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Cytotype and genotype predict mortality and recruitment in Colorado quaking aspen (*Populus tremuloides*)

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Abstract. Species responses to climate change depend on environment, genetics, and interactions among these factors. Intraspecific cytotype (ploidy level) variation is a common type of genetic variation in many species. However, the importance of intraspecific cytotype variation in determining demography across environments is poorly known. We studied quaking aspen (*Populus tremuloides*), which occurs in diploid and triploid cytotypes. This widespread tree species is experiencing contractions in its western range, which could potentially be linked to cytotype-dependent drought tolerance. We found that interactions between cytotype and environment drive mortality and recruitment across 503 plots in Colorado. Triploids were more vulnerable to mortality relative to diploids and had reduced recruitment on more drought-prone and disturbed plots relative to diploids. Furthermore, there was substantial genotype-dependent variation in demography. Thus, cytotype and genotype variation are associated with decline in this foundation species. Future assessment of demographic responses to climate change will benefit from knowledge of how genetic and environmental mosaics interact to determine species' ecophysiology and demography.

Key words: *cytotype; demography; drought mortality; forest; ploidy level;* Populus tremuloides.

Introduction

Accurate predictions of species responses to environmental change require a robust understanding of underlying demographic mechanisms across populations and individuals (Aitken et al. 2008). Species are comprised of multiple individuals, which vary in their genotypes and thus their phenotypes. Performance depends on genotype (G), environment (E), and genotype × environment interactions (G × E). As environments change, some genotypes will outperform others (Davis et al. 2005, Gienapp et al. 2008). The net effect of these factors on performance will be that some 'winner' genotypes will experience high recruitment and survival and other "loser" genotypes will experience mortality (Aitken et al. 2008) for a given type of environmental change. This reshuffling dynamic is widely acknowledged in evolutionary ecology, but largely absent from spatial ecology and earth systems models (but see Scoble and Lowe 2010, Pauls et al. 2013, Greer et al. 2016, Ikeda et al. 2017).

Cytotype, here used to indicate variation in ploidy level (the number of copies of each chromosome, e.g., diploid, $n = 2$, vs. triploid, $n = 3$), can be a key component of intraspecific genotypic variation. Within species, higher ploidy level is often associated with increased cell sizes, growth rates (Comai 2005), and stomatal sizes (Doyle and Coate 2019), and is expected to influence rates of survival and persistence in varying environments. Polyploidization is a major driver of speciation, with 15% of angiosperms and 30% of ferns thought to have arisen through ploidy level variation, eventually leading to reproductive isolation and divergence (Wood et al. 2009).

Intraspecific cytotype variation is common across a wide range of ecologically important species. For example, many grass species vary in their ploidy level (Keeler

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1998), e.g., *Deschampsia caespitosa* (tussock grass) (Rothera and Davy 1986) and *Panicum virgatum* (switchgrass) (Nielsen 1944, McMillan and Weiler 1959). Many woody plants also vary in ploidy level, e.g., *Artemisia tridentata* (big sagebrush; Pellicer et al. 2010, Richardson et al. 2012*b*), *Betula papyrifera* (paper birch; Grant and Thompson 1975), *Inga* spp. (Figueiredo et al. 2014), *Larrea tridentata* (creosote bush; Laport et al. 2012), *Olea europaea* (olive; Besnard et al. 2007), *Polylepis* spp. (Schmidt-Lebuhn et al. 2010), and *Ulmus americana* (American elm; Whittemore and Olsen 2011). While even-numbered ploidy levels may provide an evolutionarily accessible strategy for increasing genetic variation when populations confront environmental change, odd-numbered ploidy levels are expected to have low or no fertility, indicating that their persistence in populations may be due to high vegetative performance. The demographic consequences of intraspecific cytotype variation across environmental gradients remain poorly studied.

Here we explore genotypic and environmental drivers of demography using the tree species quaking aspen (*Populus tremuloides*), which often occurs in both diploid and triploid cytotypes within populations (Every and Wiens 1971, Mock et al. 2012) and even within stands (Mock et al. 2008). Quaking aspen is the most broadly distributed species in North America, occurring widely from central Mexico to northern Canada (Little 1971, DeByle and Winokur 1985). Aspen is a foundation species and the primary land cover type in much of the Rocky Mountains (Jones and Markstrom 1973). At continental scales, triploidy appears be most common in the southwestern portion of the species' range (Mock et al. 2012). Quaking aspen often grows in cohesive clones, and contiguous stands generally consist of multiple clones. The distribution of clone sizes is poorly known, but data suggest that most clones have mean diameters of ≤100 m, with limited spatial interdigitation of stems from different clones in the same stand (Kemperman and Barnes 1976, Mock et al. 2008). In the Intermountain West, large clones also occur, some covering up to 430,000 m^2 (DeWoody et al. 2008). Many of these larger clones are triploid (Bishop et al. 2019).

Over recent decades, quaking aspen has experienced high levels of dieback in the southwestern portions of its range. In some cases, forests are replaced by forests while in other cases forests convert to desert scrub or grassland (Worrall et al. 2008). The proximate cause of mortality is probably a combination of regional drought driving hydraulic failure (Anderegg et al. 2013*a*) likely in a cumulative fashion (Anderegg et al. 2013*b*, Trugman et al. 2018), and also subsequent attack by various fungi and insects (Worrall et al. 2010, Marchetti et al. 2011). Regeneration failure may be exacerbated by ungulate herbivory (Seager et al. 2013, Fairweather et al. 2014) or herbaceous competition (Bockstette et al. 2017). Much of western North America is expected to experience temperature increases of 5°–7°C over the next century, with droughts of multiple decades likely to occur (Cook et al. 2015). Consequently, climate-envelope models (not including genetic predictors) suggest that quaking aspen will largely contract to the northern Rockies and boreal zone by the end of the century (Worrall et al. 2013).

Variation in genotype and cytotype within and among aspen populations could play a critical role in aspen response to climate change. Mortality occurs patchily on landscapes (Worrall et al. 2010) consistent with selection against some genotypes. Triploids may have lower resistance to drought stress than diploids (Greer et al. 2017) in environments with lower water availability. This differential environmental response may occur because triploid aspen may grow taller and faster, have larger leaves, greater leaf nitrogen/chlorophyll, leaf mass per area, photosynthetic rates, stomatal conductance, and sensitivity to higher vapor pressure deficits (Greer et al. 2017, Flansburg 2018). Variation in performance across cytotypes also occurs in other species (Levin 1983). As such, triploids may exhibit a fast but risky ecological strategy that could fail under stressful conditions, e.g., via drought-driven embolism (Anderegg et al. 2013*b*, Tai et al. 2017), under the hypothesis (as yet untested) that triploids have larger xylem conduits. Preliminary work in a northern Arizona aspen forest is also consistent with higher mortality for triploids (Dixon and DeWald 2015). However, little is known about how these observations generalize, or how the spatial distribution of environmental stress drives mortality for older genotypes that may have originally colonized the landscape under different environmental conditions. There may also be other effects of genotype on demography beyond that of cytotype (Barnes 1975).

Here we asked how environment, cytotype, genotype, and their interactions drive the demography (survival and recruitment) of quaking aspen. We first describe the spatial structure of genotype and cytotype and their distribution along environmental gradients. We then test several complementary hypotheses explaining variation in mortality and recruitment of quaking aspen:

- $H_{\text{environment}}$: demography depends on the environment, consistent with some prior regional studies of mortality (Worrall et al. 2013, Tai et al. 2017). Under this hypothesis, stem mortality should be higher and stem recruitment lower in hotter and drier environments.
- H_{cytotype}: demography depends on the cytotype (ploidy level), consistent with a prior single-site study of mortality (Dixon and DeWald 2015). Under this hypothesis, stem mortality should be higher and stem recruitment lower for triploids.
- H_{genotype}: demography depends on genotype differences beyond the effects of cytotype. Under this hypothesis stem mortality and recruitment should vary among genotypes, across all environments (Mitton and Grant 1996).
- $H_{\text{cytotype} \times \text{environment}}$: demography depends on the environmental context for a given cytotype. Under

this hypothesis, stem mortality should be higher and stem recruitment lower for triploids in hotter and drier environments. Cytotype-dependent climate occupancies are indirectly consistent with this hypothesis (Greer et al. 2016). Prior work has also shown genotype × environment effects on fitness-related defense traits (Osier and Lindroth 2006).

To test these hypotheses, we analyzed observational demographic, genetic, and environmental data from 503 aspen permanent plots in a drought-affected region, using data for counts of small-diameter stems as proxies of recruitment and counts of dead stems as proxies of mortality.

Methods

Plots

We focused our study in southwestern Colorado, a region currently experiencing high aspen forest dieback (Worrall et al. 2008) and forecast to experience large aspen range contractions in the coming century (Rehfeldt et al. 2009, Worrall et al. 2013). The region has seen sustained drought through the 2000s (see Palmer Drought Severity Index in Appendix S1: Fig. S1). The study area comprised ~400 km^2 of land in the upper Gunnison River watershed. Watersheds included the Middle East River, Oh-Be-Joyful Creek/Slate River, Washington Gulch, and Coal Creek. Natural land cover in these areas includes aspen forest, conifer forest, subalpine meadow, and alpine scree and talus.

We established 503 plots in the summer of 2018 (Fig. 1). These plots were chosen to cover a range of spatial and environmental scales and were approximately evenly distributed among watersheds. Plots fell into three categories. The first category was *gridded*. In each watershed, we selected 70 plots (280 total) in a hand-selected region that appeared to have high clonal diversity based on heterogeneity in color of autumn foliage in Google Earth imagery. Plots were selected on a 25-m rectangular spacing pattern in these regions, each grid covering an area of ~4 ha. These plots were chosen prospectively to assess genotype boundaries and assess evidence for fine-scale spatial structuring of cytotype (unfortunately our selection yielded primarily triploid gridded plots). The second category was *random*. In each watershed, we located ~45 plots (194 total) in areas containing aspen forest cover and occurring on either non-wilderness U.S. Forest Service or private conservation land on slopes less than 40°. Areas of aspen cover within the region were hand-mapped to 5 m resolution using Google Earth imagery. GIS software was then used to pick random coordinates within regions satisfying these constraints. These plots were chosen to provide the most representative sampling of the region. The third category was *opportunistic*. In each watershed, we also selected ~7 plots (29 total) that appeared visually to have high mortality or low recruitment. These plots were chosen to improve statistical balancing of demographic variables in the dataset for later analyses. The mean nearest-neighbor distance among non-gridded plots was 209 ± 264 m (mean ± SD).

Plots were relocated from the pre-chosen coordinates if they were found to be in small clearings. In this case, the nearest forest patch (with at least 5 m buffer to the clearing) was chosen. Final plot coordinates were measured using GPS units (Geo7X, Trimble, Sunnyvale, California, USA) with nominal sub-meter resolution (in practice, 1–2 m due to topography and canopy cover).

Demography

We made a single census of all plots, with work occurring between June and August 2018. Each plot was centered at a living, standing aspen stem (minimum 5 cm diameter at breast height), which was permanently tagged. The single-year census provides time-integrated proxies of demography, via total counts of new small trees (recruitment) and total counts of standing dead trees (mortality) in each plot. This approach is not able to estimate annualized demographic rates for use in a time-series analysis, but it does effectively buffer interannual demographic stochasticity, especially within a context of sustained multi-year drought in this region. The likely timescale of these integrated proxies is ~5 yr, based on field observations of treefall and sapling growth. Example plot conditions are shown in Appendix S1: Fig. S2.

To estimate mortality, from this center tree, we recorded data for the nearest 10 adult stems (of any species, alive or standing dead). In almost all cases, plots included only quaking aspen, but a small number of plots also included some individuals of *Abies lasiocarpa*, *Picea engelmanii*, *Pinus contorta*, and/or *Salix scouleriana*. Stems were included if they had diameters at breast height (DBH) >5 cm, i.e., at a point of measurement 130 cm above ground level for the stem. Points of measurements were adjusted up or down in a small number of cases (55/5,533 trees) to avoid damaged stem segments. For each stem, we recorded the DBH, the *x*/*y* coordinates (using a laser rangefinder and electronic compass; TruPulse 360R, LaserTech, Centennial, Colorado, USA) relative to the center tree, and a health code following RAINFOR protocol (Peacock et al. 2007).

Health codes were aggregated into several categories: *healthy*, for stems that were alive, whether standing or leaning; *damaged*, for stems that were alive, but are experiencing unrecoverable damage, e.g., with a dead crown, major stem breaks, lying on the ground, or with major signs of wounding (including ooze, certain fungal cankers, large fractions of bark loss, or sparse or few leaves in the canopy), or *dead*, for stems that were standing but had no canopies, no bark, or bark with a non-green cambium (as determined by a scratch test). We summarized plot data (excluding non-aspen stems) as a count (number of trees in each health category).

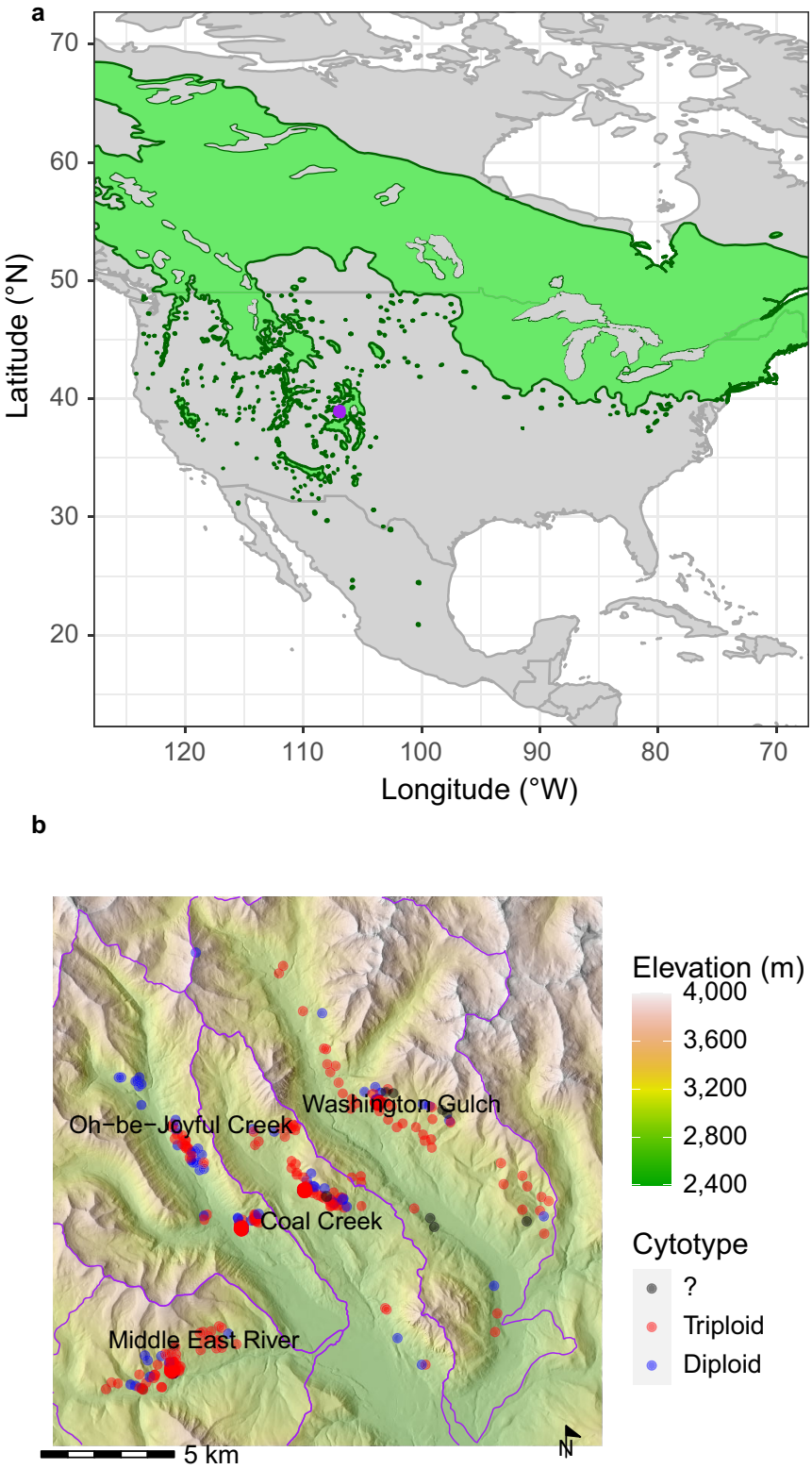

FIG. 1. (a) The geographic range of quaking aspen (green; from Little 1971) overlaid with the study region (purple). (b) Zoom of the study region, across four watersheds (purple boundaries). Plots are colored by cytotype. Large clusters of plots in each watershed indicate gridded sites (see Fig. 2 for zoom).

To estimate recruitment, we counted small stems within a 3 m radius around the focal tree for each plot. Small was defined as DBH <1 cm and ≥5 mm diameter at ground height.

Each plot always included 11 trees (center + 10 nearest), except for one mountain summit plot where we found only a single tree. Plot radius was variable and took a mean value of 5.6 ± 2.2 m (mean ± SD). Plots contained 9.0 ± 1.9 healthy stems, 0.7 ± 1.0 damaged stems, and 1.3 ± 1.5 dead stems, as well as 5.7 ± 7.8 small stems.

Environment data

We also measured a range of biotic and abiotic environmental factors at each plot at the same time that census data were collected. These factors were intended to capture information on the potential stressfulness of growth conditions.

Light availability at each plot was estimated via a hemispherical photograph from 2 m north of the focal tree (using a Canon 6D camera with 17–40 mm f/4L lens set to 17 mm focal length; ~75° × 95° field of view; Canon, Melville, New York, USA). Photos were then scored from 1 (open canopy) to 5 (closed canopy) independently by two trained individuals; any scores that differed were revised until consensus was reached. These values were then transformed to a continuous light index as 1 − (CanopyOpenness − 1)/4, where a value of 0 indicates a closed canopy and 1 an open canopy.

Cow presence was used as a metric of disturbance, as grazing is common in this region (Crawford et al. 1998). Plots that had any cow excrement, wallows, or hoof prints present were scored positive, while the absence of all factors was scored negative. This metric was collected at the time of initial visit and it is possible that there were additional cow impacts later in the season. As such, we consider this to be a metric of intense cow activity in the recent past, but lack of evidence is not necessarily indicative of cow absence. We were not able to collect data for impacts of other ungulates such as elk.

We qualitatively characterized the regolith type at each plot based on the soil coarse fraction. For each plot, three soil samples were obtained within a 3 m radius of the focal stem. Depth integrated samples were taken from 0 to 30 cm, (or to bedrock or rocks >10 cm length, whichever was reached first) using a soil corer (1055/EP, Lamotte, Chestertown, Maryland, USA) or soil knife (NJP640, Nisaku, Niigata, Japan) and composited in the field. Samples were scored qualitatively as being *soil* (only rocks <1 cm length), *scree* (some rocks >1 cm length), or *talus* (some rocks >5 cm length). Plots where trees grew directly from bedrock or large boulders had no samples collected and were scored as *talus*.

We also extracted information on rock units from USGS 1:24,000 maps. To facilitate later statistical analysis, we then coarsened the 21 observed rock units to five types aggregating units with presumed similar hydrological properties: Quaternary deposits (alluvium, outwash gravels, deltas, undifferentiated deposits, landslides, debris flows, glacial deposits), Quaternary talus and rock glacier, igneous intrusives (granite, granodiorite, gabbro, porphyry, or quartz monzonite), shales and limestones (Mancos shale and Fort Hays limestone), sandstone or conglomerate or siltstones (Dakota or Entrada or Mesa Verde or Morrison or Maroon formations).

To describe the physiography of each plot, we used a digital elevation model (1/3 arcsecond resolution) of the area produced from the National Elevation Dataset (Gesch et al. 2002). From these data, we obtained the elevation (m), and also calculated the slope (%) using a 3 × 3 kernel. We also mapped the net summer insolation (MJ/m^2) by using the digital elevation model coupled to a raytracing algorithm that simulated the diurnal progress of the sun across the terrain from 1 June 2018 to 30 September 2018. We did not obtain further gridded climate data, as in this montane region both temperature and precipitation are strongly positively correlated with elevation (Karger et al. 2017).

Genetic data

In June–August 2018, we obtained 5–10 healthy mature leaves from the lower canopy of the focal tree at each plot. Leaves were obtained from each tree using a slingshot (Big Shot, Notch Equipment, Greensboro, North Carolina, USA) following standard technique (Youngentob et al. 2016). Leaves from each of 503 sampled trees were placed in paper envelopes and dried at room temperature in silica desiccant before analysis. DNA was extracted (96 Well Synergy Plant DNA Extraction Kit, OPS Diagnostics, Lebanon, New Jersey, USA) following the manufacturer's recommendations, with final DNA elution volumes of 100 μL (Buffer AE, Qiagen, Qiagen Sciences, Germantown, Maryland, USA).

Double-digest restriction fragment-based DNA libraries were prepared using a method similar to that described by (Gompert et al. 2012), using restriction enzymes EcoRI (NEB, R0101L, New England BioLabs, Ipswich, Massachusetts, USA) and MseI (NEB, R0525L). DNA library processing is further described in Appendix S1: Text S1, Appendix S1: Table S1, and Appendix S1: Table S2. Supporting data are available through the Utah State University Digital Commons repository (Blonder et al. 2019); barcodes01-03.txt).

We conducted a reference-guided sequence assembly using ipyrad v.0.7.28 (Eaton 2014) and a *P. tremuloides* reference sequence available through PopGenIE (Sundell et al. 2015; sequence *available online*).[10] We performed two separate ipyrad runs, with different sets of parameters, one for identifying clonal membership (250 samples per locus minimum, msl_250) and one for identifying ploidy level (10 samples per locus minimum, msl_10).

[10] ftp://plantgenie.org/Data/PopGenIE/Populus_tremuloides/v1.1/FASTA/

We required retention of a greater number of samples per locus for clonal membership than for ploidy level estimation because the clonal determination is strengthened by using more common loci across samples, but ploidy level estimation is strengthened by having a greater number of loci per sample.

Cytotype was assigned based on estimates of allelic read depth proportions and levels of heterozygosity (Gompert and Mock 2017). Genotype (clonal membership) was assigned by calculating pairwise Jaccard similarities (across all SNPs), then assigning samples with similarities higher than 0.98 to the same clone. Additional details for assembly and filtering are provided in Appendix S1: Text S1 and Appendix S1: Fig. S3. The remaining parameters for these runs are provided along with the raw data and relevant scripts and results for clonal membership and ploidy determination, in Blonder et al. (2019).

Statistical analyses, inferential

We built generalized linear mixed models (GLMMs) to determine whether ploidy level and/or environment variables predicted demographic variables. For the two demographic response variables, we used a mortality proxy (count of adult dead trees plus count of adult damaged trees), and a recruitment proxy (count of small trees).

G (Genotype) was modeled as a random intercept. E (Environment) was modeled as fixed effects and included the variables: slope, insolation, elevation, rock unit, regolith texture, cow presence, and canopy openness, as well as DBH.mean. C (cytotype) was modeled as a fixed effect.

$$C = \text{Cytotype}$$

$$E = \sum_k \text{Environment}_k + \text{DBH.Mean}$$

$$G = \text{Genotype}$$

In order to test the complementary hypotheses presented in the introduction, we built several model structures based on different combinations of explanatory variables: ~ 1, $\sim E$, $\sim 1|G$, $\sim C$, $\sim E + 1|G$, $\sim E + C$, $\sim G + C$, $\sim E + C + 1|G$, and $\sim E \times C + 1|G$. We did not try to fit any models of the form $\sim E \times G$ (i.e., treating G as fixed) or $\sim E|G$ (i.e., genotype-dependent slope) due to the limited variation in environment within clones in this dataset. However, demography could vary among large genotypes that span environmental gradients.

DBH.Mean is defined as the mean DBH of all adult living trees (m). It was included in the E variables as a proxy for stand age and overall density within each plot that could potentially have had direct or cytotype-dependent effects on demography (e.g., lower recruitment has been previously reported in older stands (Rogers et al. 2010)). The DBH-age relationship is relatively strong in this species under common local environmental conditions (Beschta and Ripple 2007). Predictors were mostly uncorrelated with each other (Appendix S1: Fig. S4), except slope and insolation ($r = 0.47$). Stand density (stem basal area per unit land area) was not included due to its correlation with canopy openness ($r = 0.47$).

All continuous predictors were z-transformed prior to analysis to enable comparisons of slope estimates as effect sizes. All models were fit to the count data using a negative binomial family distribution, which assumed a linear relationship between the mean and variance of the distribution. AIC-based model comparisons indicated that other families (e.g., Poisson, normal), or quadratic mean-variance relationships were less well supported. Model residuals were formally checked for normality, homoscedasticity, and absence of correlation with predicted values, and passed in all cases. Model residuals were also checked for spatial autocorrelation using the Moran's I test using inversely weighted Euclidean distances with diagonal entries set to zero. In all cases, test P values were nonsignificant, so no spatial autocorrelation structure was included in the models.

To test hypotheses, we calculated the Akaike Information Criterion (AIC) to determine which model for each demographic response variable was best supported by the data (e.g., an $E + G$ model vs. an $E + G + C$ model). Models with $\Delta\text{AIC} < 2$ were considered having equivalent performance.

We then performed further analyses of the best-fitting model. Fixed effect confidence intervals and P values include a 95% range, calculated via the Wald method; random effect confidence intervals are calculated symmetrically as the estimate $\pm$ the square root of the conditional variance. The R^2 statistic was estimated as the fraction of variance explained by the fixed effects (marginal) or by the fixed and random effects (conditional), relative to the sum of the fixed effects variance, random effects variance, additive dispersion component, and distribution-specific variance (Nakagawa and Schielzeth 2013).

Statistical analyses, descriptive.—We summarized the distribution of ploidy level across environments, using either bar plots (categorical predictors) or density plots (continuous predictors). Differences among ploidy levels were tested using either chi-square tests (categorical variables) or linear regression (continuous variables).

To visualize the spatial structure of the genetic data at local scale, we made maps of the *gridded plots* after interpolating at 1-m spatial resolution using a k-nearest-neighbors algorithm with $k = 1$, and clipped to a 25 m radius of all data points. This approach is reasonable as prior analyses of genetic structure in aspen populations in central Utah found general homogeneity in clone identity at sub-50-m scales (Mock et al. 2008).

Statistical software.—All statistical analyses were carried out using R software (version 3.5.1). Spatial analyses

were conducted with the *class*, *geoR*, *insol*, *raster*, *gstat*, and *sp* packages; generalized linear mixed models and diagnostics with the *ape*, *car*, *glmmTMB*, and *DHARMa* packages; conditional model predictions with the *visreg* package; and other plots with the *GGAlly*, *ggspatial*, *ggplot2*, *maptools*, *RStoolbox*, and *sjPlot* packages.

Results

Spatial structure of genetics

A total of 480 trees (one per plot) were successfully classified with respect to cytotype and 487 assigned to clone (genotype). The sampled trees were mostly triploids. Of the 480 trees with genotype and cytotype assignments, 407 (85%) were triploid and 73 (15%) were diploid. Among the randomly sampled trees, there was a more even balance between cytotypes (154 triploids [72%] vs. 59 diploids [28%]; Fig. 2, Appendix S1: Fig. S5). Among the 480 successfully classified trees (including non-randomly located plots), we found 191 unique genotypes. These clones were represented by from 1 to 36 sampled trees. The majority of observed clones were represented by a single sample.

Among the randomly sampled plots with reliable data (i.e., those that are a representative sample of the landscape), 5 of 207 clones were represented by more than three samples, all of which were triploid. Within the randomly sampled plots, of the clones represented only by a single sample, 74 of 130 were triploid, 55 were diploid, and 1 was unclassifiable. This is consistent with previous findings that the larger aspen clones tend to be triploid (Mock et al. 2008).

Among the intensive grid plots where there was sufficient spatial resolution to estimate the boundaries of these genotypes, most clones were spatially homogeneous, though there were also some cases of interdigitation between genotypes at sub-25-m spatial scales (Fig. 2).

Distribution of cytotype along environmental gradients

There were some differences in the relative prevalence of diploid and triploid trees across environmental gradients (Appendix S1: Fig. S6). Triploid trees occurred more frequently on regolith classified as soil (73% vs. 49%) while diploids were more likely to occur on scree (10% vs. 4%) and talus (41% vs. 22%; χ^2 $P < 0.001$). Diploid trees were more likely to occur on shales and limestones (15% vs 6%), and igneous intrusive rock units (29% vs. 14%), while triploids were more likely to occur on Quaternary deposits (54% vs. 34%; χ^2 $P < 0.0001$). Cow presence was similar between diploid and triploid trees (7% for both; χ^2 $P = 1$). Light level varied slightly among cytotypes (χ^2 $P = 0.05$), with triploid trees marginally more common at lower canopy light index values. Diploid trees also occurred at higher elevations (mean 3,050 vs. 2,995 m; t test $P < 0.01$) and on steeper slopes (24° vs. 19°; t test $P < 10^{-4}$), while both diploid and triploid trees were found in locations with similar summer insolation (22.9 vs. 22.8 MJ m^{-2}; t test $P = 0.75$). Triploid trees also had higher mean DBH (20.6 vs. 15.7 cm; t test $P < 10^{-12}$).

Drivers of recruitment

The best-supported recruitment model included environment (E), genotype (G), cytotype (C) and cytotype × environment predictors ($C \times E$; Appendix S1: Fig. S7a). This model had an AIC value more than 5 units smaller than the next-best model, indicating strong support for it. The $E + G + C + C \times E$ model explained 17% of the variation with fixed effects alone (marginal R^2) and 42% with the random effects (conditional R^2). The significant predictors included cow presence (+1.8), slope (+0.3), DBH.Mean (−0.4), and cow presence × triploidy (+0.1) (Fig. 3). A range of other predictors also had large effects, e.g., various rock units (positive and negative) and triploidy (positive). A full model summary is reported in Appendix S1: Table S3.

Drivers of mortality

There were four mortality models with similar support (ΔAIC <2 units). Models depended on environment (E) and/or genotype (G) and/or cytotype (C) (Appendix S1: Fig. S7b). The $E + G + C$ model is presented here and the below referenced figures, as it included the most variables of interest among the best-supported set explained 6% of the variation with fixed effects alone (marginal R^2) and 30% with the random effects (conditional R^2). The predictors with confidence intervals excluding zero included scree regolith (+0.4), and slope (−0.1) (Fig. 4). Several other predictors also had large effects, e.g., shale or limestone rock unit (positive) and triploidy (positive). A full model summary is reported in Appendix S1: Table S4.

Summary of model predictions

Conditional plots of each demographic model yielded several predictions for climate change responses using proxy variables. We examined model predictions with respect to variables that may be proxies of warming (e.g., elevation and summer insolation) and drought and water holding capacity, e.g., regolith type and rock unit, as well a proxy of human impact (cow presence). This analysis suggests that with warming, recruitment would decrease more strongly for triploids than diploids at low elevations, at higher summer insolation, on scree-like regolith, and in cow-grazed plots (Fig. 5a); and that mortality would increase more strongly for triploids than diploids in general, with slightly more severe mortality on plots with higher summer insolation (Fig. 5b). That is, at plots described by variables that are proxies for hotter and drier conditions, the model predicted that triploids will die more and recruit less than diploids.

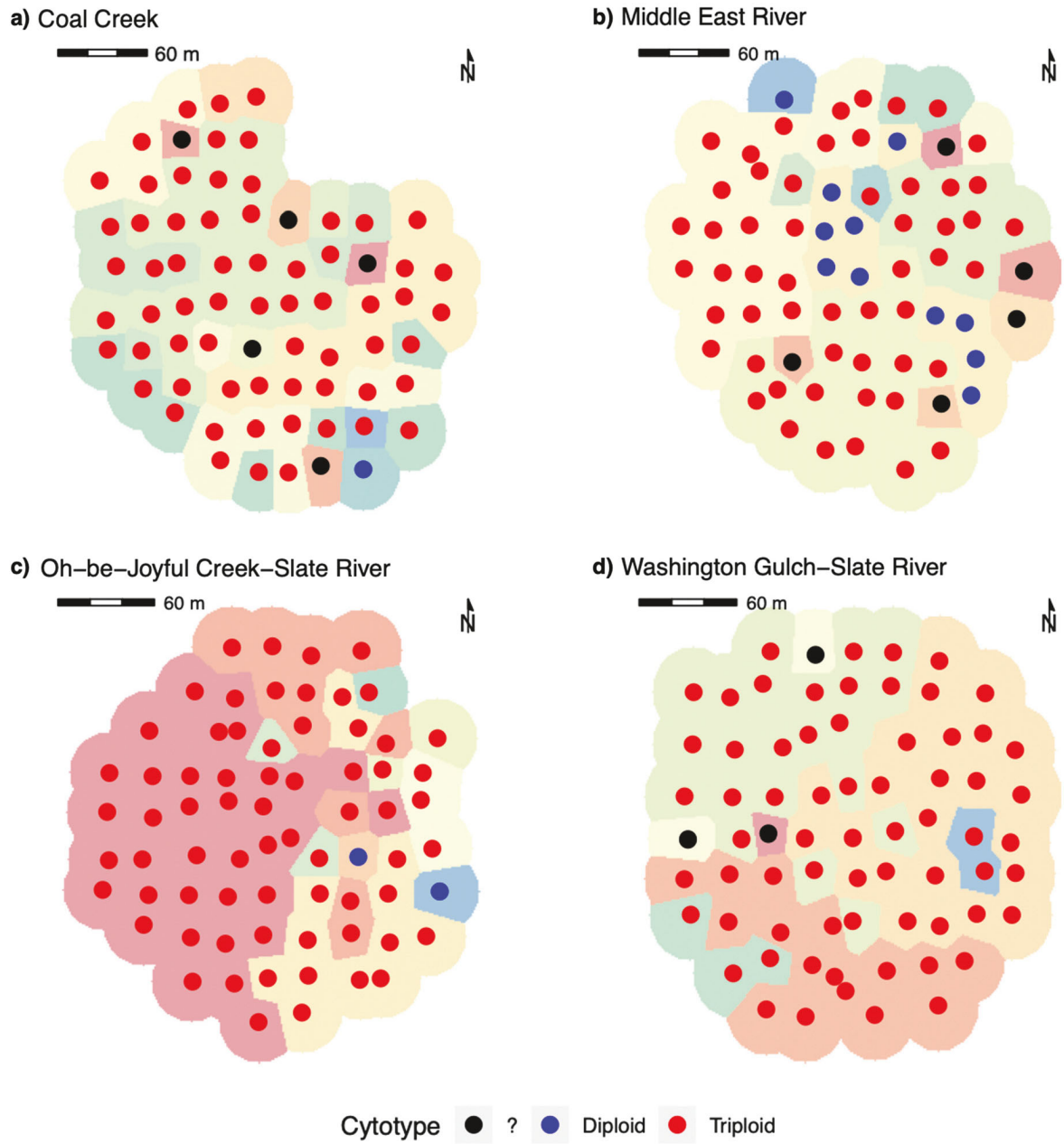

FIG. 2. Spatial distribution of cytotype and genotype in grids within four watersheds. Sampling points are located by georeferenced coordinates. Points are colored by cytotype and polygons shaded by genotype, as extrapolated using a nearest-neighbor algorithm. Colors are unique only within each panel, as no genotypes occur in multiple watersheds.

DISCUSSION

These results show that all of the hypotheses for the decline of quaking aspen in southwestern Colorado were variously supported by the model selection analyses. First, both mortality and recruitment were related to environment, supporting $H_{\text{environment}}$. There were significant effects of slope and cow presence on recruitment, as well as strong effects of slope and rock unit on mortality. Second, both mortality and recruitment were related to genotype, supporting H_{genotype}, due to the large genotype random effect variance. Third, the data also show that both mortality and recruitment are related to cytotype, supporting H_{cytotype}, with a positive effect of triploidy in both cases. Fourth, the data show that recruitment was related to interactions among cytotype and the environment, supporting $H_{\text{cytotype} \times \text{environment}}$, via a cow presence × triploidy effect. Thus, environmental variables alone provide an incomplete explanation of aspen demographic responses. The spatial mosaic of

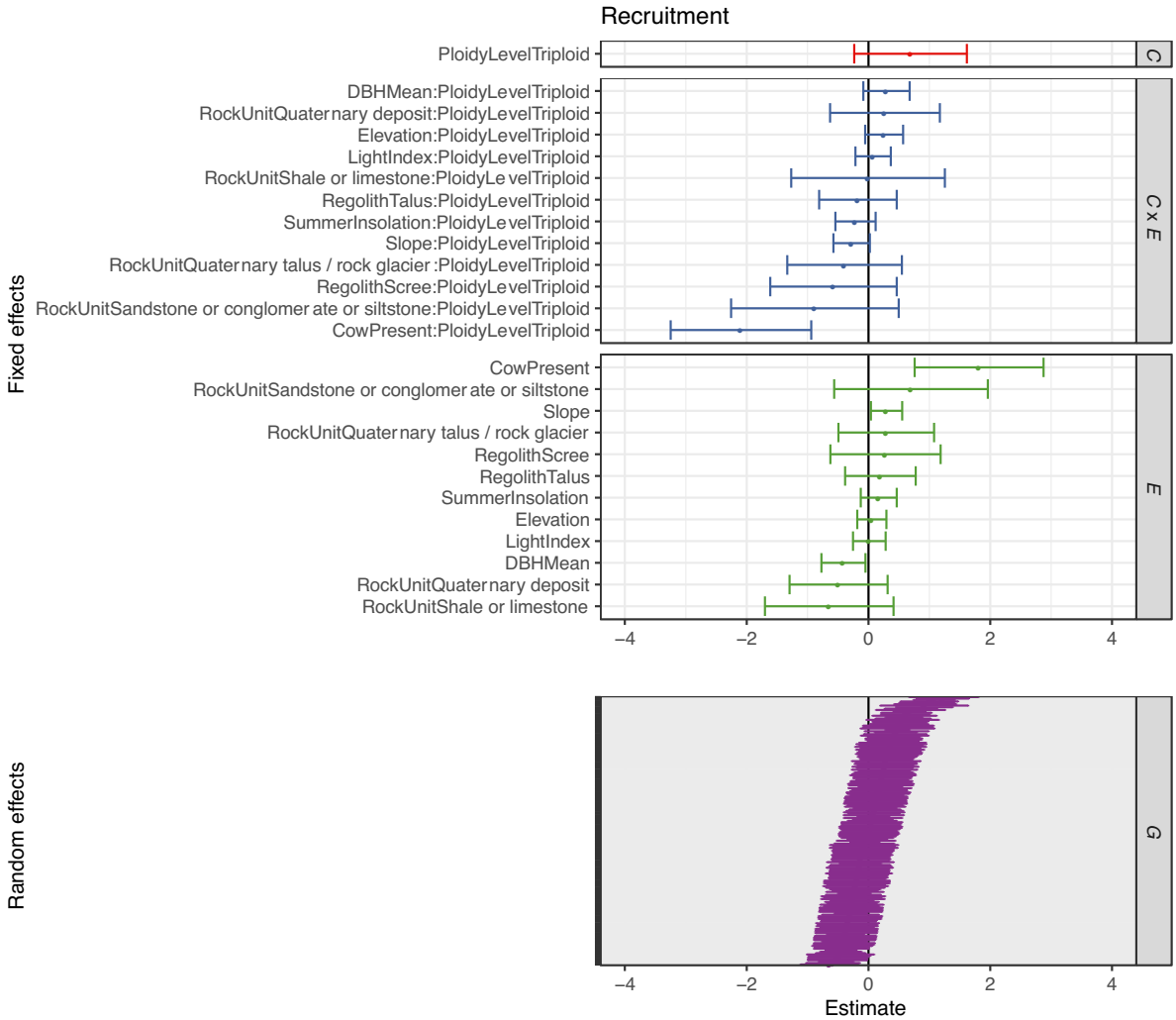

FIG. 3. Summary of the best-fitting recruitment model. Bars indicated standardized coefficient estimates and confidence intervals, and are grouped by variable type. Facets indicate cytotype effects (*C*), environment effects (*E*), genotype effects (*G*), and interactions among cytotype and environment (*C* x *E*). Labels for genotypes are omitted for visual clarity. Effects are shown on an untransformed scale and reported in tabular form in Appendix S1: Table S3.

genetic features (cytotype and genotype) as well as the spatial mosaic of environments (both abiotic and biotic) are necessary to understand the demography of this species.

Thus, these findings help extend the literature on environmental causes of aspen decline (Rehfeldt et al. 2009, Worrall et al. 2010, 2013, Huang and Anderegg 2012) and unify it with the small extant knowledge of genetic structure and genotype-dependent mortality (Mock et al. 2008, Dixon and DeWald 2015) in this ecologically dominant species. As a result, this study indicates that our understanding of demography under environmental change is enhanced by linking ecology and genetics (Metcalf and Pavard 2007, Mock et al. 2008, Dixon and DeWald 2015, Lowe et al. 2017).

These findings are consistent with prior environment-only monitoring studies of aspen decline (Worrall et al. 2010), but build on them by clarifying the genetic context in which declines may be strongest. Thus, there is a potential for broad-scale selection of genotypes in the future; however, the identities of each will vary due to the complexity of the underlying geographic/environmental mosaics, and the present-day distribution of diploids and triploids on the landscape. This conclusion provides a mechanism underlying the high spatial patchiness of aspen mortality observed in recent decades: dead patches may represent the loss of clones that occurred in newly unfavorable conditions for their cytotype. It may be that most of these losses have been triploids.

However, it is also important to acknowledge that there were large effects on demography arising from differences among genotype that were not tied to cytotype. Such genotype differences often had a larger effect than

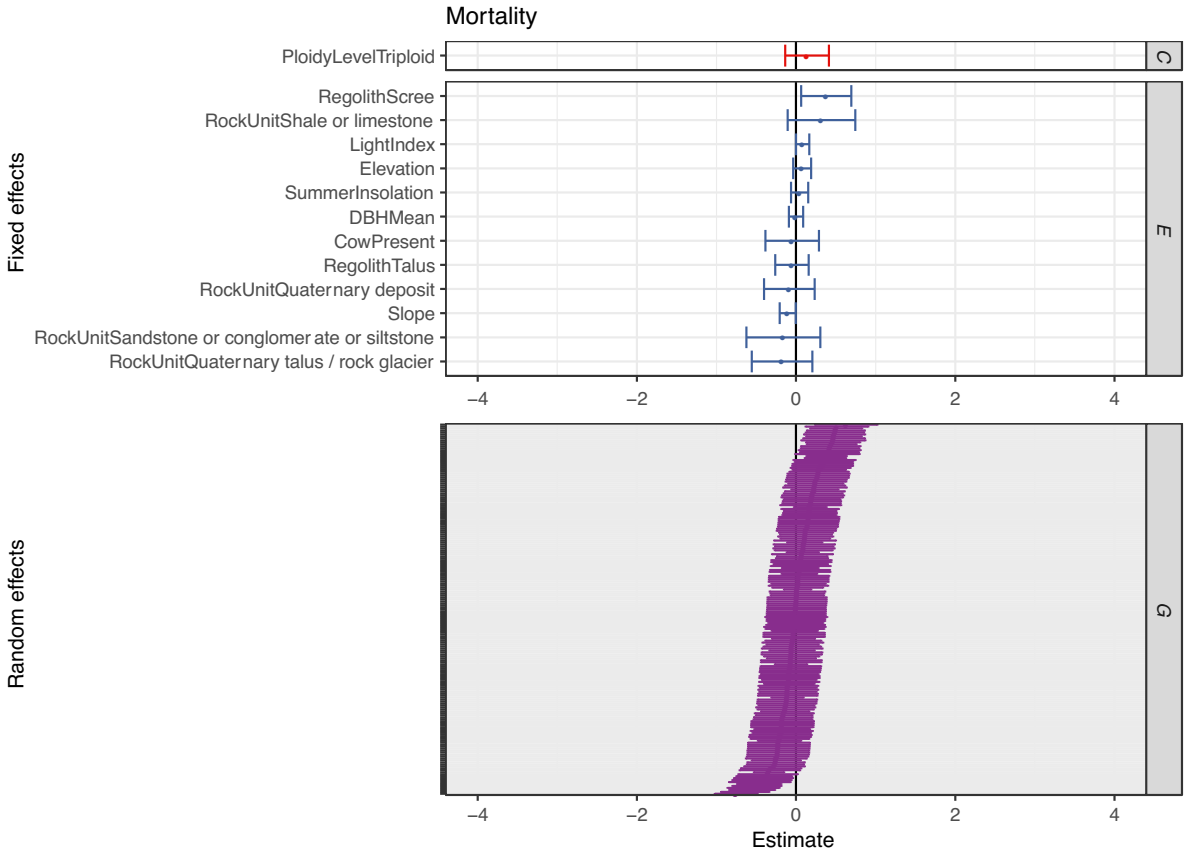

FIG. 4. Summary of a best-fitting mortality model. Bars indicated standardized coefficient estimates and confidence intervals and are grouped by variable type. Facets indicate cytotype effects (*C*), environment effects (*E*), genotype effects (*G*), and interactions among cytotype and environment (*C* x *E*). Labels for genotypes are omitted for visual clarity. Note that very similar models also fit the data similarly well. Effects are shown on an untransformed scale and reported in tabular form in Appendix S1: Table S4.

cytotype, as assessed by the high marginal R^2 values relative to conditional R^2 values for each model. These clonal effects were especially strong for recruitment. Landscapes with many genotypes may provide regional-scale insurance against population decline by increasing the chances that some genotypes may be able to offset environmentally driven declines through high population growth rates.

The current analysis suggests changes in population growth rates, but our measurement of demography proxies is not yet sufficient for such predictions. Longer-term monitoring would enable tracking of vegetative growth and reproduction rates at these plots and refining these single-year proxies of demography into direct estimates of these rates. For example, our dead tree counts certainly integrate over multiple years of mortality events. Additionally, we were not able to estimate vegetative growth from a single-year survey. Declines in growth may be an adaptive strategy to buffer against stressful environments, and could counteract some of the increased mortality and decreased recruitment observed for triploids. Longer-term data for all of these demographic rates could ultimately be used to construct integral projection models that would be able to directly predict long-term population dynamics.

The physiological basis of the variable demographic responses of diploids and triploids will require further exploration. Prior studies have demonstrated how ecophysiological traits (e.g., leaf nitrogen content, water use efficiency) vary with genotype (Blonder et al. 2013) and cytotype (Greer et al. 2017). Other earlier studies have also identified key physiological differences driven by cytotype or genotype, e.g., growth rate, branch length, leaf size, and phenology (Van Buijtenen et al. 1957, Einspahr et al. 1963, Benson and Einspahr 1967, Every and Wiens 1971). Clarifying how these traits interact with environment to determine demography across environmental gradients remains a priority (Salguero-Gómez et al. 2018). One hypothesis arises from related cytotype effects (Bennett 1971, Doyle and Coate 2019): larger genome size (i.e., higher ploidy levels) can physically cause variation in leaf cell size. This in turn may lead to changes in water use efficiency and gas exchange rates due to changes in stomatal guard cell sizes and density

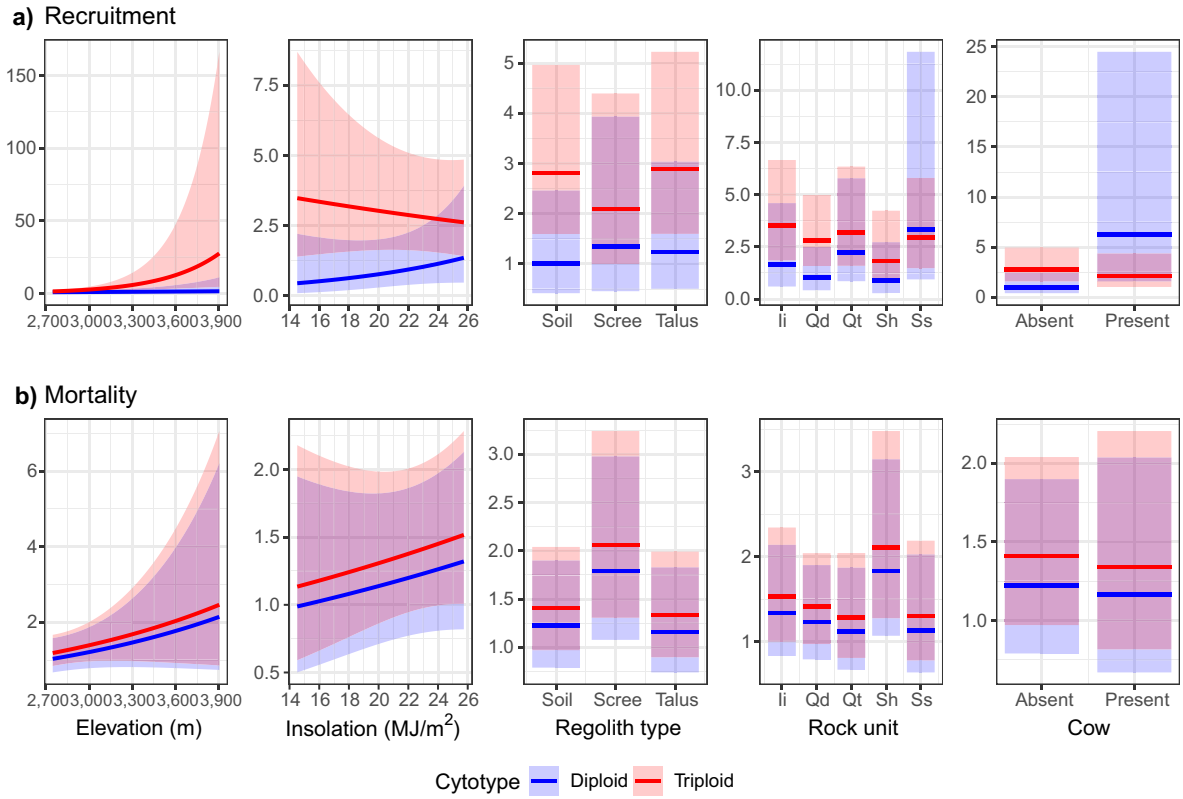

FIG. 5. Conditional predictions for a subset of predictors on (a) recruitment and (b) mortality using the best-fitting models for each. Predictions (*y*-axis values) are made while varying the *x*-axis predictor of interest and holding all other predictors to median values (continuous predictors) or modal values (categorical predictors) and assuming no random (genotype) effects. Predictions are shown as solid lines; confidence intervals as transparent bands.

(Beaulieu et al. 2008). In particular, larger genome sizes may limit the packing of cells, reducing maximum gas exchange rates (Roddy et al. 2020). However, these predictions run contrary to the observation of higher gas exchange rates in triploid relative to diploid aspen (Greer et al. 2017). Regardless, it seems clear that high gas exchange rates seen in triploids predispose them to a "fast and risky" life history (Reich 2014), which is consistent with the mortality data in this study. We hypothesize that cytotype influences stem xylem architecture, e.g., with larger and riskier conduits in triploids, or increased accumulation of cavitation damage in triploids after drought (Anderegg et al. 2013*b*) and/or defoliation (Hillabrand et al. 2018). There could also be cytotype-dependent variation in defense chemistry (Marchetti et al. 2011). Previous work has shown that calcium also promotes xylem cavitation resistance (Herbette and Cochard 2010), suggesting that variation in soil calcium availability might also interact with ploidy level to determine mortality risk. However, our data seem to show higher mortality on more calcium-rich rock types (e.g., limestones), contrary to this prediction.

We have left open the question of whether plots respond in genotypically uniform ways over time, as assumed by our models. There are two potential within-plot sources of non-uniformity: adult trees belonging to different genotypes (e.g., due to encroachment from nearby areas); new recruits having different genotypes than adults (e.g., due to sexual reproduction, or vegetative encroachment). Alternatively, it could be possible that canopy mortality of triploids does not represent true mortality of an individual. If rootstocks remain viable, resprouting of the same genotype may occur, as has been documented in Canadian aspen stands (DesRochers and Lieffers 2001). That is, canopy mortality may be an adaptive strategy to avoid stressful conditions, at least under conditions where stresses are rare, and where herbivory of resprouted growth is low. These factors all may be relevant in changing environments where strong selection may be occurring. For example, plots experiencing high adult mortality may see high subsequent recruitment, but of a novel and better-suited genotype. There is also a possibility that demographic responses differ by sex, leading to shifts in the sex ratios of populations. Aspen is a dioecious species, meaning that genotypes may be either male or female (Grant and Mitton 1979), as likely determined by an incipient sex chromosome (Pakull et al. 2009). The sex of the genotype may

influence stress physiology (Melnikova et al. 2017), and thus potentially demography. Sex-dependent climate change responses remain poorly understood. We will be exploring these possibilities in future work once we have collected further demographic data.

Other regions within the species' range may comprise different genetic clusters, and lineage-specific adaptation may occur. In such cases, the relationship between genotype and demography may differ, and the model results here may not transfer (e.g., as occurs in spruce [Prunier et al. 2012]). In aspen, several genetic clusters have been identified in western North America (Bagley et al. 2020), but analyses did not cover southwestern Colorado and the southwestern United States. Further work in this area, coupled to broader demographic surveys, are likely to provide further insight into this question.

The cytotype results are more likely to generalize across space. Triploid aspen has been observed to be more common in the southwestern part of the species' range, i.e., central and northern Mexico, Arizona, New Mexico, and Texas (Mock et al. 2012). Under current warmer (and warming) climates, triploid cytotypes may no longer confer performance benefits. We speculate that landscapes may therefore see declines of triploids and re-expansion of diploids, leading to large selective sweeps across populations.

Generalizable results from this study could help improve predictions of species distribution models and consequently the management of aspen (Rehfeldt et al. 2015). Species distribution models currently fit to environmental predictors alone could be augmented with genotypic predictors to account for heterogeneity in a species' response to environmental change (Whitham et al. 2006, Hamann and Aitken 2013, Franklin 2013). Such genetically informed niche models have already been demonstrated to dramatically improve predictive capacity for the related species *Populus fremontii* (Ikeda et al. 2017).

Genetic analyses have required time-intensive methods on field-collected samples, e.g., determination of cytotype using flow cytometry and DNA sequencing (Gompert and Mock 2017, Greer et al. 2017). However, recent work has demonstrated that remote sensing of quaking aspen cytotype (Blonder et al. 2020) and genotype (Madritch et al. 2014) can be achieved. These genetic characteristics should systematically affect individual canopy structure and chemistry, which in turn affect spectral reflectance. This method opens the possibility of upscaling genetically informed species distribution models to whole regions for quaking aspen and other species. Indeed, this study resulted in a data set that was biased toward triploids, because we had no advance knowledge to guide ground-based sampling. This issue potentially constrains interpretation of cytotype findings due to the relatively low representation of diploids. Further advances in remote sensing may make it possible to balance ground-based sampling across cytotypes or to skip ground-based sampling entirely.

Incorporating genetic information into species distribution models may improve our ability to predict responses to environmental change and to identify populations for which climate adaptation and land management may be most effective. For example, big sagebrush (*Artemisia tridentata*) is another key Western species in which cytotype variation also occurs, and for which cytotype-dependent responses to climate change exist (Richardson et al. 2012*a*, Chaney et al. 2017). This information is now informing current management and restoration efforts (Brabec et al. 2017, Richardson and Chaney 2018). Similar approaches may soon become possible for aspen, where seed sourcing is a key issue for restoration work (Landhausser et al. 2019). We hope that increasingly available genetic information will help to predict and support the response of this iconic species to environmental change.

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Literature Cited

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications 1:95–111.

Anderegg, L. D. L., W. R. L. Anderegg, J. Abatzoglou, A. M. Hausladen, and J. A. Berry. 2013*a*. Drought characteristics' role in widespread aspen forest mortality across Colorado, USA. Global Change Biology 19:1526–1537.

Anderegg, W. R., L. Plavcová, L. D. Anderegg, U. G. Hacke, J. A. Berry, and C. B. Field. 2013*b*. Drought's legacy: multiyear hydraulic deterioration underlies widespread aspen forest die-off and portends increased future risk. Global Change Biology 19:1188–1196.

Bagley, J. C., N. M. Heming, E. E. Gutiérrez, U. K. Devisetty, K. E. Mock, A. J. Eckert, and S. H. Strauss. 2020. Genotyping-by-sequencing and ecological niche modeling

illuminate phylogeography, admixture, and Pleistocene range dynamics in quaking aspen (*Populus tremuloides*). Ecology and Evolution 10:4609–4629.

Barnes, B. V. 1975. Phenotypic variation of trembling aspen in western North America. Forest Science 21:319–328.

Beaulieu, J. M., I. J. Leitch, S. Patel, A. Pendharkar, and C. A. Knight. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. New Phytologist 179:975–986.

Bennett, M. 1971. The duration of meiosis. Proceedings of the Royal Society B 178:277–299.

Benson, M. K., and D. W. Einspahr. 1967. Early growth of diploid, triploid and triploid hybrid aspen. Forest Science 13:150–155.

Beschta, R. L., and W. J. Ripple. 2007. Wolves, elk, and aspen in the winter range of Jasper National Park, Canada. Canadian Journal of Forest Research 37:1873–1885.

Besnard, G., C. Garcia-Verdugo, R. Rubio De Casas, U. A. Treier, N. Galland, and P. Vargas. 2007. Polyploidy in the olive complex (*Olea europaea*): Evidence from flow cytometry and nuclear microsatellite analyses. Annals of Botany 101:25–30.

Bishop, M., T. J. Furniss, K. E. Mock, and J. A. Lutz. 2019. Genetic and spatial structuring of *Populus tremuloides* in a mixed-species forest of southwestern Utah, USA. Western North American Naturalist 79:63–71.

Blonder, B., et al. 2021. Cytotype and genotype predict mortality and recruitment in Colorado quaking aspen (*Populus tremuloides*). Dryad data set. https://doi.org/10.6078/D1898W.

Blonder, B., et al. 2020. Remote sensing of ploidy level in quaking aspen (*Populus tremuloides* Michx.). Journal of Ecology 108:175–188.

Blonder, B., C. Violle, and B. J. Enquist. 2013. Assessing the causes and scales of the leaf economics spectrum using venation networks in *Populus tremuloides*. Journal of Ecology 101:981–989.

Blonder, B., J. A. Walton, and K. E. Mock. 2019. Ploidy and clonal membership in *Populus tremuloides* from RADseq data. Digital Commons. https://digitalcommons.usu.edu/all_datasets/93/

Bockstette, S. W., B. D. Pinno, M. F. Dyck, and S. M. Landhäusser. 2017. Root competition, not soil compaction, restricts access to soil resources for aspen on a reclaimed mine soil. Botany 95:685–695.

Brabec, M. M., M. J. Germino, and B. A. Richardson. 2017. Climate adaption and post-fire restoration of a foundational perennial in cold desert: Insights from intraspecific variation in response to weather. Journal of Applied Ecology 54:293–302.

Chaney, L., B. A. Richardson, and M. J. Germino. 2017. Climate drives adaptive genetic responses associated with survival in big sagebrush (*Artemisia tridentata*). Evolutionary Applications 10:313–322.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Nature Reviews Genetics 6:836.

Cook, B. I., T. R. Ault, and J. E. Smerdon. 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. Science Advances 1:e1400082.

Crawford, J. L., S. P. Mcnulty, J. B. Sowell, and M. D. Morgan. 1998. Changes in aspen communities over 30 years in Gunnison County, Colorado. The American Midland Naturalist 140:197–206.

Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to changing climate. Ecology 86:1704–1714.

DeByle, N. V., and R. P. Winokur 1985. Aspen: ecology and management in the western United States. USDA Forest Service General Technical Report RM-119. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.

DesRochers, A., and V. J. Lieffers. 2001. The coarse-root system of mature *Populus tremuloides* in declining stands in Alberta, Canada. Journal of Vegetation Science 12:355–360.

DeWoody, J., C. A. Rowe, V. D. Hipkins, and K. E. Mock. 2008. "Pando" Lives: molecular genetic evidence of a giant aspen clone in Central Utah. Western North American Naturalist 68:493–497.

Dixon, G. B., and L. E. DeWald. 2015. Microsatellite survey reveals possible link between triploidy and mortality of quaking aspen in Kaibab National Forest, Arizona. Canadian Journal of Forest Research 45:1369–1375.

Doyle, J. J., and J. E. Coate. 2019. Polyploidy, the nucleotype, and novelty: The impact of genome doubling on the biology of the cell. International Journal of Plant Sciences 180:1–52.

Eaton, D. A. R. 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30:1844–1849.

Einspahr, D. W., J. Van Buijtenen, and J. Peckham. 1963. Natural variation and heritability in triploid aspen. Silvae Genetica 12:51–58.

Every, A. D., and D. Wiens. 1971. Triploidy in Utah aspen. Madroño 21:138–147.

Fairweather, M. L., E. A. Rokala, and K. E. Mock. 2014. Aspen seedling establishment and growth after wildfire in central Arizona: an instructive case history. Forest Science 60:703–712.

Figueiredo, M. F., R. Bruno, A. E. Barros e Silva, S. Nascimento, I. G. Oliveira, and L. P. Felix. 2014. Intraspecific and interspecific polyploidy of Brazilian species of the genus Inga (Leguminosae: Mimosoideae). Genetics and Molecular Research 13:3395–3403.

Flansburg, A. L. 2018. Trembling aspen triploid advantage has a physiological basis with geographic caveats. University of Wisconsin, Wisconsin, Madison, USA.

Franklin, J. 2013. Species distribution models in conservation biogeography: developments and challenges. Diversity and Distributions 19:1217–1223.

Gesch, D. B., G. A. Evans, M. J. Oimoen, and S. Arundel. 2002. The national elevation data set. Photogrammetric Engineering and Remote Sensing. Volume 68. Pages 83–110. American Society for Photogrammetry and Remote Sensing, Sioux Falls, South Dakota, USA.

Gienapp, P., C. Teplitsky, J. Alho, J. Mills, and J. Merilä. 2008. Climate change and evolution: disentangling environmental and genetic responses. Molecular Ecology 17:167–178.

Gompert, Z., and K. E. Mock. 2017. Detection of individual ploidy levels with genotyping-by-sequencing (GBS) analysis. Molecular Ecology Resources 17:1156–1167.

Gompert, Z., L. K. Lucas, C. C. Nice, J. A. Fordyce, M. L. Forister, and C. A. Buerkle. 2012. Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. Evolution 66:2167–2181.

Grant, M. C., and J. B. Mitton. 1979. Elevational gradients in adult sex ratios and sexual differentiation in vegetative growth rates of *Populus tremuloides* Michx. Evolution 33:914–918.

Grant, W., and B. Thompson. 1975. Observations on Canadian birches, *Betula cordifolia*, *B. neoalaskana*, *B. populifolia*, *B. papyrifera*, and *B.× caerulea*. Canadian Journal of Botany 53:1478–1490.

Greer, B. T., C. Still, G. L. Cullinan, J. R. Brooks, and F. C. Meinzer. 2017. Polyploidy influences plant–environment interactions in quaking aspen (*Populus tremuloides* Michx.). Tree Physiology 38:630–640.

Greer, B. T., C. Still, G. T. Howe, C. Tague, and D. A. Roberts. 2016. Populations of aspen (*Populus tremuloides* Michx.) with

different evolutionary histories differ in their climate occupancy. Ecology and Evolution 6:3032–3039.

Hamann, A., and S. N. Aitken. 2013. Conservation planning under climate change: accounting for adaptive potential and migration capacity in species distribution models. Diversity and Distributions 19:268–280.

Herbette, S., and H. Cochard. 2010. Calcium is a major determinant of xylem vulnerability to cavitation. Plant Physiology 153:1932–1939.

Hillabrand, R. M., V. J. Lieffers, E. H. Hogg, E. Martínez-Sancho, A. Menzel, and U. G. Hacke. 2018. Functional xylem anatomy of aspen exhibits greater change due to insect defoliation than to drought. Tree Physiology 39:45–54.

Huang, C.-Y., and W. R. L. Anderegg. 2012. Large drought-induced aboveground live biomass losses in southern Rocky Mountain aspen forests. Global Change Biology 18:1016–1027.

Ikeda, D. H., T. L. Max, G. J. Allan, M. K. Lau, S. M. Shuster, and T. G. Whitham. 2017. Genetically informed ecological niche models improve climate change predictions. Global Change Biology 23:164–176.

Jones, J., and D. Markstrom 1973. Aspen: an American wood. United States Department of Agriculture, Forest Service, Washington, D.C., USA.

Karger, D. N., O. Conrad, J. Böhner, T. Kawohl, H. Kreft, R. W. Soria-Auza, N. E. Zimmermann, H. P. Linder, and M. Kessler. 2017. Climatologies at high resolution for the earth's land surface areas. Scientific data 4. 170122.

Keeler, K. H. 1998. Population biology of intraspecific polyploidy in grasses. Pages 183–206 *in* G. P. Cheplick, editor. Population biology of grasses. Cambridge University Press, Cambridge, UK.

Kemperman, J. A., and B. V. Barnes. 1976. Clone size in American aspens. Canadian Journal of Botany 54:2603–2607.

Landhausser, S. M., B. D. Pinno, and K. E. Mock. 2019. Tamm Review: Seedling-based ecology, management, and restoration in aspen (*Populus tremuloides*). Forest Ecology and Management 432:231–245.

Laport, R. G., R. L. Minckley, and J. Ramsey. 2012. Phylogeny and cytogeography of the North American creosote bush (*Larrea tridentata*, Zygophyllaceae). Systematic Botany 37:153–164.

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

Little, D. L. 1971. Atlas of United States trees. Miscellaneous publication 1146. United States Department of Agriculture, Forest Service, Washington, D.C., USA.

Lowe, W. H., R. P. Kovach, and F. W. Allendorf. 2017. Population genetics and demography unite ecology and evolution. Trends in Ecology & Evolution 32:141–152.

Madritch, M. D., C. C. Kingdon, A. Singh, K. E. Mock, R. L. Lindroth, and P. A. Townsend. 2014. Imaging spectroscopy links aspen genotype with below-ground processes at landscape scales. Philosophical Transactions of the Royal Society B 369:20130194.

Marchetti, S. B., J. J. Worrall, and T. Eager. 2011. Secondary insects and diseases contribute to sudden aspen decline in southwestern Colorado, USA. Canadian Journal of Forest Research 41:2315–2325.

McMillan, C., and J. Weiler. 1959. Cytogeography of *Pancium virgatum* in central North America. American Journal of Botany 46:590–593.

Melnikova, N. V., E. V. Borkhert, A. V. Snezhkina, A. V. Kudryavtseva, and A. A. Dmitriev. 2017. Sex-Specific Response to Stress in Populus. Frontiers in Plant Science 8:1827.

Metcalf, C. J. E., and S. Pavard. 2007. Why evolutionary biologists should be demographers. Trends in Ecology & Evolution 22:205–212.

Mitton, J. B., and M. C. Grant. 1996. Genetic variation and the natural history of quaking aspen. BioScience 46:25–31.

Mock, K. E., et al. 2012. Widespread triploidy in western North American aspen (*Populus tremuloides*). PLoS ONE 7: e48406.

Mock, K. E., C. Rowe, M. B. Hooten, J. Dewoody, and V. Hipkins. 2008. Clonal dynamics in western North American aspen (*Populus tremuloides*). Molecular Ecology 17:4827–4844.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Nielsen, E. L. 1944. Analysis of variation in *Panicum virgatum*. Journal of Agricultural Research 69:327–353.

Osier, T. L., and R. L. Lindroth. 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. Oecologia 148:293–303.

Pakull, B., K. Groppe, M. Meyer, T. Markussen, and M. Fladung. 2009. Genetic linkage mapping in aspen (*Populus tremula* L. and *Populus tremuloides* Michx.). Tree Genetics & Genomes 5:505–515.

Pauls, S. U., C. Nowak, M. Bálint, and M. Pfenninger. 2013. The impact of global climate change on genetic diversity within populations and species. Molecular Ecology 22:925–946.

Peacock, J., T. R. Baker, S. L. Lewis, G. Lopez-Gonzalez, and O. L. Phillips. 2007. The RAINFOR database: monitoring forest biomass and dynamics. Journal of Vegetation Science 18:535–542.

Pellicer, J., S. Garcia, M. Canela, T. Garnatje, A. A. Korobkov, J. Twibell, and J. Vallès. 2010. Genome size dynamics in *Artemisia* L. (Asteraceae): following the track of polyploidy. Plant Biology 12:820–830.

Prunier, J., S. Gerardi, J. Laroche, J. Beaulieu, and J. Bousquet. 2012. Parallel and lineage-specific molecular adaptation to climate in boreal black spruce. Molecular Ecology 21:4270–4286.

Rehfeldt, G. E., D. E. Ferguson, and N. L. Crookston. 2009. Aspen, climate, and sudden decline in western USA. Forest Ecology and Management 258:2353–2364.

Rehfeldt, G. E., J. J. Worrall, S. B. Marchetti, and N. L. Crookston. 2015. Adapting forest management to climate change using bioclimate models with topographic drivers. Forestry: An International Journal of Forest Research 88:528–539.

Reich, P. B. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. Journal of Ecology 102:275–301.

Richardson, B. A., and L. Chaney. 2018. Climate-based seed transfer of a widespread shrub: population shifts, restoration strategies, and the trailing edge. Ecological Applications 28:2165–2174.

Richardson, B. A., J. T. Page, P. Bajgain, S. C. Sanderson, and J. A. Udall. 2012*a*. Deep sequencing of amplicons reveals widespread intraspecific hybridization and multiple origins of polyploidy in big sagebrush (*Artemisia tridentata*; Asteraceae). American Journal of Botany 99:1962–1975.

Richardson, B. A., J. T. Page, P. Bajgain, S. C. Sanderson, and J. A. Udall. 2012*b*. Deep sequencing of amplicons reveals widespread intraspecific hybridization and multiple origins of polyploidy in big sagebrush (*Artemisia tridentata*; Asteraceae). American Journal of Botany 99:1962–1975.

Roddy, A. B., et al. 2020. The scaling of genome size and cell size limits maximum rates of photosynthesis with implications for ecological strategies. International Journal of Plant Sciences 181:75–87.

Rogers, P. C., A. J. Leffler, and R. J. Ryel. 2010. Landscape assessment of a stable aspen community in southern Utah, USA. Forest Ecology and Management 259:487–495.

Rothera, S. L., and A. J. Davy. 1986. Polyploidy and habitat differentiation in Deschampsia cespitosa. New Phytologist 102:449–467.

Salguero-Gómez, R., C. Violle, O. Gimenez, and D. Childs. 2018. Delivering the promises of trait-based approaches to the needs of demographic approaches, and vice versa. Functional Ecology 32:1424–1435.

Schmidt-Lebuhn, A. N., J. Fuchs, D. Hertel, H. Hirsch, J. Toivonen, and M. Kessler. 2010. An Andean radiation: polyploidy in the tree genus *Polylepis* (Rosaceae, Sanguisorbeae). Plant Biology 12:917–926.

Scoble, J., and A. J. Lowe. 2010. A case for incorporating phylogeography and landscape genetics into species distribution modelling approaches to improve climate adaptation and conservation planning. Diversity and Distributions 16:343–353.

Seager, S. T., C. Eisenberg, and S. B. S. Clair. 2013. Patterns and consequences of ungulate herbivory on aspen in western North America. Forest Ecology and Management 299:81–90.

Sundell, D., C. Mannapperuma, S. Netotea, N. Delhomme, Y. Lin, A. Sjödin, Y. Van de Peer, S. Jansson, T. R. Hvidsten, and N. R. Street. 2015. The plant genome integrative explorer resource: PlantGen IE. org. New Phytologist 208:1149–1156.

Tai, X., D. S. Mackay, W. R. L. Anderegg, J. S. Sperry, and P. D. Brooks. 2017. Plant hydraulics improves and topography mediates prediction of aspen mortality in southwestern USA. New Phytologist 213:113–127.

Trugman, A. T., M. Detto, M. K. Bartlett, D. Medvigy, W. R. L. Anderegg, C. Schwalm, B. Schaffer, and S. W. Pacala. 2018. Tree carbon allocation explains forest drought-kill and recovery patterns. Ecology Letters 21:1552–1560.

Van Buijtenen, J., P. N. Joranson, and D. W. Einspahr 1957. Naturally occurring triploid quaking aspen in the United States. Proceedings of the Society of American Foresters Annual Meeting, Syracuse, New York, USA.

Whitham, T. G., et al. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nature Reviews Genetics 7:510.

Whittemore, A. T., and R. T. Olsen. 2011. *Ulmus americana* (Ulmaceae) is a polyploid complex. American Journal of Botany 98:754–760.

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.

Worrall, J. J., L. Egeland, T. Eager, R. A. Mask, E. W. Johnson, P. A. Kemp, and W. D. Shepperd. 2008. Rapid mortality of Populus tremuloides in southwestern Colorado, USA. Forest Ecology and Management 255:686–696.

Worrall, J. J., S. B. Marchetti, L. Egeland, R. A. Mask, T. Eager, and B. Howell. 2010. Effects and etiology of sudden aspen decline in southwestern Colorado, USA. Forest Ecology and Management 260:638–648.

Worrall, J. J., G. E. Rehfeldt, A. Hamann, E. H. Hogg, S. B. Marchetti, M. Michaelian, and L. K. Gray. 2013. Recent declines of Populus tremuloides in North America linked to climate. Forest Ecology and Management 299:35–51.

Youngentob, K. N., C. Zdenek, and E. van Gorsel. 2016. A simple and effective method to collect leaves and seeds from tall trees. Methods in Ecology and Evolution 7:1119–1123.

Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2438/full

Open Research

All data and code (Blonder et al. 2021) necessary to replicate the study are available on Dryad at https://doi.org/10.6078/D1898W. All genetic data (Blonder et al. 2019) are archived at the Utah State University Digital Commons: https://digitalcommons.usu.edu/all_datasets/93/