors Flat, Utah of the United States using previously published methods to screen for cytotype ($2x$ or $4x$; Richardson et al., 2012). In Ephraim, Utah, common garden plants were not cytotyped due to high mortality. The study included a total of 19 diploid and 20 tetraploid populations. All 19 diploid populations were determined to be
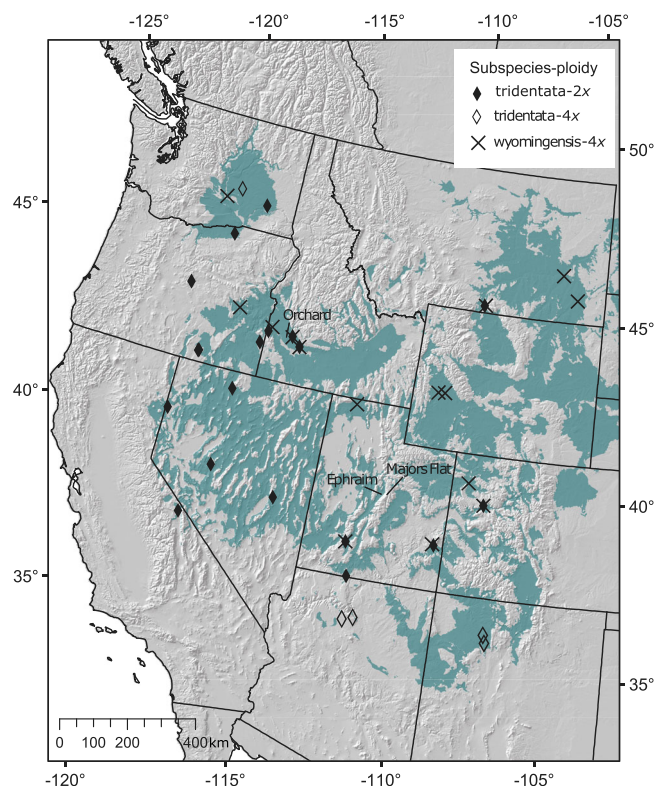
tridentata–2*x* based on growth rates and stature. Tetraploid populations were evaluated for growth habit to determine subspecies (i.e., *wyomingensis* or *tridentata*–4*x*; McArthur and Sanderson, 1999a). Fifteen of the 20 tetraploids populations had a prostrate growth habit, indicative of *wyomingensis*. Five had an upright growth habit, indicative of *tridentata*–4*x* (Figure 1; Appendix S1). Seeds from the populations were cleaned using previously published methods (Richardson et al., 2015) and grown in a greenhouse for 3 months before outplanting. Seed were sown in February 2010 on top of the soil surface in 6-inch (15.2 cm) Ray

FIGURE 1 Geographic origins of studied big sagebrush (*Artemisia tridentata*) populations. Subspecies/ploidy are shown by symbols (see legend). Common gardens Ephraim, Orchard, and Majors Flat are labeled. Blue fill, indicating the historical (1960 to 1990) climatic niche of Wyoming big sagebrush (*A. tridentata* subsp. *wyomingensis*), is shown for reference (adapted from Still and Richardson, 2015). Basin big sagebrush (*A. tridentata* subsp. *tridentata*) largely overlaps this distribution at this spatial scale

Leach Cone-tainers (Stuewe and Sons, Corvallis, OR, USA) and misted periodically to ensure the upper soil layer remained moist. Misting occurred until germination and was followed by regular periodic watering. Seedlings were grown in a soil mix of 1:1 peat to sand. Three hours of supplement light were provided. A completely randomized design was used at three common gardens (Ephraim, Majors Flat, and Orchard) that have varied environments (Table 1). All gardens contained loamy soils >1 m in depth. Due to variation in seed yield and germination of the wild collections, representation of each population varied in each garden from 2 to 11 individuals per population with an average of over eight plants per population. Full details on the common garden design were described by Chaney et al. (2017).

Data collection

Aboveground plant growth was determined by height and crown area during the 2011 and 2012 growing seasons. Plant heights were measured from the ground to the highest, living, nonflowering stem tip. Crown areas were calculated from overhead digital photos. Photos were analyzed by cropping leaves and stems from a white background placed under the plant using the color threshold feature in ImageJ v1.45 (Schneider et al., 2012). Cropped pixel area was scaled to a known standard on each photo. Heights and crown areas were used to estimate volume based on an ellipsoid volume equation from measurements that occurred within 2 weeks of each other. This method has been shown to be an accurate predictor of aboveground biomass in big sagebrush (Cleary et al., 2008). To minimize any greenhouse or planting effect on growth, we began measurements for the base growth volume (m^3) in October 2010 after the first growing season. Yearly growth measurements ($m^3\ yr^{-1}$) were completed in late May 2011 and 2012 at the gardens, which captured most of the growth that occurs in the spring.

Seed yield was estimated for two randomly selected plants per population at the Majors Flat and Orchard gardens during the 2012 and 2013 growing seasons. The Ephraim garden was omitted from seed yield collections due to increasing rates of mortality associated with winter temperatures (Chaney et al., 2017). In August, two flowering

TABLE 1 Geographic and climatic attributes of the common gardens (see mapped locations in Figure 1). Climate data are based on values from 2010 to 2013 water years, except for MAP estimates at Majors Flat, which is based on 30-year model interpolation (ClimateNA, Wang et al., 2016)

Garden	Lat.	Long.	Elev. (m)	MCMT (°C)	MWMT (°C)	MAP (mm)	Dominant subspecies	Climate
Ephraim	39.37	−111.57	1686	−9.7	21.2	277	*tridentata*	Cool-dry
Majors Flat	39.33	−111.52	2105	−0.4	18.6	507	*vaseyana*	Cool-wet
Orchard	43.32	−115.99	974	−2.9	25.0	261	*wyomingensis*	Warm-dry

Notes: Lat. = latitude, Long. = longitude, Elev. = elevation above sea level, MCMT = mean coldest month temperature, MWMT = mean warmest month temperature, MAP = mean annual precipitation.

stems of approximately the same size were fitted with bags (13 × 45 cm) constructed with mosquito netting and tied below the inflorescence stem with string. The bags allowed for open wind-disseminated pollination (0.8 × 0.8 mm aperture) while serving as a reservoir to collect seeds as they were dislodged from the inflorescence. The remaining un-bagged flowering stems were counted and used to estimate whole-plant seed yield. The bags were then harvested in early December 2012 and 2013 after seed ripening. Contents of each bag were cleaned to remove all chaff, and the seeds were weighed. An estimate of total seed mass (g) per plant was calculated by multiplying the mean mass from the bags by the total number of flowering stems on the plant. The accuracy of the seed yield estimates relies on having con-sistent seed masses within a plant sample (i.e., between flowering stalks). To test this accuracy, we performed ana-lysis of variance to assess whether within plant subsampling had a significant effect in explaining the variance. Sub-sampling was not a significant source of variance ($P = 0.82$, Appendix S2). Seed produced from inter-cytotype pollina-tion could also inflate seed masses, since $4x$ seed is on average $1.36\times$ heavier than $2x$ seed (Richardson et al., 2015). To evaluate whether inter-cytotype pollination was a factor in seed mass, 900 seeds from six diploid plants at Orchard and Majors Flat gardens were germinated and screened for cytotype via flow cytometry using published methods (Richardson et al., 2012). Only two of the 900 plants had increased genome size values that support inter-cytotype pollination, suggesting these events are rare (B. Richardson, unpublished data).

As mentioned above, individual seed mass varies among cytotype and to a smaller degree between populations (Richardson et al., 2015). To estimate fecundity, we calcu-lated plant seed yield estimates (i.e., number of seeds pro-duced per plant) by dividing the mean mass for the population seed yield by the mean mass for single seeds from the population. These data can be found at GitHub: https://github.com/brichardsonfs/seed_yield. We used seed yield in the analyses below, referred to as seed yield hereafter.

Climate data

Twenty-nine climate variables were obtained from a single point location at each population using the ClimateNA v 5.21 software package (Wang et al., 2016). The climate variables, based on the magnitude and timing of tempera-ture, precipitation, and interactions, were generated from 30-year weather data collected between 1961 and 1990 and downscaled to 1-km^2 resolution. More information on cli-mate variables and data downscaling and interpolation was given by Wang et al. (2016) and is also available at https://adaptwest.databasin.org/pages/adaptwest-climatena/. Climate variables were evaluated as predictors of trait varia-tion using linear regression prior to general linear mixed model (GLMM) analysis (see below).

Statistical analyses

A GLMM was developed for each trait using glmmTMB v 1.01 (Brooks et al., 2017) within the R statistical framework v 4.0.1 (R Core Team, 2020). Our seed data collection contained instances of zero data, especially for tetraploid populations at the Orchard garden. We believe this is biologically relevant information. Therefore, a Tweedie (log) distribution model was used to model seed yield and growth data to account for potential zero in-flation. Zero inflation could be caused by either genetic or environmental effects; therefore, it was applied to both fixed and random effects. Cytotype and population-source climate variables were treated as fixed effects with later variables used to infer climatic adaptation. Random effects were assigned to experimental design attributes. These attributes included populations, year, and garden in a full factorial design. For assessing environmental variation from GxE, garden and year were a separate random effect from the population interaction with garden and year.

We used a three-step process to assess growth and seed yield in relation to climate variables of population origin. First, Pearson correlation coefficients (r), calculated from the response variable population means and source climate variables, were used to guide the initial selection of potential predictor variables. We selected the top three climate vari-ables based on the correlation coefficient (r) to include in the GLMM. Second, predictors were run separately or together in the GLMM if collinearity was low ($r < 0.7$). Third, selection of the best fitting GLMM was determined by the ANOVA function using the Akaike information criterion (AIC). Reported P values in the models were cal-culated with the Wald statistic. To assess GLMM compo-nents and fit, we calculated the conditional and marginal R^2 based on the methods of Nakagawa et al. (2017) using sjPlot package v2.8.4 (Lüdecke 2020). Conditional R^2 (R^2c) ac-counts for the goodness-of-fit of fixed and random effects, and marginal R^2 (R^2m) accounts for the goodness-of-fit of only fixed effects. R^2 values are described as percentages in the results. R scripts can be found at GitHub for growth (https://github.com/brichardsonfs/growth) and seed yield (https://github.com/brichardsonfs/seed_yield).

RESULTS

Growth and seed yield

Growth varied greatly among subspecies, ploidy, and en-vironments (i.e., common gardens, Table 2). Plant growth over two consecutive years ranged widely from –0.38 to 7.6 m^3 year^{-1}. On average, the greatest growth was observed at the cool-dry Ephraim garden and the least growth at the warm-dry Orchard garden. Over the 2 years, Ephraim plants were on average over $3\times$ the size of Orchard plants (Figure 2A). Diploid *tridentata* displayed

TABLE 2 Results of (A) growth and (B) seed yield generalized linear mixed effect model. Confidence interval (95%) are provided in parentheses below estimates. Climate variable predictors include PAS = precipitation as snow for growth, DD0_wt = winter degree-days <0°C and PPT_sm = summer (June to August) precipitation for seed yield. Subspecies = subsp

(A) Growth (m^3 $year^{-1}$)

Fixed effects

Growth = 0.12 + subsp + PAS(−0.012)

Predictors	Estimates	p
tridentata−2x (Intercept)	0.12	0.794
	(−0.76 to 1.00)	
tridentata−4x	−0.64	**<0.001**
	(−0.80 to −0.48)	
wyomingensis−4x	−0.15	**<0.001**
	(−1.28 to −1.02)	
PAS	−0.02	**<0.001**
	(−0.02 to −0.01)	

Zero-inflated model

(Intercept)	−24.62	0.996

Random Effects

residual	0.39
garden:year	1.16
pop(garden:year)	0.1
N_{garden}	3
N_{year}	2
N_{pop}	39
Observations	1878
Marginal R^2/Conditional R^2	0.17/0.80

(B) Seed yield

Fixed effects

Seed yield = 12.29 + subsp + DD0_wt(−0.026) + PPT_sm(−0.015)

Predictors	Estimates	p
tridentata-2x (Intercept)	12.29	**<0.001**
	(10.34 to 14.24)	
tridentata-4x	−2.27	**<0.001**
	(−3.24 to −1.30)	
wyomingensis-4x	−2.91	**<0.001**
	(−3.62 to −2.19)	
DD0_wt	−0.03	**0.037**
	(−0.05 to −0.00)	
PPT_sm	−0.015	**0.012**
	(−0.03 to −0.00)	

(Continues)

TABLE 2 (Continued)

(B) Seed yield

Fixed effects

Seed yield = 12.29 + subsp + DD0_wt(−0.026) + PPT_sm(−0.015)

Zero-inflated model

(Intercept)	−19.74	0.989

Random effects

residual	0.92
garden:year	3.42
pop(garden:year)	2.39
N_{garden}	2
N_{year}	2
N_{pop}	39
Observations	573
Marginal R^2/Conditional R^2	0.34/0.91

consistently greater growth than tetraploids (*tridentata* and *wyomingensis*) across gardens. Averaged across years and gardens, *tridentata*−2x grew about 40% larger than *tridentata*−4x and 72% larger than *wyomingensis* (Figure 2A; Appendix S3).

Seed yield varied greatly between the Majors Flat and Orchard gardens, and overall, yields were higher at Majors Flat and among *tridentata*−2x at both gardens (Figure 2B). Seed yield data were not collected for the Ephraim garden due to sharply declining survival by 2012 caused by limited cold hardiness in warm-adapted populations (Chaney et al., 2017; Lazarus et al., 2019). Individual plant seed yield ranged from 0 to 1.3 million seeds per year. Seed yield by year showed different patterns between gardens. Unlike the growth data where growth was consistent or increased in the second year, seed yield declined from 2012 to 2013 at Majors Flat (Figure 2B). Subspecies-cytotype seed yield patterns were similar to growth, with *tridentata*−2x being the more fecund, regardless of garden (Figure 2B). However, differences in subspecies-cytotype were markedly different between gardens. At the Majors Flat garden, seed yield of *tridentata*−2x was 66% and 79% greater than that of *tridentata*−4x and *wyomingensis*, respectively. At the Orchard garden, differences in diploid and tetraploid seed yields expanded: *tridentata*−2x produced >98% more seed than the tetraploid subspecies did (Appendix S3).

Population mean growth and seed yield responses showed a general decline from cool-dry or cool-wet gardens to the warm-dry garden (Figure 3A,B). However, exceptions to the trend were found for seed yield among several populations of *tridentata*−2x that exhibited stable or increasing seed yield at the Orchard garden (Figure 3B). Overall, the population means for growth and seed yield from each garden were positively correlated ($r = 0.7$, $p < 0.0001$). Regression analysis showed that 1 m^3 of growth equates to
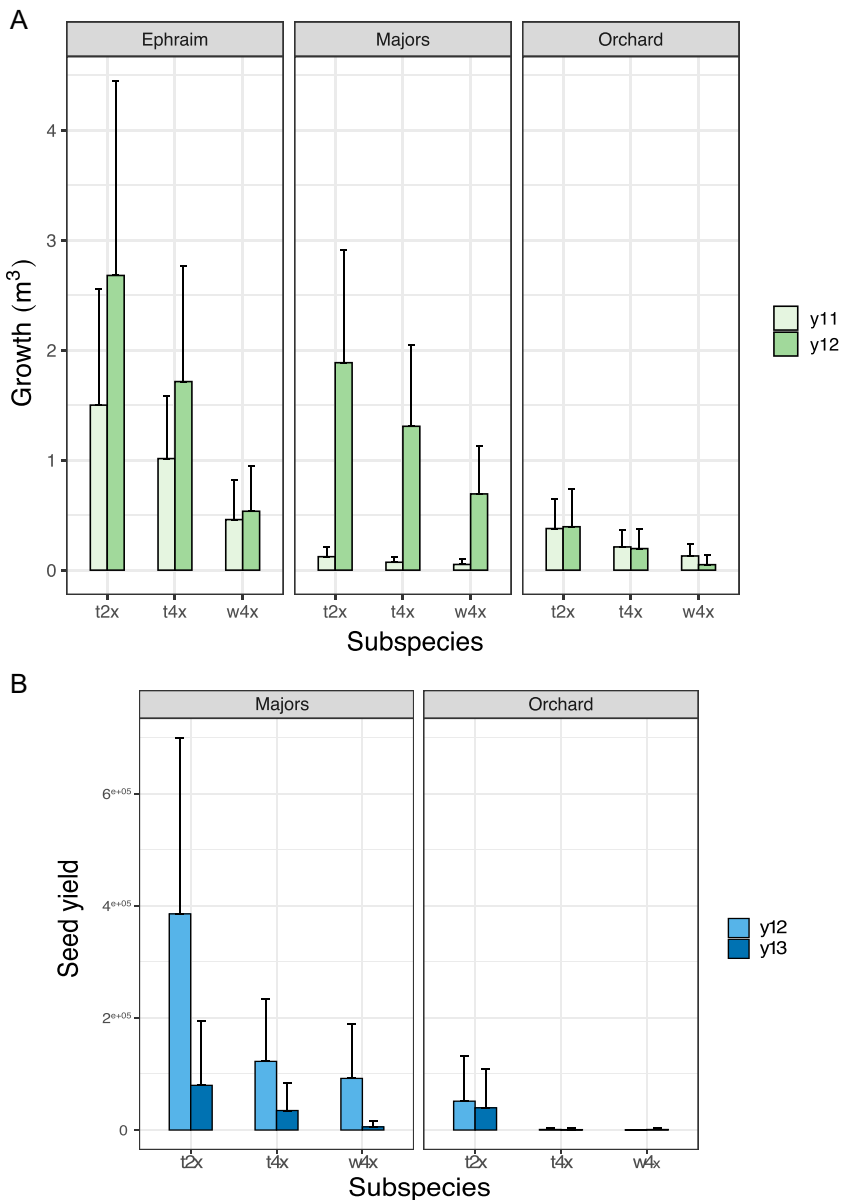
A

B

an increase of about 98,000 seeds for *tridentata–2x* (Figure 4). Subspecies and cytotype did not have a significant effect on the regression slope between seed yield and plant size (Appendix S4).

Mixed-effects models

General linear mixed models explained 80% and 91% of the variation in growth and seed yield, respectively (Table 2A,B). As indicated from the observed data, environment exhibited a strong effect on growth and seed yields (Figure 2). Random effects explained 65% and 57% of the variation in growth and seed yield, respectively. Broken down into individual components, environment (i.e., garden:year) and GxE (i.e., population:[garden:year]), the majority of the random effect variance can be attributed to

environmental effects. Environmental variance accounted for 69% of the random effect in growth and 51% in seed yield. GxE variance attributed 7% among growth and 36% among seed yield (Table 2). More detailed environmental effects were evident between garden and year. Specifically, the warm-dry garden, Orchard, had strong negative effects on growth and seed yield intercepts. Growth effects on traits were positive for the cool-dry garden, Ephraim, and cool-wet garden, Majors Flat (Appendix S5a). Yearly effects were consistent for Ephraim and Orchard gardens but mixed at Majors Flat (Appendix S5a and b).

Fixed effects were assigned to genetic attributes: subspecies-cytotype and population-source climate. Together, these attributes explained 17% of the variation in growth and 34% in seed yield (marginal R^2, Table 2A and B). Within these fixed effects, the majority of explained variation was ascribed to subspecies-cytotype. Tetraploidy
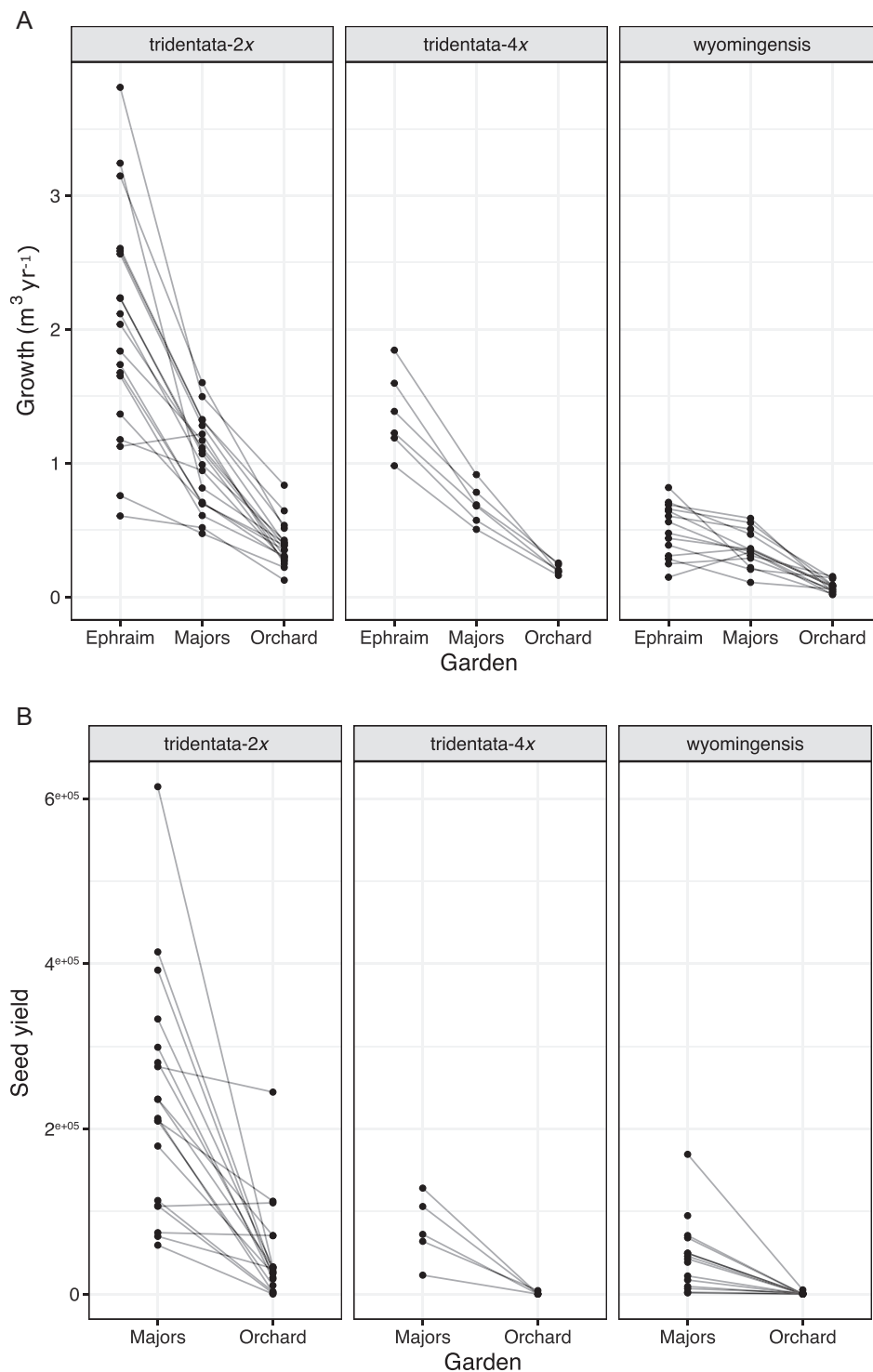
FIGURE 3 Populations means for growth (A) and (B) seed yield of big sagebrush (dots) plotted for the common gardens and subspecies-cytotypes. Lines connect the same populations at different gardens. Possible gene–environment interactions are indicated by intersecting lines. Average environmental conditions for the gardens can be found in Table 1

(i.e., *tridentata*–*4x* and *wyomingensis*) showed strong negative effects on the intercepts of both traits (Appendix S6). When climate variables in each model were removed, they accounted for up to 5% of the variation (Appendix S7).

Environment appeared to have varied effects on growth and seed yield. Growth within *tridentata*–*2x* showed a

negative association across gardens with precipitation as snow, an interaction of winter precipitation and temperatures (Figure 5). However, the response of seed yield showed an inconsistent response between gardens for diploids. Significant correlation ($r = -0.6$, $P = 0.006$) was found between *tridentata*–*2x* seed yield at the Orchard

garden and summer precipitation (June to August), but no association was found at the Majors Flat garden (Figure 6). Tetraploid seed yield was near zero at Orchard and, therefore, too low to draw any association. Diploid populations that experience less summer precipitation support higher seed yields at this garden, whereas populations originating from locations with higher summer precipitation produced less seed (Figure 6).

DISCUSSION

Subspecies- and ploidy-derived trait variation

Studies have shown that genome duplication can create profound functional changes across plant systems (Warner and Edwards, 1993; Corneillie et al., 2019). In big sagebrush, formation of tetraploids from diploid progenitors result in novel trait variation, slower growth, and lower fecundity compared to diploids. Trait differences between subspecies and cytotypes were evident regardless of the common garden environment (Figure 2, Table 2). Richardson et al. (2012) have shown that these tetraploid populations are derived from separate diploid lineages, suggesting that separate genome-duplication events result in similar altered growth and fecundity. Convergence of phenotypes, chromosome arrangements, and gene expression patterns after independent genome-duplication events appear to be common in polyploid species complexes (Buggs et al., 2012; reviewed by Soltis et al., 2016). Interpopulation variation within ploidy levels can be in part explained by climatic adaptation (Table 2), but another potential source of genetic trait variation could be from subspecific introgression (i.e., subsp. *tridentata* and *vaseyana*) either as the initial genome
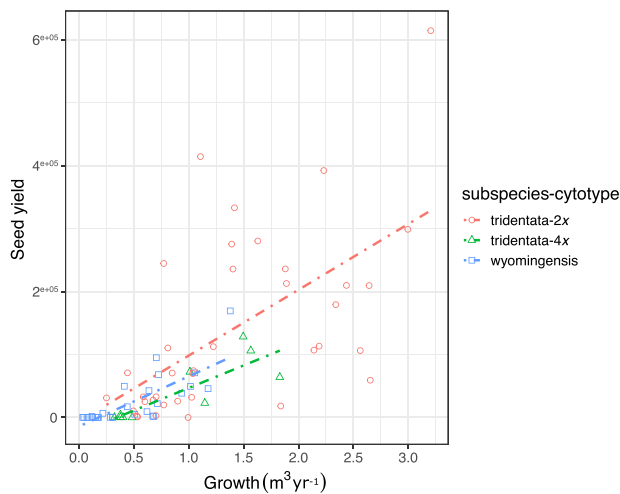

FIGURE 4 Relationship between growth (m^3) and seed yield of big sagebrush ($p < 0.0001$, Pearson correlation coefficient $r = 0.7$). Data from population means are plotted from two gardens (Majors Flat and Orchard). Subspecies and cytotype are labeled. See Appendix S4 for more detail
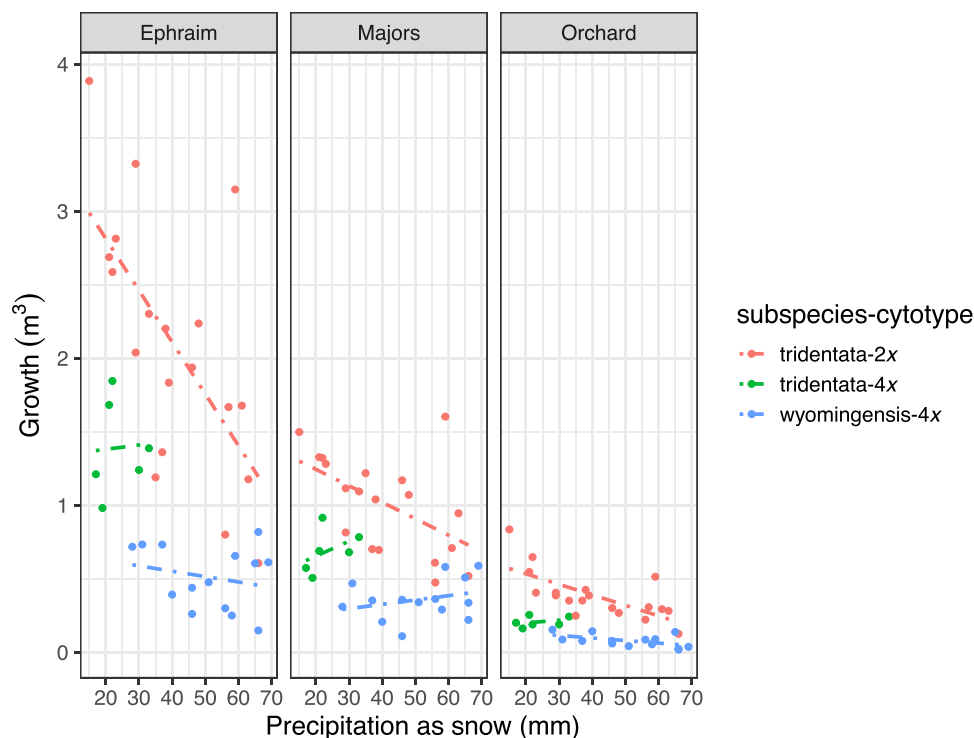
FIGURE 5 Relationships between mean growth and precipitation as snow (mm) at the geographic origin of each population, for each garden and subspecies-cytotype. All garden and subspecies-cytotype regressions were significant ($r > -0.54$, $P > 0.017$) for *tridentata*−2x. No correlation was found for 4x subspecies except *wyomingensis* at Orchard ($r = -0.54$, $P = 0.04$)
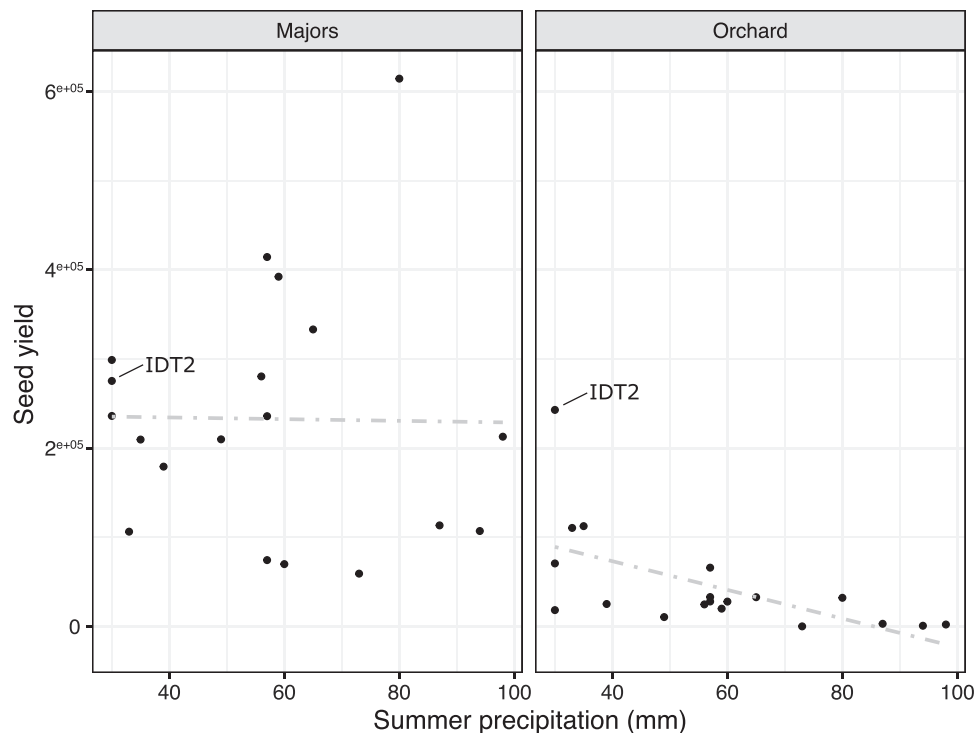
FIGURE 6 Relationship of mean seed yield to the amount of summer precipitation (mm) at the geographic origin of each population of *tridentata*–2*x* at the Orchard garden (*r* = −0.6, *P* = 0.006) and the Majors Flat garden (*r* = 0.02, *P* = 0.91). IDT2, near Mountain Home, Idaho, is the local population at the Orchard garden, and had the highest seed yield

duplication event or after polyploidization. Diploid subspecies *tridentata* and *vaseyana* are known to introgress along ecotones (Freeman et al., 1991). Similar processes of introgression are likely among tetraploid lineages (e.g., *wyomingensis* and *vaseyana*–4*x*) based on the admixture found in nuclear amplicon sequences (Richardson et al., 2012).

Big sagebrush trait variation is associated with genome duplication. Similar to a previous study (McArthur and Welch, 1982), our data support that subspecies-cytotype has a substantial effect on growth across environments, accounting for 16% of variation across environments. Seed yield variation showed similar patterns. Subspecies-cytotype accounted for 29% of the total variation (Appendix S7). Interpretation of the seed yield data is more complicated than growth, given that gardens were open-pollinated. A potential source of error in seed yield data is incompatibility among genetically divergent sources that may lead to aborted seed and reduced yield. However, incompatibility in this species is unlikely within ploidal levels since introgression among subspecies is common (McArthur et al., 1988). Moreover, if incompatibility were prevalent, a decoupling between growth and seed yield would be expected. Instead, we found a strong association (Figure 4, *r* = 0.7). Nevertheless, inter-cytotype incompatibility could have affected seed yields and could be a source of error.

Genome duplication appears to have varied influence on traits of big sagebrush. In contrast to the concomitant responses of growth, seed yield (this study) and seed size (Richardson et al., 2015) to genome duplication, subspecies

and cytotype were not predictors of phenology and survival traits. Instead, the majority of genetic variation was explained by climate (Chaney et al., 2017; Richardson et al., 2017). Varied influence of genome duplication on traits has been documented in other species (Oswald and Nuismer, 2011; Balao et al., 2011). Several genetic and epigenetic mechanisms could underlie these differential effects on traits. For example, genome duplication has been shown to differentially affect secondary metabolite pathways, including big sagebrush (Jaeger et al., 2016). Lavania et al. (2012) have found that changes in some pathways have consequences to plant size in *Cymbopogon* (Poaceae). Gene sensitivity to duplication may depend on the type of the cellular function pathway (i.e., metabolic versus developmental), the proximity to transposable elements, and the type of methylation that may affect nearby transposable elements (Shi et al., 2015).

Tetraploid plants in this study have converged on a strategy of reduced aboveground growth and seed production. Questions remain as to whether the trait modifications are predominantly a result of genome duplication or whether environmental selection has shaped growth and seed yield in tetraploids. Studies documenting the change in gene expression and molecular mechanisms in model plant systems (e.g., Shi et al., 2015) appear to support genome duplication as the source of many trait modifications. In big sagebrush, observations of first generation tetraploids (i.e., seedling derived from diploids), suggest tetraploid-derived modification of growth (B. Richardson et al., unpublished data), but more detail study is needed.

Gene and gene–environment interaction

Growth and seed yield variation attributed to interpopulation climatic adaptation was relatively low (<4%, Appendix S7), which contrasts with a higher level of climatic (and local) adaptation evident in survival and phenology traits from long-term common gardens (Chaney et al., 2017; Richardson et al. 2017; Germino et al., 2019). However, a climatic adaptation signal in growth and seed yield does appear to be site-specific among populations as observed in the relationship between GxE random effect values and climate (Figure 3B). GxE seed yield variation among diploids at the warm-dry garden reflected a strong association with summer precipitation ($r = -0.6$, $P = 0.006$). Populations that experience lower summer precipitation, like that of the local population, IDT2, had increased seed yield at the Orchard garden, whereas populations that experience higher summer precipitation had poor seed yield (Figure 6). These poor performing populations tended to be located in the eastern portion of big sagebrush distribution where summer monsoons and rain events are more common. Further study using multiple common gardens or treatments would be needed to better understand the GxE in both traits. Nevertheless, population seed yield differences, especially among *tridentata*–2x at the warm-dry Orchard garden, support adaptation to seasonality of precipitation. Slight differences in seed yield could have large affects in fitness over time, and seed transfer across this climate gradient should be limited. While seed yield is not incorporated into the big sagebrush seed transfer (see Climate Smart Restoration Tool, climaterestorationtool.org), the summer precipitation gradient was also found to be a predictor in survival variation (Chaney et al., 2017), reflecting strategies in freezing resistance (Lazarus et al., 2019), and incorporated into seed transfer zones (Richardson and Chaney, 2018).

Strategies to niche differentiation

We propose that the trait variation observed here between big sagebrush subspecies and cytotypes suggests different ecological strategies for survival. The C-S-R theory (Grime, 1988) is used to categorize species as having different ecological specializations based on their traits, specifically growth strategies oriented toward performance in environments where selective pressures are distinctly characterized by competition (C), stress (S), or ruderal (R) conditions. Rarely has this concept been used below the species level (Astuti et al., 2018). Traits of the diploid subspecies *tridentata* are consistent with a competitive strategy with fast growth and high fecundity and plasticity, and traits of *wyomingensis* and *tridentata*–4x are consistent with a stress-tolerating strategy with slower growth and lower fecundity. It is also plausible that the moderate growth of and fecundity *tridentata*–4x (Figure 2) represents a trade-off between a competition and stress strategies, when compared to *tridentata*–2x. However, the smaller sample size in this group makes such inference limited.

While C-S-R theory appears to reflect the general dichotomy between ploidy, there are nuanced trait trade-offs below the ground. Welch and Jacobsen (1988) found faster seedling root growth in *wyomingensis* compared to *tridentata*, and recently, Zaiats et al. (2020) showed that *wyomingensis* had more extensive lateral roots and better uptake of surface water, indicating root growth habits between these subspecies are likely adapted to the depths of the soil profile. Given that these subspecies have largely sympatric distributions and large disparities in fecundity regardless of environment (Figure 1B), it is possible that one subspecies may have an advantage depending on the longevity of soil water into the growing season, which is in part governed by soil depth and type (Schlaepfer et al., 2011). Soils with limited moisture likely provide a more stressful environment and strong selection at the seedling stage favoring *wyomingensis*. Established stands of *wyomingensis* may competitively exclude *tridentata* based on the uptake of soil surface water. In hindsight, our study design may have negated observing seedling advantages in *wyomingensis* because plants were first grown in the greenhouse before outplanting and the garden locations have deeper soil profiles (>1 m). Moreover, since our study examined growth and seed yield for 2 years in a plant that has a lifespan of approximately 20 to 35 years (Perryman et al., 2001), it remains unclear whether these large differences among subspecies and cytotype remain as stands age. If the strategy of *wyomingensis* and *tridentata*–4x is a long-term strategy, greater seed production may occur episodically, later in the lifespan of tetraploids compared to diploids.

CONCLUSIONS

Big sagebrush is a landscape dominant species that resides over much of the cold deserts of western North America. This dominance can be attributed in part to a dual life-history strategy afforded by polyploidy and enabling niche differentiation. Tetraploidy in *wyomingensis* is associated with substantially lower aboveground growth rates and seed yield that are consistent with a conservative, stress-tolerating strategy. This strategy appears to provide this subspecies an edge in more arid areas and in shallow soils. Higher growth and fecundity associated with *tridentata*–2x likely promotes competitive and colonizing strategy. An understanding of these dual strategies can help determine where and how these subspecies are seeded on the landscape to increase restoration success.

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AUTHOR CONTRIBUTIONS
B.A.R. designed the study, analyzed the data, and drafted the manuscript. M.J.G., M.V.W., and S.B. helped interpret the results and revised the manuscript.

DATA AVAILABILITY STATEMENT
The data sets and R scripts are available from GitHub. Growth: (https://github.com/brichardsonfs/growth). Seed yield: (https://github.com/brichardsonfs/seed_yield)

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information tab for this article.

Appendix S1. Population information including subspecies, ploidy, and sample size (*N*) at each common garden (Ephraim, Majors Flat, and Orchard).

Appendix S2. Analysis of variance table of seed yield to determine whether within-plant collections (subsamples) explained significant variation.

Appendix S3. Plant yearly mean growth, seed mass and seed count values for subspecies-cytotype over 2 years at each common garden.

Appendix S4. Linear regression model of seed yield population means (seed count per year) predicted by growth volume (m3 per year) and subspecies-cytotype.

Appendix S5. Random effects, garden and year, on the model intercept for (a) growth and (b) seed yield.

Appendix S6. Conditional (fixed) effects, subspecies-cytotype and climate, on the model intercept for (a) growth and (b) seed yield.

Appendix S7. Summary of AIC (Akaike information criterion), marginal (fixed effects) and conditional variation (fixed and random effects) explained from mixed effect models with and without population climate predictors for growth and seed yield.