Received: 6 September 2023 | Revised: 3 March 2024 | Accepted: 15 March 2024

DOI: 10.1002/ecs2.70010

ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

ARTICLE
Climate Ecology

Testing source elevation versus genotype as predictors of sugar pine performance in a post-fire restoration planting

Emily V. Moran[1] | **Rainbow DeSilva**[1,2] | **Courtney Canning**[3] | **Jessica W. Wright**[3]

[1]Department of Life and Environmental Sciences, University of California, Merced, Merced, California, USA

[2]Department of Environmental Studies, San Jose State University, San Jose, California, USA

[3]Pacific Southwest Research Station, USDA Forest Service, Placerville, California, USA

Correspondence
Emily V. Moran
Email: emoran5@ucmerced.edu

Funding information
National Science Foundation, Grant/Award Number: ACI-1548562; USDA Forest Service, Grant/Award Number: 20-JV-11272138-050; NIH, Grant/Award Number: 1S10OD010786-01

Handling Editor: Sunshine A. Van Bael

Abstract

Climate change is motivating a reassessment of how seeds are selected for reforestation, as rapid environmental change can lead to local maladaptation in trees. Genetic association studies and past seed source climate both have the potential to help identify appropriate planting stock, but these techniques have not been compared and tested as part of an operational planting program. In this study, we combined an analysis of single nucleotide polymorphisms (SNPs) associated with environmental gradients in sugar pine (*Pinus lambertiana*) with an analysis of post-fire seedling survival and growth in a restoration experiment. Our genotype–environment association (GEA) tests of 92 individuals from varying climates within CA revealed 829 SNPs (out of 300,604) with significant association with climate gradients, especially April snowpack. Of these, 323 either had annotations that suggested potential functional importance or were identified by two different methods. We then built Bayesian models of survival and growth for all seedlings in a separate post-fire planting experiment, to test the relative predictive ability of source elevation (a common proxy for source climate) versus the proportion of seedling alleles expected to be locally advantageous based on GEA. Across three sites within the King Fire scar in Eldorado National Forest in 2017, 2018, and 2019, 1774 seedlings were planted. Of these, 206 had enough green needles in 2020 to allow sample collection, and 161 were successfully genotyped. We found that source elevation was generally better at predicting seedling performance than genotype indices, perhaps because of the limited scope of the association analysis. Seed sources from 500 to 1800 ft (152.4–548.6 m) lower in elevation, and one seed zone further south generally performed as well or better than local seed sources. This result, and those of similar previous studies, suggest that "climate matching" using past climate information for existing seed sourcing units is a reasonable starting point for finding seedlings suited to already-altered planting site climate conditions. However, further tests with more extensive genomic and

performance data may improve the utility of genotype information for seed selection.

KEYWORDS
assisted migration, Bayesian model, climate matching, forest restoration, genetic association analysis, genomic selection, *Pinus lambertiana*, seed source

INTRODUCTION

The stability and productivity of forest ecosystems across the globe are threatened by ongoing climate change (Alberto et al., 2013; IPCC, 2013). Both elevated "background" mortality rates (van Mantgem et al., 2009) and abrupt large-scale tree mortality events due to increasing drought and other stressors related to climate change have already been observed (Clifford et al., 2013; Fettig et al., 2019; O'Brien et al., 2017). In the previous decade, worldwide investment in ecological restoration topped US $2 trillion/year; In order to realize the full value of those efforts, selecting seed sources most likely to survive and thrive is crucial (Prober et al., 2015). However, local tree populations are likely to become increasingly maladapted as evolutionary adaptation and dispersal lag behind rapid global change (Aitken et al., 2008; Kuparinen et al., 2010; Wilczek et al., 2014). Some countries and regions are already officially recommending non-local seed sourcing for reforestation (O'Neill et al., 2008), within specified limits.

However, large climate transfer distances (the climate difference between source and planting sites) could potentially reduce seedling performance either directly (through frost damage when moving seedlings to higher latitudes or elevations, for example) or through altered biotic interactions in the new environment (Grady et al., 2015; Kranabetter et al., 2015). However, what constitutes too large a climate transfer is not known for most species, and projections of where suitable climates for a species will be in 50 or 100 years may identify areas that are currently still unsuitable. Thus, testing of seed sourcing techniques is needed to further refine seed transfer guidelines.

In the mid-20th century, seed zones for forestry were developed in recognition of local adaptation in trees, to identify areas where seeds could be transferred with minimal risk of productivity loss (Buck et al., 1970; Johnson et al., 2004). In California, seeds for reforestation are traditionally sourced from within the same seed zone and the same 500 ft (150 m) elevational band as the planting site (Buck et al., 1970; Johnson et al., 2004). However, substantial changes in temperature and precipitation patterns have already been observed in California (Rapacciuolo et al., 2014; Reidmiller et al., 2018). Even as drought-induced tree mortality events (Fettig et al., 2019) and increasingly large and severe wildfires (Miller et al., 2009) increase the need for restoration plantings, seed zones identified in the 20th century may no longer serve to identify the best-performing seed sources, requiring new methods to select appropriate germplasm (Williams & Dumroese, 2013).

One option for climate-adapted forest restoration is to use "climate matching": choosing seed populations adapted to climatic conditions that are similar to current or future conditions at the planting site (McKenney et al., 1999; O'Neill et al., 2017; Prober et al., 2015; Ruiz-Talonia et al., 2014). However, in some cases different populations might be predicted to perform better under current versus near-future conditions (Grady et al., 2015; Gross et al., 2017), or there is no close analog at any site in the past for planting site conditions (Boiffin et al., 2017; Williams & Jackson, 2007). In addition, populations suited to the planting site climate might not be adapted to the soil, photoperiod (Way & Montgomery, 2015), or species interactions (Bucharova, 2017; Grady et al., 2015; Kranabetter et al., 2015) in the new location. It has been suggested that planting a mix of local and non-local seed sources might help buffer against this possibility (Prober et al., 2015). Using genetic markers to select local source trees whose offspring are likely to perform better under predicted future climate conditions, but which are likely adapted to other local environmental conditions, is another possible approach.

Genotype–environment association (GEA) methods have emerged as powerful tools to identify the climatic drivers of local adaptation and provide insights into the genomic basis of local adaptation (de Villemereuil et al., 2014; Forester et al., 2018; Hoban et al., 2016; Sork, 2018). Genomic selection, already being applied in a variety of agricultural systems (Poland et al., 2012; Xavier et al., 2016), could accelerate tree-breeding programs by helping to identify parent trees with desirable characteristics (Beaulieu et al., 2014; Grattapaglia & Resende, 2011; Resende et al., 2012). Genetic association studies seek to identify genetic variants that are statistically associated with environmental gradients (GEA) (Eckert et al., 2010; Frichot et al., 2013; Prunier et al., 2011) or

desirable phenotypic traits (genotype–phenotype association; GPA) (Holliday et al., 2010; Neale & Savolainen, 2004). Such markers can then be used predictively to guide genomic selection.

Genetic selection approaches have been used in many forest tree species, but until recently have been based on a limited number of loci (Beaulieu et al., 2014; Brawner et al., 2012; Isik et al., 2016), and thus could not truly be called "genomic selection." A full genome sequence can aid in discovering more potentially important genetic variation (Rigault et al., 2011; Zimin et al., 2017), but creating an annotated genome sequence can be a slow process for tree species like conifers that have extremely large genomes (De La Torre et al., 2014). However, even without a genome-wide set of markers, it can be possible to make useful predictions about performance. Jaramillo-Correa et al. (2015) found that in *Pinus pinaster* an increase in the average population frequency of just 18 single nucleotide polymorphisms (SNPs) associated with hot/dry conditions from 0.25 to 0.6 increased predicted survival in a garden at the hot/dry end of this species' range by about 10%. This suggests that using a higher number of environmentally associated loci might further improve predictions of which individuals or populations will thrive under particular environmental conditions. For example, a prior *Arabidopsis* study (Hancock et al., 2011) found that individuals with more "locally advantageous" SNPs in the upper 1% of those associated with climate variation (out of ~215,000 SNPS tested) had higher reproductive success. Genomic selection studies using open-pollinated conifer families and <7000 intragenic SNPs have also yielded promising results. For example, one study in *Picea glauca* found that SNPs linked to wood and growth traits through GPA could predict these traits in common gardens outside the training set with 90% the accuracy of full pedigree information (which would not be obtainable for wild-collected seed) if training and validation sets shared half-sibs, and about half the accuracy if they did not (Beaulieu et al., 2014).

Sugar pine, *Pinus lambertiana* Douglas, is an ecologically important tree species in California with high carbon storage and sequestration potential (Burns & Honkala, 1990; Tomback & Achuff, 2010), and it is also one of the few conifers with a fully sequenced genome (Stevens et al., 2016). Conservation concerns around sugar pine are growing due to the combined impacts of white pine blister rust (WPBR) (Tomback & Achuff, 2010) and declines related to changing fire regimes and climate (Maloney et al., 2011). In addition, sugar pine suffered extensive mortality due to the severe drought period between 2012 and 2016 (Fettig et al., 2019; Griffin & Anchukaitis, 2014). These factors combined with the continued proliferation of high-intensity wildfires in California are increasing the need for restoration of sugar pine (Stewart et al., 2021).

Numerous genetic studies in this species center around understanding genetic resistance to WPBR (Sniezko et al., 2020; Vázquez-Lobo et al., 2017), with comparably few investigating the extent of climate-associated genomic variation across the range of this species (Eckert et al., 2015; Vangestel et al., 2016). Neutral genetic structure across the range of sugar pine is weak and populations generally exhibit patterns of isolation by distance, with evidence of a genetic subdivision between northern and southern populations (Liston et al., 2007; Vangestel et al., 2016). Yet, in laboratory experiments, seedlings showed high variation in growth rates based on location of seed origin (Harry et al., 1983), suggesting local adaptation of trees to the growing conditions of their home environment. In an association study using candidate genes, Vangestel et al. (2016) found nine loci with potential links to local adaptation to drought. Moreover, some sugar pine populations within the Tahoe Basin show signatures of local adaptation to gradients of water availability at fine spatial scales (Eckert et al., 2015). Selection operating in the decades between seed and pollen dispersal and maturity may therefore create differences in ecologically relevant genetic variation between sites even when differentiation at neutral loci remains low due to high initial gene flow.

This study aimed to evaluate the ability of climate-associated genetic markers versus seed source elevation to explain variation in sugar pine seedling performance at three post-wildfire restoration planting sites. This requires first a GEA analysis using tree genotypes with known locations/source climates, and then a model-based comparison of the predictive ability of seedling genotype versus source elevation. Because the genotypes used for the association analysis had already been grafted into a Forest Service orchard, the information gained from this study has the potential to be put to use rapidly in designing seed lots for restoration plantings.

MATERIALS AND METHODS

The King Fire restoration planting area

U.S. Forest Service seed sources are categorized and stored by 500-ft (152.4-m) elevation bands within a seed zone (SZ), where the elevation label indicates the upper edge. We will therefore use these bands as elevation categories throughout this paper. The planting experiment was established within the 28,710-ha burn scar of the King Fire, which occurred in 2014 (Figure 1). Three 4.9–5.3 ha sites within the Georgetown Ranger District of the Eldorado National Forest were planted in 2017, 2018, and 2019 (Table 1). The sites are located at elevations of 4400 to 4800 ft (1341.1–1463 m) within SZ 526. Thus, the

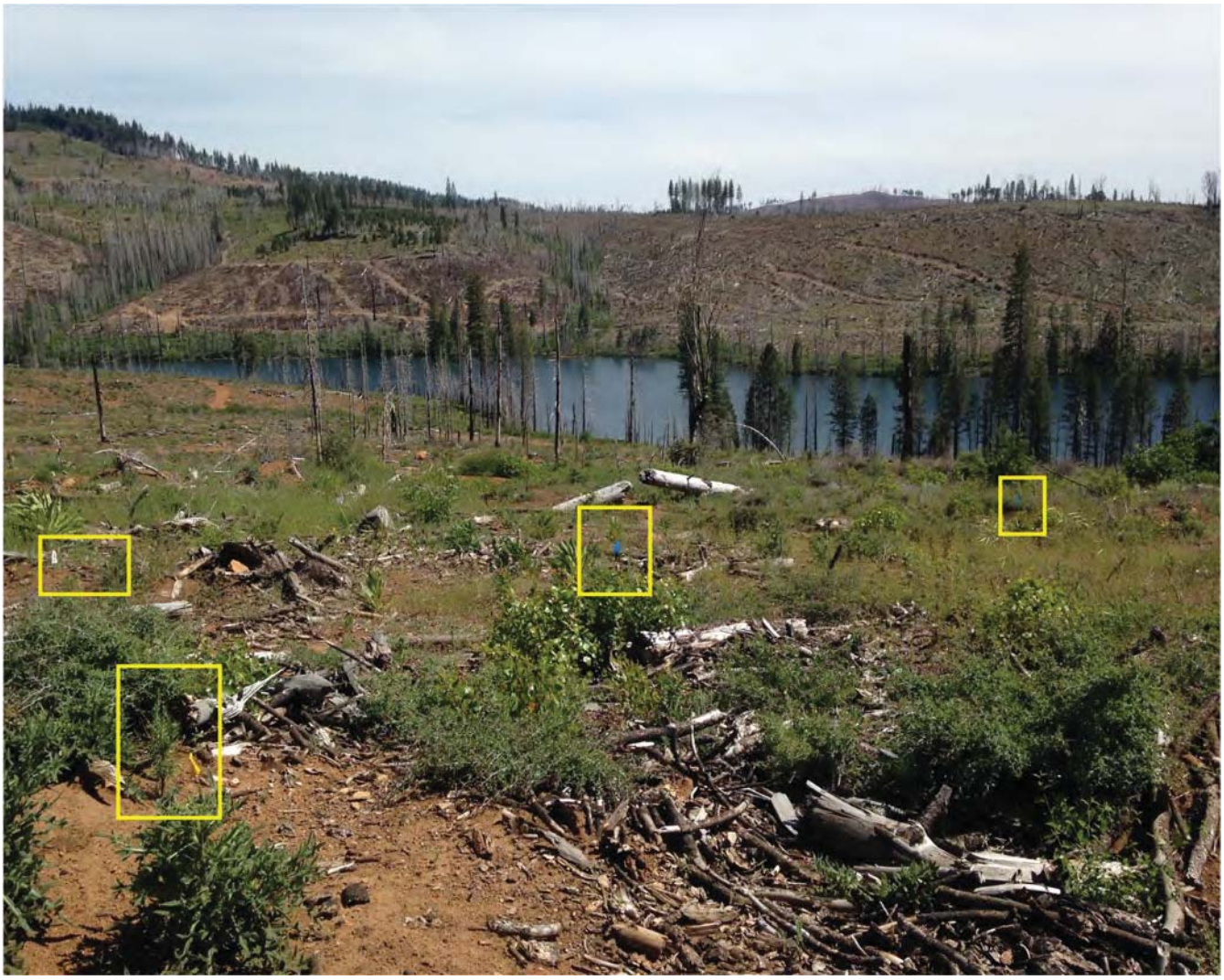

FIGURE 1 King Fire planting area, 2019. Planted seedlings in foreground are indicated by yellow boxes. Photo credit: Emily Moran.

TABLE 1 Seedling sample sizes: 1774 seedlings were initially planted.

Year in which trees were alive	2017 site	2018 site	2019 site	All sites
2017	662 (initial planting)	NA	NA	662
2018	397	668 (initial planting)	NA	1065
2019	342	437	444 (initial planting)	1223
2020	287	357	293	937
2021	183	330	289	802
2022	166	216	247	629
No. trees successfully genotyped in 2020	41	55	65	161

Note: The total seedling-years included in all-seedling models = 4689.

planting sites are on the upper end of the 4500-ft band—which will be referred to as "local"—or the lower end of the 5000-ft (1524-m) band.

Source trees were wild individuals identified as major gene-resistant for WPBR (Kinloch, 2003). Planted sources included genotypes from the 4500-, 5000-, 5250-, and 5500-ft (1371.6-, 1524-, 1600.2-, 1676.4-m) bands within the local SZ 526, as well as a range of other SZs further south (Figure 2). The mean annual temperature and precipitation of these sources in 1940–1969 versus the

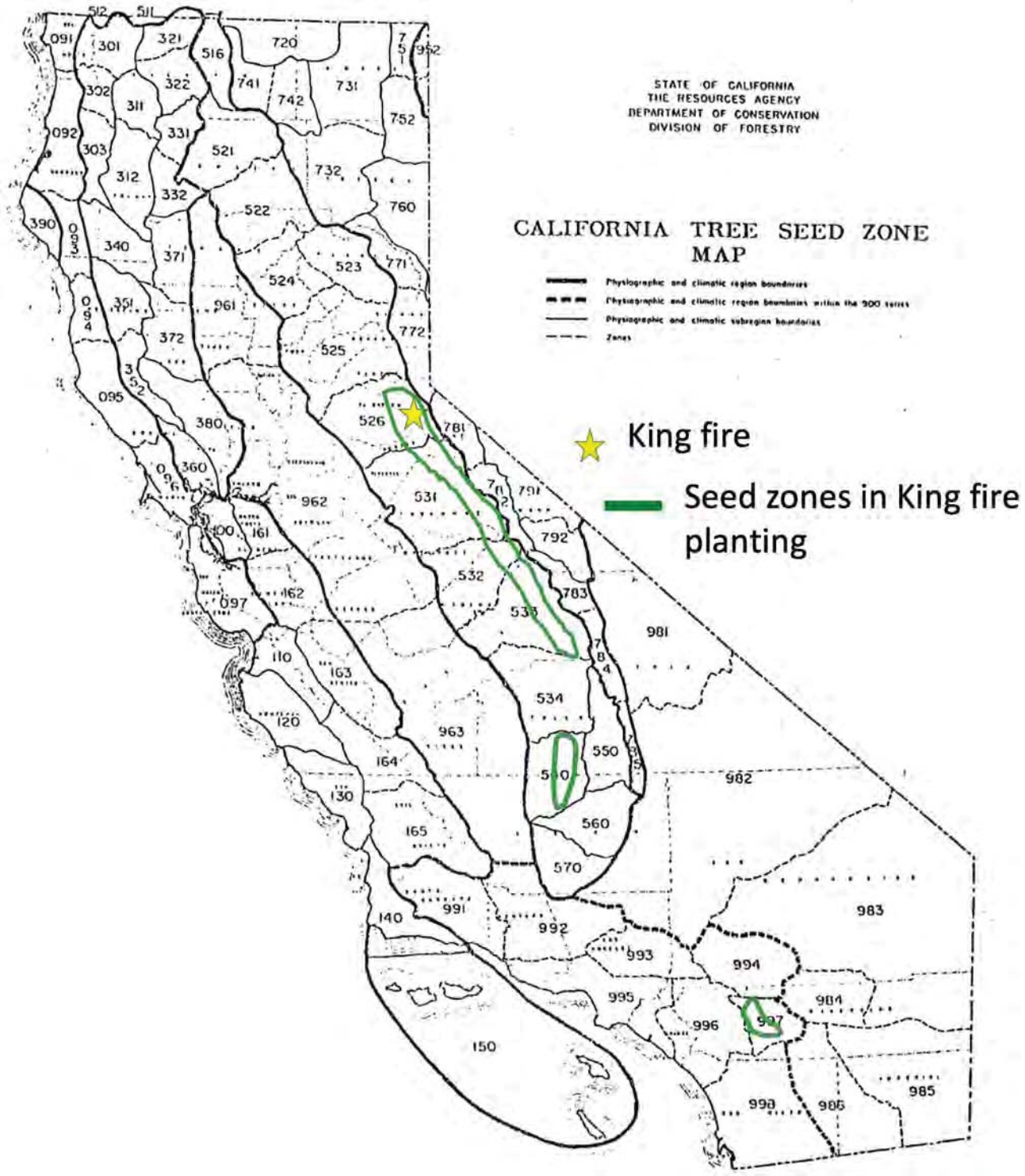

FIGURE 2 Seed zones included in King Fire restoration plantings. Original seed zone map published in Buck et al. (1970); reprinted by permission of CalFire.

current climate of the planting area is shown in Figure 3. The numbers of each seed source planted is shown in Appendix S1: Table S1. A "climate matching" approach would recommend choosing seed from areas where the mid-20th century climate (before anthropogenic climate change rapidly accelerated) best matches current or projected conditions at the planting site, but none of these sources match both temperature and precipitation. The hottest source areas were represented by 3000- and 4000-ft (914.4- and 1219.2-m) bands in SZ 531, one zone

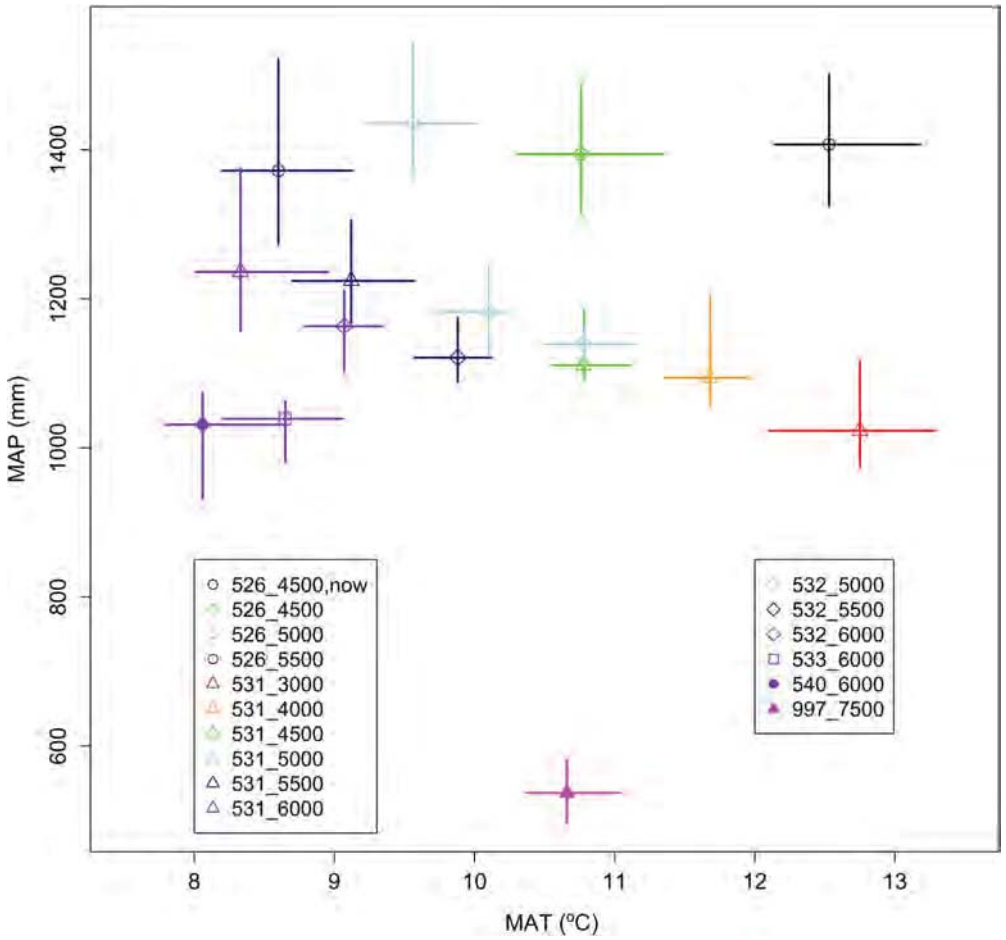

FIGURE 3 Mean annual precipitation (MAP) and mean annual temperature (MAT) 1940–1969 for each seed-zone/elevation source area, relative to current planting area MAP and MAT (black). Dots indicates medians, lines indicate first to third quartile range. Numbers are based on Climate-Adapted Seed Tool|Reforestation Tools (https://reforestationtools.org/climate-adapted-seed-tool/). Elevation bands within seed zones are given in feet, as that is how they are classified by the US Forest Service; conversions to meters are given in throughout the text. Seed zone geographical locations are shown in the map in Figure 1. "526_4500, now" indicates current conditions (2020) for the planting sites, which are within seed zone 526's 4500-ft (1371.6-m) elevation band. All other numbers are for seed source areas, in the mid-20th century (1940–1969). Note that past MAT for lower-elevation seed sources 531_3000 and 531_4000 (914.4 and 1219.2 m) most closely match the current planting site MAT, while past conditions for the local seed source (526_4500; 1371.6 m) match the current MAP but were several degrees cooler.

south of the planting area; high summer heat means these sites would also be effectively more arid than cooler sites with the same precipitation due to evapotranspiration. The higher elevation sources, regardless of SZ, all represented 1.5–4.5°C lower mean annual temperatures than the planting area currently experiences, and those from more southern SZs also had lower precipitation, with the high-elevation source from the far south in the Transverse Range (SZ 997, 7500 ft; 2286 m) having the overall lowest mean annual precipitation. As shown by the bars representing the 5th–9th percentiles in Figure 3, however, average climate conditions within a SZ elevation band can vary by nearly as much as the mid-20th century versus current conditions for a given site, which is why we wished to test whether SNPs associated with climate variation could improve predictions of seedling performance.

Our goal was to evaluate tree performance in an operational setting; Therefore, we used approaches that would be effective in those conditions, and not what would be considered more "traditional" for a research planting (planting in a grid pattern, rectangular "blocks," clearing all competing vegetation, etc.). Traditional research plantings are also typically smaller in scale and not designed to restore a functional forest landscape, as operational plantings are.

Seed-lots were tagged with colored zip-ties so they could be identified after planting, and haphazardly mixed by requiring that each of the 10 seedlings in a planting bag had a unique zip-tie tag. Neither the nursery staff

sorting the seedlings into bags nor the planters were aware of which seed lot each color corresponded to. Seedlings were planted using standard operational planting techniques, with 250 trees/ha spaced approximately 6.1 m apart. A colorful flag was placed next to each seedling and in the fall after planting each seedling was also given a unique numbered metal tag. The height and initial survival were also recorded at this time (hereafter called "year 0.5").

During each subsequent summer after planting until 2021 the height, basal diameter, and proportion of live needles were recorded for each seedling that could be located. Because of shrub and weed growth, seedlings were sometimes missed in the underbrush and then found again a year or two later; these were known to be the same seedlings due to their tag number and size. In summer 2020, needle samples were collected, whenever this could be done without undue injury to the seedling. These samples were labeled and dried over silica gel for later genetic analysis. The August 2021 survey of the 2019 planting site had to be stopped before it was completed due to a major fire in the area that threatened the site. However, the planting area itself did not burn, and another field team returned in November to finish the survey.

Sugar pine genotyping

We originally planned to sequence 611 planted sugar pine seedlings from the restoration planting, 5–7 per mother tree, and use their genotypes to reconstruct the maternal genotypes, which could, in turn, be used for a GEA analysis. However, mortality from planting to the time of needle collection proved to be extremely high for the sugar pines, and such genotype reconstruction was not possible. Suspecting this to be the case, we also collected needles from 92 trees at the USDA Forest Service, Foresthill seed orchard (39°05′06.4″ N, 120°43′51.6″ W). The sugar pine grafted saplings at the seed orchard were too young to contribute seed to the field planting but had precisely known source locations for which climate data could be obtained. The genotypes within the Foresthill orchard were grown from scion collected from across the Sierra Nevada and northern California, grafted to a common rootstock, and pre-screened for WPBR resistance (Kinloch et al., 1996). These 92 samples, all from different locations, were used in GEAs in order to identify loci associated with climate variation, which we will hereafter refer to as "candidate loci." The geographic distribution of these samples is shown in Figure 4. We also collected needles for DNA extraction from 206 planted seedlings from the three restoration planting sites for use in the survival and growth models.

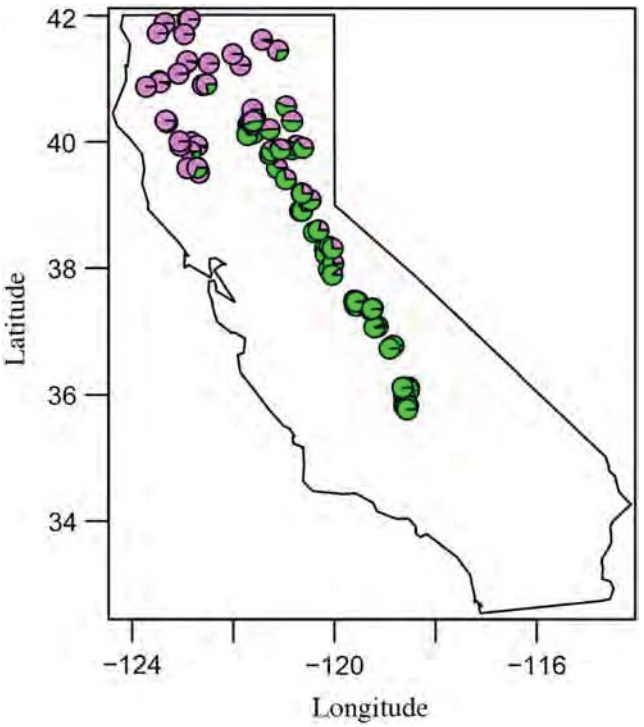

FIGURE 4 The 92 sugar pine genotypes used in the climate association grouped as two populations: One in northern California (purple) and one in the southern Sierra Nevada Mountains (green), with some introgression in the middle. Figure based on admixture analysis with $K = 2$, with circles representing individual trees and their degree of admixture.

DNA sequencing, SNP calling, and filtering

We chose to use a genotyping-by-sequencing (GBS) approach that has previously been shown to yield tens of thousands (Chen et al., 2013; Pan et al., 2015) to millions of SNPs (Shu & Moran, 2023) in conifers, depending on the number of individuals sequenced. It is also cost-effective relative to approaches such as full genome resequencing. Not all of these markers will fall within genes, and many are likely to be neutral, yet some may also represent important regulatory variation not represented in gene-based sequence capture approaches (Elshire et al., 2011). As this is a reduced-representation approach, not all variants in the genome will be identified.

High-purity genomic DNA was extracted from the leaf tissues of all 298 individuals—the 92 Foresthill trees and the 206 restoration-planting seedlings—using Macerney-Nagle DNA isolation kits. DNA was subsequently sent to the UC Davis Genome Center for library preparation and GBS. In brief, DNA was fragmented using the methylation sensitive enzyme ApeKI, the resulting product was ligated to barcoded adapters, cleaned and purified to remove small fragments

(<250 base pairs [bp]), and increase DNA concentration. Using an Illumina HiSeq 4000 platform, 48-plex GBS libraries were then sequenced (100-bp single-end reads).

We used the Stacks v.2.59 pipeline (Rochette & Catchen, 2017) for reference genome alignment and SNP calling. Sequence data were aligned to the sugar pine reference genome (v1.5: https://treegenesdb.org/FTP/Genomes/Pila/v1.5/) to obtain read position. Further details are provided in Appendix S2. Individuals with >80% missing data were removed, and SNPs were filtered using VCFtools (Danecek et al., 2011) to remove loci with more than 50% missing data and minor allele count <5. The resulting dataset consisted of 300,604 bi-allelic SNPs genotyped across 253 individuals (92 from Foresthill + 161 restoration planting seedlings) with an average of ~26% missing data. These genetic analyses were conducted using XSEDE computing resources (Towns et al., 2014).

Climate data

For both the association analysis and the modeling of seedling survival and growth, we used climate data obtained from the California Basin Characterization Model (CA BCM: 270 m resolution) (Flint et al., 2013). This model downscales climate data to a 270-m resolution taking into account topography, soils, and underlying geology. Publicly released data for this model can be found at the California Climate Commons (http://climate.calcommons.org/bcm) or the USGS (https://www.usgs.gov/centers/california-water-science-center/science/basin-characterization-model-bcm#data). As 2021 data had not yet been released at the time of this analysis, we directly requested monthly climate sequences for the locations of the individual trees used in the GEA analysis, and the three planting locations.

For the association analysis, we used 30-year climate averages between 1921 and 1950 for the source location of each of the Foresthill genotypes. We chose this 20th century time period because it better approximates the conditions under which the wild trees that were grafted into the orchard became established and underwent selection as seedlings and saplings. From these data, we extracted 15 climate variables (Appendix S3). After a principal components analysis (PCA; Appendix S3: Figure S1), we chose to retain five climate variables that contribute strongly to PC1 and PC2 but have relatively low correlations with one another (Appendix S3: Table S1): climatic water deficit (CWD, a measure of aridity equal to actual minus potential evapotranspiration); minimum January temperature (tmn); maximum July temperature (tmx); winter precipitation (pptw); and April 1 snowpack (pck4). Temperatures are in degrees Celsius; precipitation, CWD, and snowpack are in millimeters. The strongest negative correlations were between April snowpack and July maximum temperature and CWD ($r = -0.67$ and -0.66, respectively), and the strongest positive correlation between July maximum and January minimum temperatures. These pairs were retained, while more strongly correlated pairs such as July maximum and summer (June, July, August) maximum temperature were not, due to the likelihood of having different biological effects.

Planting site climate

For the seedling growth and survival models, we obtained annual values for CWD, July maximum temperature, January minimum temperature, total annual precipitation, and April snowpack for 2016–2021 from the Basin Characterization Model. Of the years since the first planting, 2018, 2020, and 2021 were particularly low in total precipitation and snowpack, while only 2019 was both wet and cool (Figure 5). However, the CWD ranking is reversed relative to what one might expect based on this, with the 2019 site exhibiting higher aridity; this is likely due to topographic variation (aspect, soil depth, etc.) between the sites.

Genotype–environment association

We used two complementary approaches, latent factor mixed models (LFMMs) and redundancy analysis (RDA), to identify loci associated with climate variation. LFMM is a univariate approach that tests for associations between individual genomic loci and environmental factors after accounting for population structure (Caye et al., 2019; Frichot et al., 2013). Because genes may not act independently, we also used RDA, an extension of multiple regression to multivariate response variables which has shown higher power to detect signals of polygenic adaptation (Forester et al., 2018; Legendre & Legendre, 2012). In simulation tests, both approaches perform well under various sampling designs and demographic scenarios and demonstrate a good balance between high power and low false-positive rate (de Villemereuil et al., 2014; Forester et al., 2018; Frichot et al., 2013).

We used LFMM2 in the R package "lfmm" (Caye et al., 2019). The ideal number of "latent factors" (populations) to represent geographic structure was estimated using PCA and admixture analyses (Appendix S2). Given that LFMM analysis at $K = 2$ and $K = 3$ provided

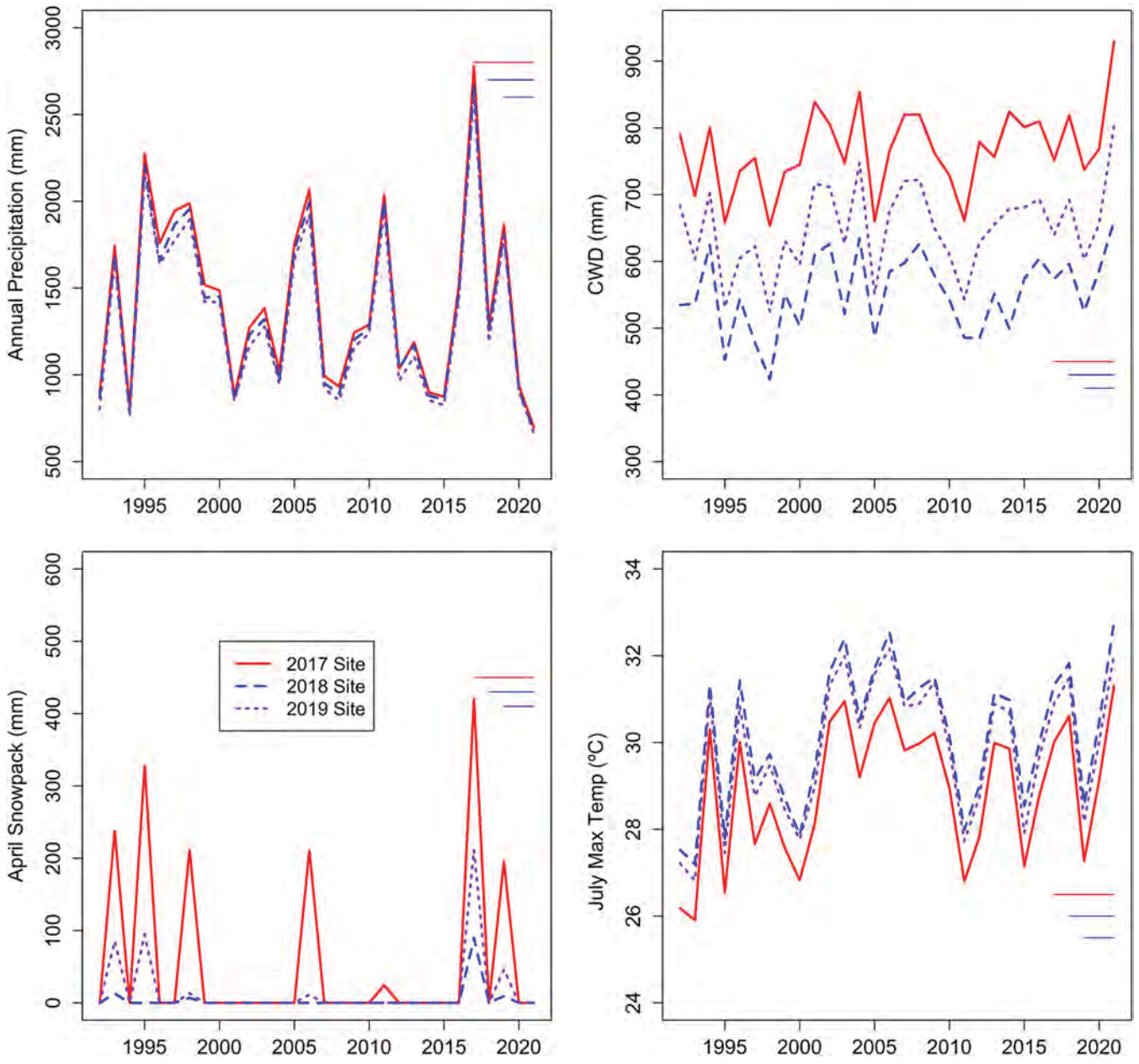

FIGURE 5 Weather conditions at the 2017, 2018, and 2019 planting sites 1992–2021. The experimental periods for each site are indicated by horizontal lines in red (2017 site), blue (2018 site), and purple (2019 site). There has been an increase in July maximum temperature and climatic water deficit (CWD) over this period, but no directional trend in total precipitation or April snowpack.

nearly identical results and two genetic clusters were also found by several prior analyses covering this same region of the species range (Liston et al., 2007; Vangestel et al., 2016), we present the results for $K = 2$ (Figure 4, admixture analysis), in which significant associations were determined based on a false discovery rate of $q = 0.05$ after applying a Benjamini–Hochberg p-value adjustment as outlined in François et al. (2016). An RDA model was constructed using the five selected climate variables and latitude. We chose only latitude to represent geographic space as latitude and longitude were highly correlated in our study area due to the orientation of the mountain range ($|r| < 0.9$; see Figure 4). This model was used to assess the total amount of variation in genomic data explained by latitude and climate variables combined. We then conducted partial RDA where the effects of climate were assessed after removing the effects of geographic space. Using the first constrained axis of the partial RDA, we identified outlier loci, as candidates for selection, by applying a ±3.5 SD (two-tailed p value of 0.0005) cut off on the SNP loadings (Carvalho et al., 2021). As both approaches require complete data, any

missing alleles were imputed using the population cluster assignments and the "impute" function in the LEA R package (see Appendix S2).

Annotation of candidate loci

To gain insights into the potential significance of these loci we obtained flanking sequences of all outlier SNPs from the sugar pine reference genome. We then used the online BLASTn database to obtain functional annotations using an e-value cutoff of 5×10^{-5} (Appendix S4). Before inclusion in statistical models, loci in linkage disequilibrium were thinned using VCFtools (Danecek et al., 2011) to remove SNPs with $R^2 > 0.8$ (Appendix S2). We also removed one locus where allele frequency could not be calculated within conditions similar to the planting site, as described below. Three hundred and five candidate loci were left for further analysis. The numbers of each seed source genotyped at these loci by site are shown in Appendix S1: Table S1.

Identification of "locally advantageous" alleles

Allele frequency at each climate-associated locus was first calculated for the entire Foresthill sample (trees used for GEA; $N = 92$). We then identified tree genotypes that came from climates that, for 1921–1950 were within 0.2 SD of the average planting site climate in 1981–2010 for each of the climate variables. If the frequency for an allele was higher in trees from climates similar to the planting site than it was for the entire GEA set of genotypes from across California, this was considered a candidate for an allele that would potentially boost performance in similar environment, or "locally advantageous allele" for short. The number of these alleles for that locus (0, 1, or 2) was calculated for each individual.

For each set of climate-associated loci found through GEA to be linked to a particular climate variable, we calculated the proportion of locally advantageous alleles (FG; short for "favored genotype") for each individual in two ways:

$$\mathrm{FG1} = \frac{2h_o + h_e}{2\mathrm{T.g}}, \quad (1)$$

$$\mathrm{FG2} = \frac{h_o + h_e}{\mathrm{T.g}}, \quad (2)$$

where T.g is the number of loci genotyped for that individual, h_o is the number of those loci homozygous for the favored allele, and h_e is the number of loci heterozygous for the favored allele. FG1 assumes that allele effects tend to be additive, with the effect of two copies of the favored allele being twice as good as having a single copy. FG2, on the other hand, reflects whether an individual has the locally favored allele or not, rather than how many copies it has.

Survival and growth analyses

Because seedlings were sometimes missed for a year or two and then discovered to be alive in a subsequent survey, we interpolated missing data as explained Appendix S1. To further investigate the effects of source elevation or genotype in the context of other factors influencing seedling survival and growth, we used a similar Bayesian generalized linear model framework in Moran et al. (2019) and Moran, Reid, et al. (2017). This model structure (Appendix S5) allows us to "borrow strength." For example, the posterior of the intercept parameter is informed by all seedlings, while that of the age effects parameter is informed by all seedlings that reach that age. The survival models use a logit link to the linear equation, with the resulting zero-to-one parameter θ being the probability of survival in a given year. If a seedling lives, $Y = 1$, if it dies $Y = 0$.

$$\begin{aligned} Y &\sim \mathrm{Bernoulli}(\theta) \\ \mathrm{logit}(\theta) &= \frac{\log(\theta)}{1 - \log(\theta)} = \mathrm{Int} + X\beta + \varepsilon \\ \varepsilon &\sim \mathrm{Normal}\left(0, \sigma^2\right). \end{aligned}$$

We used a linear mixed model for annual height growth increment, using only data from living individuals.

$$\begin{aligned} Y &\sim \mathrm{Int} + X\beta + \varepsilon \\ \varepsilon &\sim \mathrm{Normal}\left(0, \sigma^2\right). \end{aligned}$$

The linear equation for both processes includes an error term ε representing additional variation in individual survival/growth beyond that captured by the predictors. "Int" indicates the intercept. This annual-increment modeling approach that has been used to model survival and growth has been used in a number of prior studies including Moran et al. (2019) and Lee and Ibáñez (2021), and lends itself well to parameterizing simulation models with annual time increments (Moran et al., 2021).

All predictor variables considered in the model versions we tested are listed in Table 2. Discrete variables were coded as indicator values in the X matrix: 1 if an individual seedling belongs to that group and 0 if it does not. Other variables such as last year's height, local or

TABLE 2 Predictor variables considered in survival and growth models. Abbreviations shown in parentheses.

Variable class	Members of class	Units	Discrete or continuous?
Intercept (Int)	NA	NA	NA
Site/planting year (Site)	Sites planted in 2017, 2018, and 2019 (Site17, Site18, Site19)	NA	Discrete
Year since seedling planting (Age)	First through fifth year seedling (Yr0.5, Yr1, Yr2, Yr3, Yr4)	NA	Discrete
Previous-year height (Ht)	NA	cm	Continuous
Source elevation band (Elev)	3000 ft, 4000 ft, etc.[a] (30, 40, 45, 50, 52, 55, 60, 75)	NA	Discrete
Source seed zone (SZ)	(526, 531, 532, 533, 540, 997)	NA	Discrete
Source elevation within seed zone (SZ_Elev)	(526_45, 526_50, 526_52, 526_55, 531_30, 531_40, 531_45, 531_50, 531_55, 531_60, 532_50, 532_55, 532_60, 533_60, 540_60, 997_75)	NA	Discrete
Planting site and source elevation interaction (SZ × Elev)	(17_30, 17_40, 17_45, 17_50, 17_55, 17_60, 18_45, 18_50, 18_52, 18_55, 18_60, 19_45, 19_50, 19_55, 19_60, 19_75)	NA	Discrete
Annual site climate (Clim)	Min January temp (Tmn); Max July temp (Tmx); Total precipitation (Ppt); April 1st snowpack (Pck4); or climatic water deficit (CWD)	°C for temp; mm for others	Continuous
Proportion "locally favored" alleles (Geno)	Two different calculations: FG1 (Tmn.FG1, Tmx.FG1, Ppt.FG1, Pck4.FG1, and CWD. FG1) or FG2 (Tmn.FG2, Tmx.FG2, Ppt.FG2, Pck4.FG2, and CWD.FG2)	NA	Continuous

[a]Band measurements were conducted in feet; 1 ft = 0.3048 m.

source climate, and FG were treated as continuous. Because sites were planted in separate years, we were not able to separate site from planting year effects; these are simply referred to as "Site" effects. Both site and seedling years-since-planting (Age) were included in all models, as differences in site-level survival are common, and much of the mortality in seedlings occurs in the first year of establishment (Beckage et al., 2005; Moran et al., 2019). Height in previous year (Ht) and local site climate variables in current year (Clim) were included in some model versions, because larger seedlings have different growth and survival rates than small ones, and current local conditions can strongly influence performance (Moran et al., 2019). Climate variables tested included maximum July temperature (JMax) and CWD in the same summer as the seedling observation in question, minimum January temperature in the preceding winter (JMin), snowpack in the preceding April (Pck4), and total precipitation from September of the preceding year through October of the current year (Ppt) for the all-seedling survival and growth. We also tested different seed source indicators, including SZ, source elevation band (Elev), and source elevation band within SZ (SV_Elev). Interactions between source elevation and planting site were also tested, because if populations are adapted to different climates, then different sources could have different responses to the planting sites.

Growth and survival models without genotype as a predictor were fit to all seedling data across all years. To more fairly compare models with and without genotype given that genotypes were not available for all seedlings, we fit a set of growth models to just the genotyped seedlings across all years, and a set of survival models to the most recent year of survival data for seedlings that were genotyped in 2020. It should be noted that, as a result, the amount of data used to fit the "all seedling" growth and survival models is greater than for the models of growth of genotyped seedlings or of one year of survival for genotyped seedlings. This is particularly visible in the range of planting site annual climate experienced by these subsets of seedlings that is used to fit the model. April 1 snowpack ranged from 0 to 420.32 mm, but for genotyped seedlings from 0 to 195.34, and in the most recent year all genotyped seedlings experienced 0 mm of snowpack (Table 3). This variable was therefore omitted from genotyped seedling survival models. Ranges for other climate variables are shown in Table 3.

Most prior distributions were centered on zero, as we had little information from previous studies indicating expected directionality of effects. The exceptions were the intercepts (which were given prior means that corresponded to 92% survival or 5 cm of annual height growth), age effects on survival (which were given more negative

TABLE 3 Ranges of site annual climate variables over all seedling-years (used in all-seedling models), all years for genotyped seedlings (used in genotyped seedling growth models), and for genotyped seedlings 2020–2021 (used in genotyped seedling survival models).

Climate variable	All seedling-years	Genotyped seedlings, all years	Genotyped seedlings, 2020–2021
April 1 snowpack (Pck4) (mm)	0 to 420.32	0 to 195.34	0
Total precipitation (Ppt) (mm)	664.33 to 2778.71	664.33 to 1866.11	664.33 to 696.03
Climatic water deficit (CWD) (mm)	525.19 to 929.36	525.19 to 929.36	660.4 to 929.36
July max temperature (Tmx) (°C)	27.27 to 32.80	27.27 to 32.80	31.31 to 32.80
January min temperature (Tmn) (°C)	−1.8 to 3.5	0.29 to 2.59	0.29 to 1.27

values for years 1 and 2 than for older seedlings), current height effects on growth and survival (which were given a small positive value) and "locally favored allele" effects (which were given a very small positive value). Regardless, all priors were broad and overlapped zero. Full prior distribution information is provided in Appendix S5. We also tested different prior configurations, such as centering all on zero; this information is also in the Appendix.

As in Moran et al. (2019), we used predictive loss (Gelfand & Ghosh, 1998) as a metric for model selection. Predictive loss (here called Dm) can be easily calculated from the output of a Gibbs sampler and, unlike AIC or BIC, does not require one to specify model dimension (Clark, 2005). Predictive Loss is the residual sum of squares (G) plus the predictive variance (P) as a penalty term. As with AIC or BIC, lower values of Dm indicate better model fit, but the absolute values will tend to be higher with larger datasets and with continuous rather than 0/1 response variables, as seen in the *Results*. Therefore, one should not directly compare Dm for models using different datasets or response variables.

RESULTS

GEA analyses

Using LFMM and RDA, we identified 829 outlier loci as potential candidates of importance for local adaptation to climate. Our LFMM results indicated 474 loci with significant associations to one of the five environmental variables. The vast majority of the candidate loci (458) showed significant associations with April 1 snowpack (Pck4). Four of these loci were also associated with July maximum temperature (Tmx). Apart from this, there was no overlap in which loci were associated with different climate variables. A total of 16 loci were associated with CWD (13), January minimum temperature (2; Tmn), or annual precipitation (1; Ppt). The partial RDA model (pRDA) identified 383 loci with significant association to climatic variation. These outlier loci were most strongly correlated with Tmx (224), CWD (69), Pck4 (44), Tmn (40), and Pptw (6). Permutation tests found that the pRDA model and its first axis were significant (Model: $\Pr(>F) = 0.047$; Axis 1: $\Pr(>F) = 0.021$). A full RDA model which included five climate variables and latitude explained 8% of the genomic variation and was highly significant ($\Pr(>F) = 0.001$). This level of prediction by environmental variables is fairly typical for this type of analysis (Capblancq & Forester, 2021), and substantially higher than the 1.5% observed in a range-wide GEA of sugar pine that was conducted simultaneously to our study (Weiss et al., 2022).

Using BLASTn, we obtained functional annotation for 323 of the outlier loci. Although the insights on functional significance were limited for many loci, as 130 were identified only as mRNA, meaning the SNP was in a gene that was transcribed, but the function of that gene was not known. However, the loci with more detailed annotations included functions related to metabolism, abiotic and biotic stress response, nutrient transport, as well as other diverse functions (Appendix S4). Three hundred and forty loci were either identified by both association methods ($N = 28$) or had annotations indicating that they were in a transcribed gene. These were thinned to remove strongly linked loci (LD > 0.8), leaving 305 loci for inclusion in models.

Survival and growth observations

In all three planting years, the highest observed mortality occurred between the spring planting and the summer/fall census when metal tags were installed. Mortality trajectories were similar for the 2017 and 2018 plantings, but a somewhat higher proportion of seedlings survived in the less arid 2019 planting site (Figures 5 and 6). Variation was observed in survival between seedlings from different source elevations. At the 2017 site, seedlings from 500 to 1800 ft (152.4–548.6 m) lower in elevation survived as much or more than seedlings from the "local" 4000–4500-ft

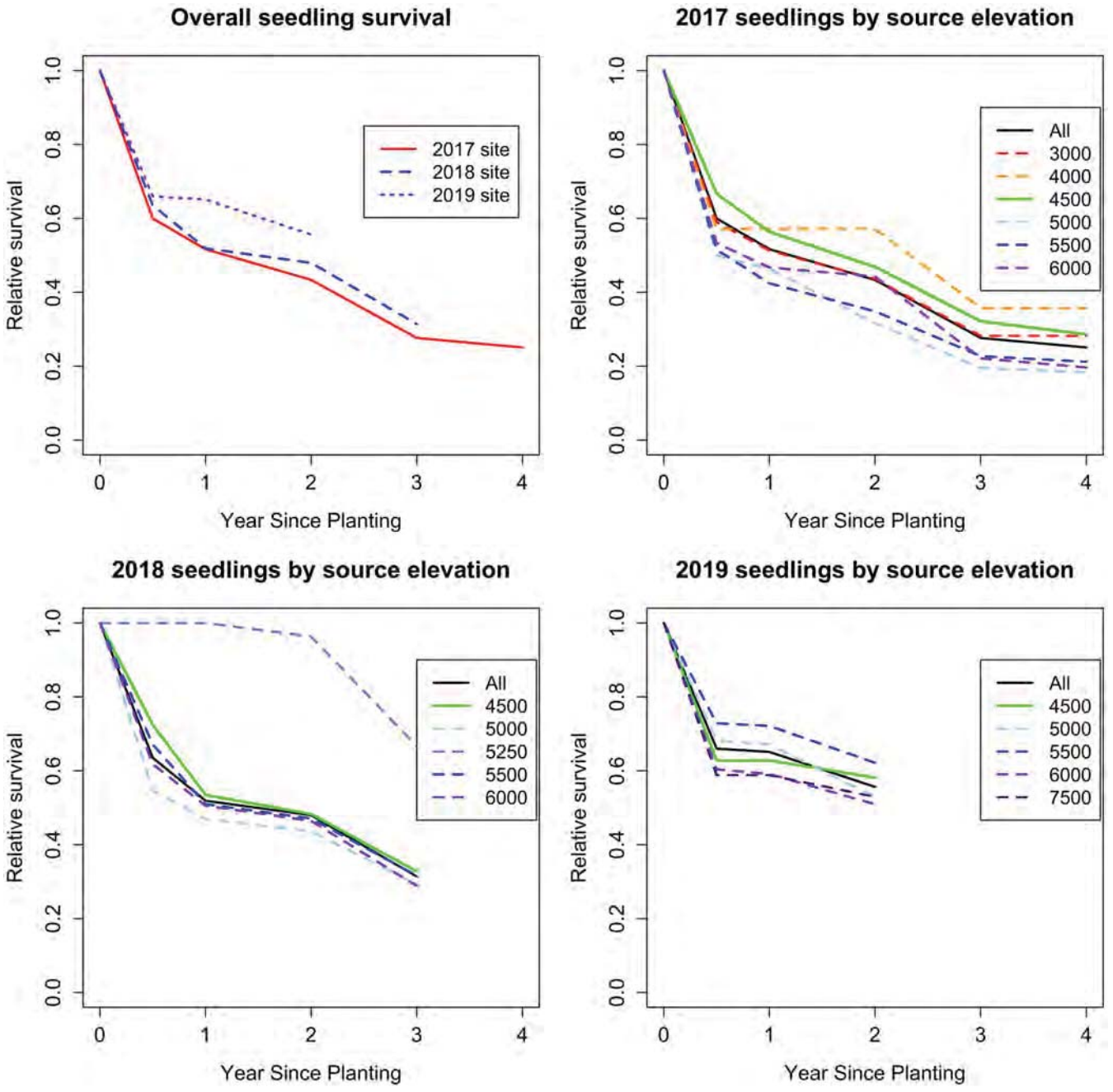

FIGURE 6 Observed proportion of sugar pine seedlings alive over time. The top left panel shows overall survival for all three sites. The other three panels show survival by source elevation for each planting site/year. "4500" in the "by elevation" panels indicates the local seed source, lower and higher numbers indicate other seed source elevations. "All" indicates the average across all seed sources.

(1219.2–1371.6-m) elevation band. While the 27 seedlings from the 5250-ft (1600.2-m) band performed surprisingly well at the 2018 site, this is likely a fluke of small sample size; in general sourcing seeds from the 5500-ft (1676.4-m) band and above led to lower survival.

Observed height growth trajectories were also similar across sites, though somewhat surprisingly average height was a bit higher in the 2017 site, which had higher CWD (Figure 5) and higher mortality, than in the less arid 2019 site that had lower mortality (Figure 7). However, there was variation between seedlings from different source elevations. Seedlings from the very low 3000-ft (914.4-m) source performed about as well as the "local" 4500-ft (1371.6-m) source in the 2017 site, while those from above 5500 ft (1676.4 m) only grew about 60% as much as average. Very high

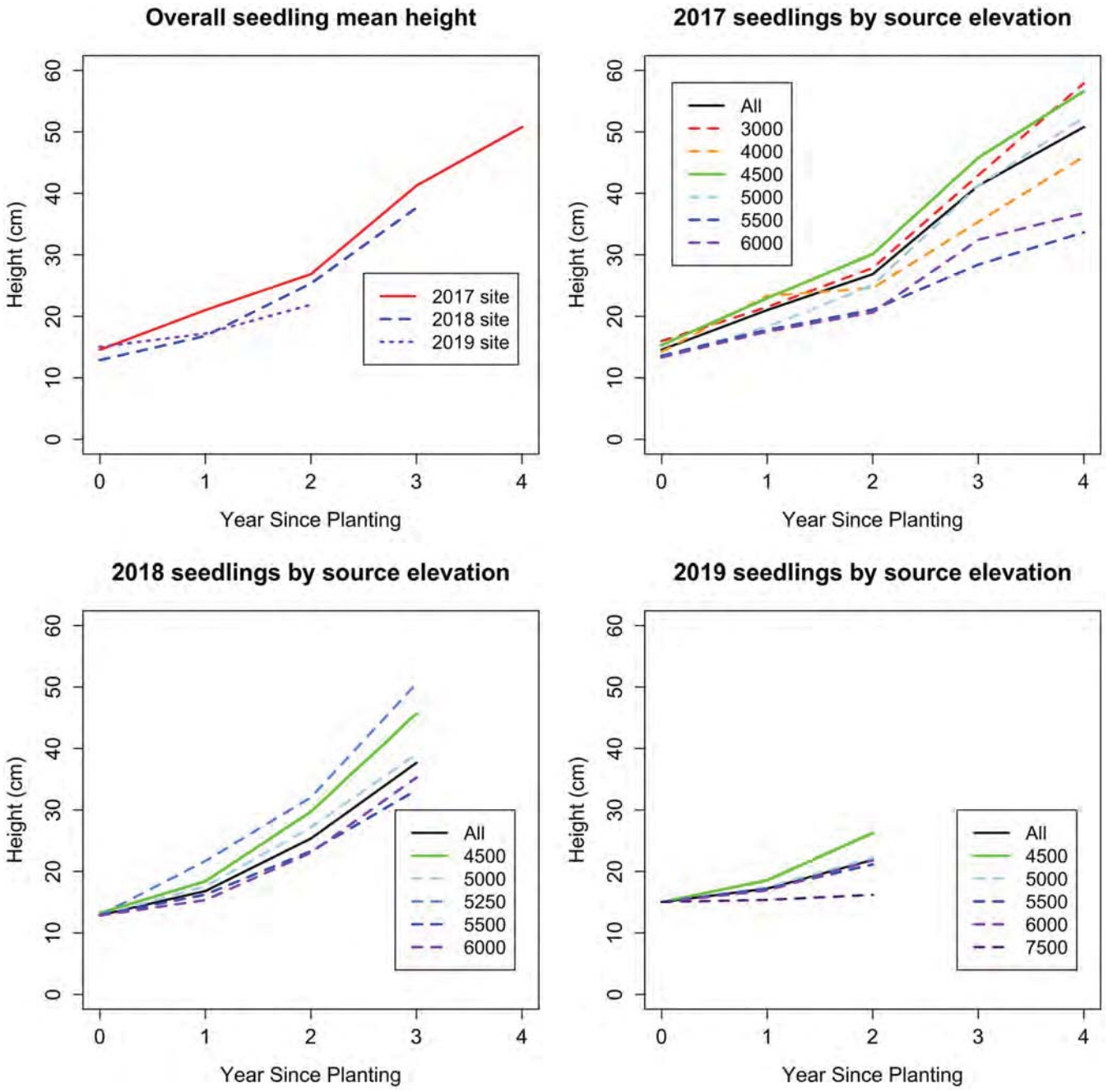

FIGURE 7 Observed average height of sugar pine seedlings over time. The top left panel shows average height for all three sites. The other three panels show height by source elevation for each planting site/year. "4500" in the "by elevation" panels indicates the local seed source, lower and higher numbers indicate other seed source elevations. "All" indicates the average across all seed sources.

elevation seedlings were also the slowest growing at the 2018 and 2019 planting sites.

Survival models

Among the all-year-all-seedling survival models, the best overall included an intercept, site, seedling age, seedling height, and source elevation (model S2; Table 4). The next best included intercept, site, seedling age, seedling height, and either seed-zone-elevation combination and January minimum temperature (S9-D), source elevation × site interactions and April snowpack (S8-A), or just source elevation and April snowpack (S7-A). Effect sizes of these variables on logit(θ), where θ is the annual survival probability, are shown in Figure 8.

Across models, previous-year seedling height was positively associated with survival, and the posterior

TABLE 4 All-seedling survival models.

Model name	Includes	Dm
S1	Intercept, Site, Age, Ht	1568.611
S2	***Intercept, Site, Age, Ht, Elev***	***1558.166***
S3	Intercept, Site, Age, Ht, Site × Elev	1566.453
S4	Intercept, Site, Age, Ht, SZ	1566.426
S5	Intercept, Site, Age, Ht, SZ_Elev	1570.861
S6	Intercept, Site, Age, Ht, Clim (A) Pck4; (B) Ppt; (C) Tmx; (D) Tmn; (E) CWD	(A) 1571.242; (B) 1573.903; (C) 1568.222; (D) 1569.752; (E) 1565.7
S7	**Intercept, Site, Age, Ht, Elev, Clim** (A) **Pck4**; (B) Ppt; (C) Tmx;(D) Tmn; (E) CWD	(A) **1559.734**; (B) 1569.133; (C) 1561.855; (D) 1562.727; (E) 1562.11
S8	**Intercept, Site, Age, Ht, Site × Elev, Clim** (A) **Pck4**; (B) Ppt; (C) Tmx; (D) Tmn; (E) CWD	(A) **1559.538**; (B) 1567.912; (C) 1560.884; (D) 1560.733; (E) 1561.363
S9	**Intercept, Site, Age, Ht, SZ_Elev, Clim** (A) Pck4; (B) Ppt; (C) Tmx; (D) **Tmn**; (E) CWD	(A) 1561.999; (B) 1605.535; (C) 1563.036; (D) **1559.117**; (E) 1560.006

Note: Best-fit models are indicated in bold; the best of these appears in italic boldface. See Table 2 for explanations of abbreviations.

distribution for the height parameter usually did not overlap zero (Figure 8). Survival was lowest for seedlings of the 2017 planting year/site and highest for 2019. Survival was lower in the first summer after planting (Yr0.5) and third year after planting. Survival from the first fall to the second summer (Yr1) tended to be higher, perhaps because the initial transplanting-shock and first-summer mortality had already happened. The estimated effect of the 5250-ft (1600.2-m) source were most positive, but survival was also slightly increased by seedlings being from the lower-elevation 4000-ft (1219.2-m) seed source from more southerly SZ 531. The estimated effects of 5000- and 6000-ft (1524- and 1828-m) sources tended to be the most negative. S8A estimated the effects of the 3000- and 4000-ft (914.4- and 1219.2-m) sources to be positive at the 2017 site, the only one in which they were planted. These effects are consistent with the patterns seen in the raw data (compare Figures 5 and 7).

Based on the S7 models, higher planting site total precipitation, April snowpack, and January minimum temperature reduced annual survival, while higher July maximum temperature and CWD increased it. Of these, precipitation was always the weakest predictor. However, annual climate had a relatively weak effect on survival probability, as shown in Appendix S6: Figure S1, where all other predictors are held constant and only site annual climate is varied.

The overall predictive ability of the four best-fit models was similar, as shown by the comparison of observed number of survivors in each year-site combination to the number predicted by the fitted models (Figure 9). Here, we used the real predictor values for each seedling-year observation, and ran 15 simulations using the fitted parameter values, including the error term. In all cases points clustered around the 1:1 line and the R^2 for predicted as a function of observed was always >0.96. The simplest model, S1 (intercept, site, age, height), and the worst-fitting model, S6-B (intercept, site, age, height, precipitation), also had strong predictive ability by this measure, with adjusted R^2 of 0.964 and 0.943, respectively (Appendix S6: Figure S2), which only considers overall seedling survival. However, that does not mean that source is unimportant; we can see the effects of seed source in both the raw data (Figure 7), in how including source reduces predictive loss even when it greatly increases the number of parameters, and in how source influences survival when other factors are held constant (Appendix S6: Figure S3). For survival from 2020 to 2021 of genotyped seedlings, three of the best-fit models included intercept, site, seedling age, seedling height, source elevation × site interactions and climate (Table 5): CWD (Sg7-E), Precipitation (Sg7-B), and January minimum temperature (Sg7-D). As with the all-seedling models, previous-year height had a strong positive effect on survival (Appendix S6: Figure S4). In the Sg7 models, all the climate variables had a negative effect on survival, and this effect was larger than in the all-species models (Appendix S6: Figure S4). However, it should be remembered that with only one year of data, the planting site and climate effects cannot be fully disentangled, and a model that included all the same predictors except climate (S3) was the third best-fitting model. Among the elevation × site effects, 5000_2019 and 4500_2018 were the most positive (neither 95% CI overlapping zero), while 5000_2018 and 4500_2019 were the most negative. Both 3000- and 4000-ft (914.4- and 1219.2-m) sources were estimated to have positive effects in the 2017 site, though both posteriors very broadly overlapped zero.

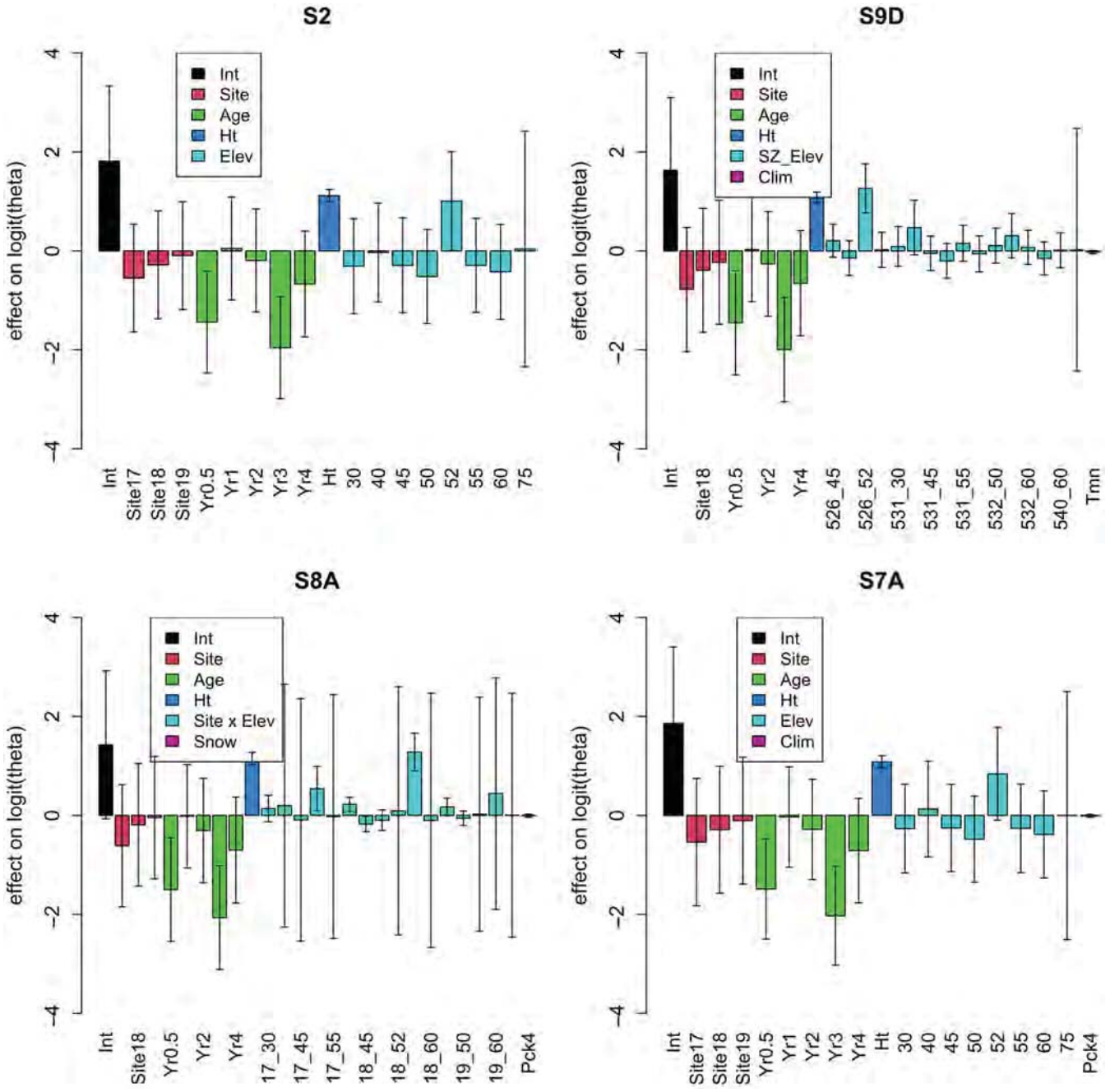

FIGURE 8 Effect sizes of various all-seedling survival model predictors on logit(theta), where theta is the annual survival probability. The height effect is the result of multiplying the fitted height parameter by average previous-year height across all seedling-year observations. Climate effects likewise indicate the multiplication of fitted climate parameter by average site annual climate values across all seedling-year observations. Error bars indicate SE of Gibbs sampler outputs. See Table 2 for predictor value abbreviations.

The models, including a genotype index but not climate (Sg8), performed similarly to those including either SZ or SZ-elevation combinations but not climate. Models including both a genotype index and annual climate tended to perform somewhat worse. For Sg8, effects of FG1 were estimated to be negative except for loci associated with winter minimum temperature and those associated with precipitation. The same was true of FG2 except only the winter minimum temperature allele index was positive. As with the all-seedling models, the overall predictive ability of the four best-fit models was similar, as shown by the comparison of observed number of survivors in each year-site combination to the number predicted by the fitted models (Appendix S6: Figure S5).

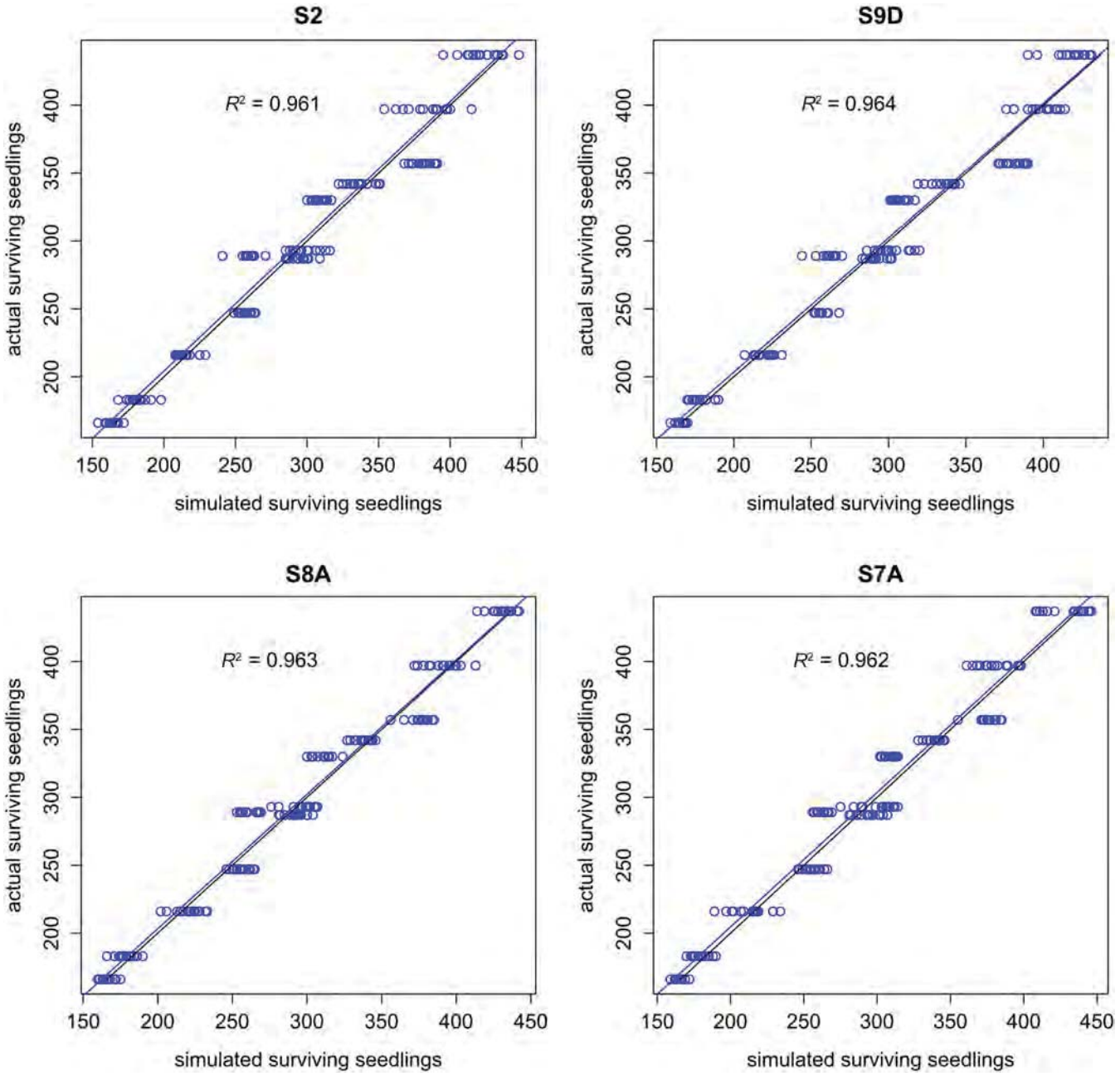

FIGURE 9 The *x*-axes show the output of 15 simulations of annual site-level seedling survival using the real predictor values for all seedling-year observations and the fitted parameter values, including the error term, for each of the indicated survival models. The *y*-axes show actual observed annual site-level survival. The blue lines and R^2 values show the relationships of predicted to observed survival. Black lines show 1:1 relationships.

Growth models

Among the all-year-all-seedling growth models (Table 6), the best overall included intercept, site, seedling age, seedling height, source elevation, and precipitation (G7-B) or April 1 snowpack (G7-A), or models that include the same variables except for source elevation (G6-B and G6-A). Current seedling height was positively associated with growth, as with survival (Figure 10). Unlike survival, however, the 2019 year/site had the most negative effect on growth, 2018 the most positive. Seedlings had the largest height growth increment in their second years, while year 4 had a strong negative effect on height growth. The 4000-, 5500-, and 6000-ft (1219.2-, 1676.4-, and 1828.8-m) sources had the most negative effects, the 5250-ft (1600.2-m) source the most positive. Site annual climate more strongly influenced growth than survival; higher precipitation and April snowpack were associated with lower growth, while

TABLE 5 Survival models for genotyped seedlings, 2020–2021.

Model name	Includes	Dm
Sg1	Intercept, Site, Age, Ht	44.4
Sg2	Intercept, Site, Age, Ht, Elev	44.616
Sg3	**Intercept, Site, Age, Ht, Site × Elev**	**42.484**
Sg4	Intercept, Site, Age, Ht, SZ	44.983
Sg5	Intercept, Site, Age, Ht, SZ_Elev	43.636
Sg6	Intercept, Site, Age, Ht, Clim (B) Ppt; (C) Tmx; (D) Tmn; (E) CWD	(B) 44.676; (C) 44.989; (D) 45.232; (E) 44.476
Sg7	**Intercept, Site, Age, Ht, Site × Elev, Clim** (B) **Ppt**; (C) Tmx; **(D) Tmn**; (E) ***CWD***	(B) **41.913**; (C) 42.642; (D) **42.511**; (E) ***41.537***
Sg8	Intercept, Site, Age, Ht, Geno (1) FG1; (2) FG2	(1) 45.138; (2) 44.369
Sg9	Intercept, Site, Age, Ht, Geno, Clim (B) Ppt; (C) Tmx; (D) Tmn; (E) CWD; (1) FG1; (2) FG2	(B1) 45.006; (B2) 44.093; (C1) 45; (C2) 43.625; (D1) 44.335; (D2) 44.037; (E1) 44.944; (E2) 45.237

Note: Notation is as in Table 4, but Geno indicates genotype indices FG1 or FG2.

TABLE 6 Growth models for all seedlings.

Model name	Includes	Dm
G1	Intercept, Site, Age, Ht	190,676.6
G2	Intercept, Site, Age, Ht, Elev	189,781.4
G3	Intercept, Site, Age, Ht, Site × Elev	190,095.2
G4	Intercept, Site, Age, Ht, SZ	190,238.7
G5	Intercept, Site, Age, Ht, SZ_Elev	190,440.3
G6	**Intercept, Site, Age, Ht, Clim** (A) **Pck4**; (B) **Ppt**; (C) Tmx; (D) Tmn; (E) CWD	(A) **189,690.4**; (B) **189,548.4**; (C) 190,467.1; (D) 190,258.3; (E) 190,612.2
G7	**Intercept, Site, Age, Ht, Elev, Clim** (A) **Pck4**; (B) ***Ppt***; (C) Tmx; (D) Tmn; (E) CWD	(A) **189,020**; (B) ***188,890***; (C) 189,793; (D) 189, 709.5; (E) 189,722

Note: Notation as in Table 4.

higher January minimum temperature was associated with higher growth (Appendix S6: Figure S6).

As for the survival models, we tested the predictive ability of growth models by running 15 simulations using the fitted parameter values for the four best models, including the error term, and calculating average height growth increment for each site-year combination. In all cases, points clustered around the 1:1 line and the R^2 for predicted as a function of observed was always >0.95 (Figure 11). For growth of genotyped seedlings (Table 7), the best-fit models included intercept, site, seedling age, seedling height, SZ and elevation and either precipitation (Gg7-B), July maximum temperature (Gg7-C) or April 1 snowpack (Gg7-A); intercept, site, seedling age, seedling height, and SZ (Gg4); or just intercept, site, seedling age, and seedling height (Gg1). As with the all-seedling growth models, previous year height was strongly associated with higher growth, while being planted at the 2019 site, or being in the fourth year since planting was negatively associated with growth.

The models including a genotype index tended to perform worse than similar models without a genotype index. The effects of all the FG1 and FG2 genotype indices were negative. In the models including SZ-elevation, with (G7) or without (G5) climate, the seed combinations with the most positive effects on growth were 526_5250, 531_5000, and 531_4000, while the ones with the most negative effects was 540_6000. April snowpack, precipitation, and July maximum temperature had a negative effect on growth.

That the four best-fit models included either one of two different metrics of source (SZ_Elev, or just SZ) or no source metric and either one of two different climate variables or no climate variable suggests that there was not enough data to distinguish between the effects of these

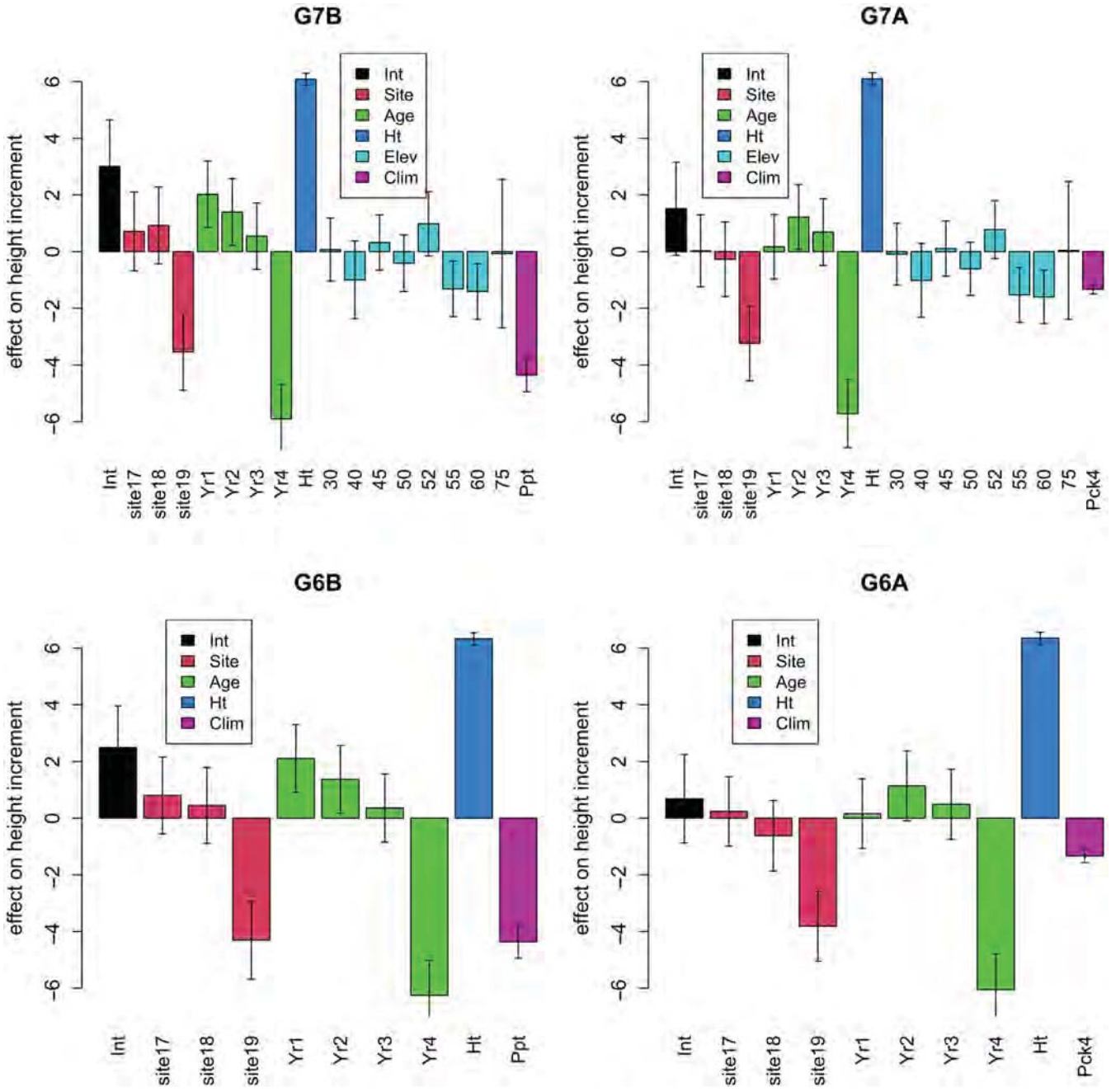

FIGURE 10 Effect sizes of various all-seedling growth model predictors on the annual height increment. The height effect is the result of multiplying the fitted height parameter by average previous-year height across all seedling-year observations. Climate effects likewise indicate the multiplication of fitted climate parameter by average site annual climate values across all seedling-year observations. Error bars indicate SE of Gibbs sampler outputs. See Table 2 for predictor value abbreviations.

factors. However, it is interesting to note that estimates of the effect of source being within SZ 531 (which is to the south of the planting location) tended to be more positive than the effect of local SZ 526 sources.

Estimated σ^2 (the error parameter) for these models was higher than for the other model sets. Unsurprisingly, then, simulations of growth based on the fitted parameters yielded lower R^2 values than for the other model sets. Even so, R^2 values for the four best-fit models ranged between 0.459 and 0.517 (Appendix S6: Figure S8).

DISCUSSION

Climate relationships in GEA and seedling performance models

April 1 snowpack being the climate variable associated with the highest number of SNPs in the LFMM analysis and a frequent useful predictor of survival and growth in the models is interesting in light of California's Mediterranean climate. High April snowpack can

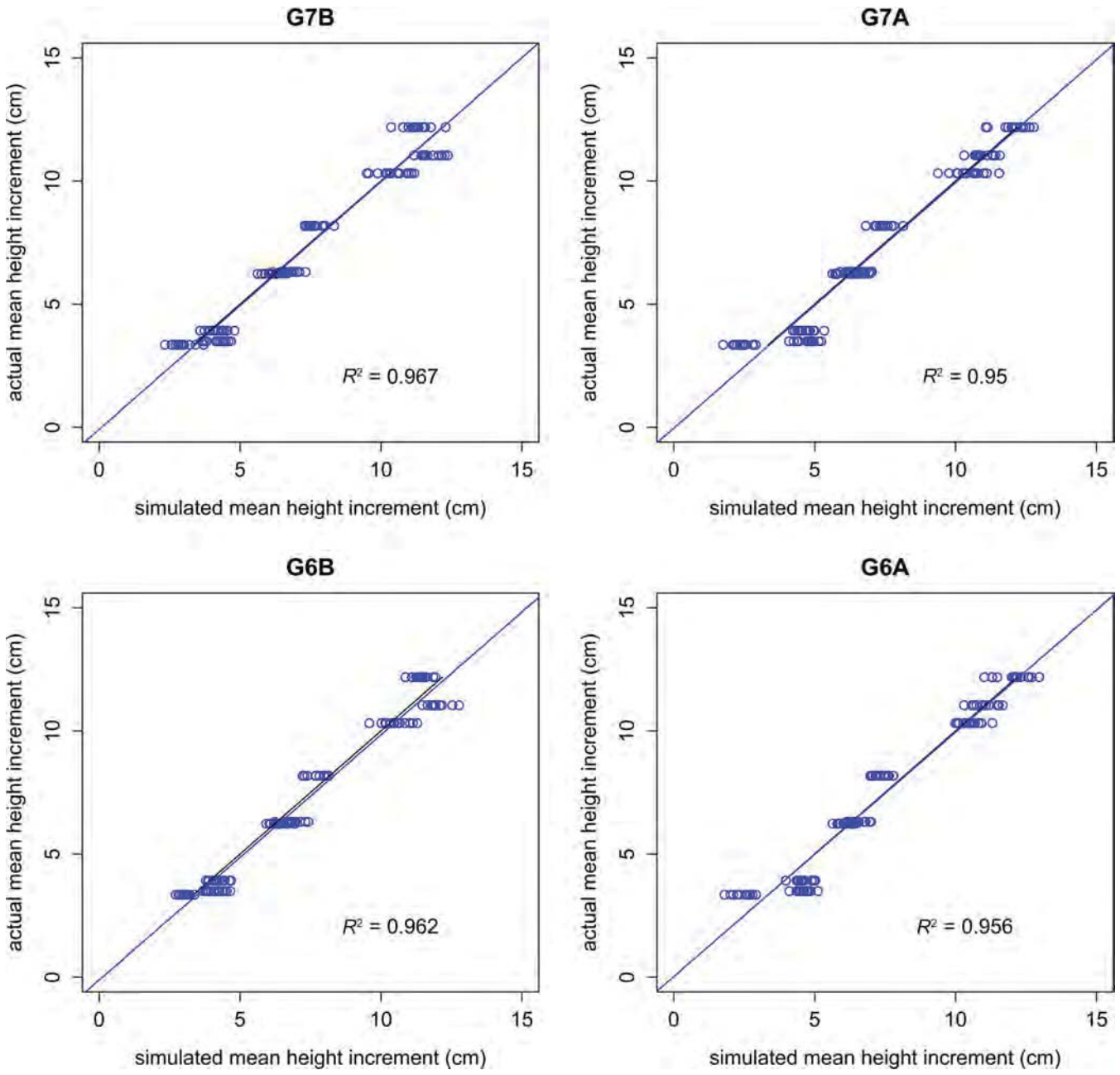

FIGURE 11 The *x*-axes show output of 15 simulations of mean annual height growth increment for each site and year using the real predictor values for all seedling-year observations and the fitted parameter values, including error term, for each of the indicated growth models. The *y*-axes show actual mean annual height growth increment for each site and year. Blue lines and R^2 values show the relationships of predicted to observed survival. Black lines show 1:1 relationships.

reflect a combination of high winter precipitation, low winter temperatures, and low spring temperatures. For seedlings, being buried in snow can buffer against extreme cold temperatures and, as it melts, the snowpack also provides moisture longer into the spring than the same amount of water that arrives as rain (Bales et al., 2011). This can be very important for seedling establishment (Andrus et al., 2018). However, deep snowpack may also have negative effects such as delaying the start of growth or increasing susceptibility to fungal infection (Renard et al., 2016). April snowpack was also the top climate variable in a GEA study of the co-occurring ponderosa pine (Shu & Moran, 2023). However, the snowpack has been declining in the Sierra Nevada and much of the Western USA, and further losses are predicted (Fyfe et al., 2017; Sun et al., 2019), which suggests that selective pressures on these populations are shifting.

TABLE 7 Growth models for genotyped seedlings.

Model name	Includes	Dm
Gg1	**Intercept, Site, Age, Ht**	**31,312.23**
Gg2	Intercept, Site, Age, Ht, Elev	31,362.93
Gg3	Intercept, Site, Age, Ht, Elev x Site	31,374.76
Gg4	**Intercept, Site, Age, Ht, SZ**	**31,326.26**
Gg5	Intercept, Site, Age, Ht, SZ_Elev	31,371.87
Gg6	Intercept, Site, Age, Ht, Clim (A) Pck4; (B) Ppt; (C) Tmx; (D) Tmn; (E) CWD	(A) 31,373.5; (B) 31,359.76; (C) 31,403.27; (D) 31,394.2; (E) 31,374.64
Gg7	**Intercept, Site, Age, Ht, SZ_Elev, Clim** (A) **Pck4**; (B) **Ppt**; (C) **Tmx**; (D) Tmn; (E) CWD	(A) **31,327.04**; (B) ***31,305.98***; (C) **31,325.36**; (D) 31,331.91; (E) 31,344.49
Gg8	Intercept, Site, Age, Ht, Geno (1) FG1 (2) FG2	(1) 31,419.28; (2) 31,348.77
Gg9	Intercept, Site, Age, Ht, Geno, Clim (A) Pck4; (B) Ppt; (C) Tmx; (D) Tmn; (E) CWD; (1) FG1; (2) FG2	(A1) 31,358.77; (A2) 31,360.74; (B1) 31,414.6; (B2) 31,372.33; (C1) 31,429.15; (C2) 31,446.2; (D1) 31,413.26; (D2) 31,442.66; (E1) 31,413.04; (E2) 31,337.65

Note: Notation as in Table 4.

Maximum summer temperature emerged as important in the RDA analysis of genotype-environment relationships. This climatic factor is also likely to have strong selective effects because higher summer temperatures increase soil drying and can directly damage seedlings (Goulden & Bales, 2019; Hikosaka et al., 2006; Kolb & Robberecht, 1996). In a previous study, we found that higher summer temperatures were associated with reduced survival and reduced probability of transitioning to a taller height class for naturally recruited seedlings of many Sierra Nevada tree species, including sugar pine (Moran et al., 2019).

In the all-seedling growth models, planting site annual climate had a fairly strong impact, with both high precipitation and high April 1 snowpack being associated with reduced height growth increment (Figure 10; Appendix S6: Figure S6). This is not likely to be a direct water-logging effect, given that soils at these sites are well-drained, but may be related to higher risk of pathogen transmission in wet years mentioned above, or to the fact that high precipitation years also tend to be cloudier and/or have shorter growing seasons (Renard et al., 2016). Cloud cover can reduce photosynthetically active radiation (PAR) by 30%, which can reduce water stress in the summer (Williams et al., 2008) but might also reduce spring growth. Many of the seed sources for this experiment came from sites that were drier than current conditions at the planting site (Figure 3) and they may not have been well-adapted to wetter environments. This latter hypothesis would be consistent with the positive effects of prior January minimum temperature having a positive effect on growth increment (Appendix S6: Figure S6), although models including this variable had higher predictive loss. In the genotyped seedling growth analyses precipitation and July maximum temperature were estimated to have negative effects (Appendix S6: Figure S7). However, models lacking climate effects performed as well or better, suggesting that more data might be needed to capture site annual climate effects with confidence.

Site annual climate variables had among the smallest effect sizes in the all-seedling survival models (Figure 8; Appendix S6: Figure S1), and a model that omitted them (S2) was the best-performing. In the genotyped seedling survival model, CWD had a strong negative effect, precipitation and January minimum temperature weaker negative effects (Appendix S6: Figure S4). However, as this is based on only one year of survival data, these results should be viewed with caution, since it is not possible to fully disentangle site effects from climate effects.

Effects of seedling source on performance

Planting year/site effects and seedling source effects likely cannot be fully disentangled from each other, since not all sources were planted each year at each site. Source elevation, elevation × site, or elevation-SZ effects did not always follow a clear pattern (e.g., with effects on survival being related to elevation or latitude in a linear way). For instance, the effects of the 7500-ft (2286-m) source from SZ 997 in the Transverse Range were generally not as negative as the 5500- and 6000-ft (1676.4- and 1828.8-m) sources from more northern SZs (Figures 7

and 9; Appendix S6). This may be because the 7500-ft (2286-m) source adapted to much drier and warmer conditions than the other high-elevation sources, though not as warm as the planting site (Figure 3). None of the sources, including the "local" source, were a perfect match for current conditions. Overall, source areas that prior to rapid climate change were as warm as the planting site were also drier, while those that were as wet were significantly colder (Figure 3).

That being said, low-elevation seed sources likely adapted to an environment that was as warm as the planting site but somewhat drier (SZ 531, 3000–4500 ft [914.4–1371.6 m]; Figure 3) tended to perform about as well or better overall than those from the local SZ and elevation (SZ 526, 4500 ft). For example, in the all-seedling survival models, the effect of the 4000-ft (1219.2-m) seed source (either overall or as 4000-ft-at-2017-site or SZ 531-4000-ft band) was consistently more positive than the local seed source (Figure 8), while in the all-seedling growth models the 3000-ft seed source had an effect size and direction similar to the local seed source (Figure 10). This was the case even though the year in which the lower-elevation seeds sources were establishing (2017) was not the hottest or driest during the experimental period (Figure 5). These results suggest that the strategy of including seed sources from the next-lowest-latitude SZ and/or up to 1500 ft (457.2 m) lower in elevation will not significantly decrease success of restoration plantings of this species, and may even increase it. This is perhaps unsurprising given that local mean annual temperatures in many potential planting sites most resemble locations 1000–1500 ft (304.8–457.2 m) downhill in the mid-20th century, before rapid warming began (Figure 3; Climate-Adapted Seed Tool|Reforestation Tools, https://reforestationtools.org/climate-adapted-seed-tool/).

This finding is consistent with a previous operational planting study in California that included fewer seed sources but more species (Young et al., 2020), and an older sugar pine provenance study that found seedlings from the southern Sierra Nevada performed as well or better than local seed sources at two northern Sierra Nevada planting sites (Kitzmiller, 2004). While a previous study found that sugar pine had lower photosynthesis, lower water use efficiency, and less variation in both metrics than other co-occurring conifer species (Grulke, 2010), that was based on naturally-recruited seedling in their home sites. The variation in performance between seed sources in our experiment suggest that it may be worth examining between-population variation in the plasticity of such physiological processes in response to varying climate, rather than just variation between populations growing in different environments across the range.

Why did genetic predictors not improve models of seedling performance in this study?

Potential reasons that the proportions of alleles at climate-associated loci expected to be locally favored were not always positively associated with seedling survival or growth include:

1. The small number of trees included in the association analysis or potential over-corrections for population structure in RDA models may have led to some spurious associations or to important causative variation being missed.
2. Some of the genotypes included in the association analysis come from areas of the northern Sierra Nevada, Klamath Mountains, and Coast Ranges not included in the planting experiment, and comprising a different genetic cluster, while the planting experiment included some seedlings from the Transverse Ranges, a mountain chain whose sugar pine population was not included in the association analysis. It also represents a third partially-interbreeding population in addition to the Sierra Nevada and northern populations (Weiss et al., 2022). Results of an association analysis may not transfer from one area of a species range to another.
3. Reduction of genetic information to a single index for each set of associations may muddy the effects of individual alleles. Positive effects were more often estimated for the indices representing fewer loci. However, including an effect for each locus would have resulted in trying to fit a model with nearly as many parameters as observations!
4. Overall small sample size of planted seedlings that survived to be genotyped and could thus be included in the models. The smaller the sample size, the fewer parameters can be fitted with confidence.

It is important to note that using latitude to correct for neutral population structure in our RDA models likely represents a conservative approach to identifying adaptive loci. Given the highly north–south distribution of the sampled trees, using latitude as our metric of geographic distance, may result in our analyses missing some of the adaptive signal for loci associated with environmental gradients that vary latitudinally. Yet, simulation studies have shown that RDA is a powerful method to detect signals of selection when population structure and selective gradients are correlated (Capblancq et al., 2018). However, this potential over-correction is a potential explanation why our models were not greatly improved by the addition of genomic data.

What can we learn from the genetic analyses?

Unbeknownst to us, another team of researchers was simultaneously conducting a range-wide GEA of *P. lambertiana* (Weiss et al., 2022). This study, which used somewhat different methods, makes for a useful point of comparison. Because they used a different climate downscaling dataset (Climate_NA), April snowpack was not included as a variable, but other moisture-related climate variables emerged as important, including mean summer precipitation and mean annual precipitation. As in our study, overlap in SNPs identified by univariate and RDA methods was modest, and gene annotations included transcription factor, protein kinases, and genes previously found to be upregulated in plants by biotic or abiotic stressors.

We were limited in the genotypes we could include in the association analysis. However, the similarity in the types of gene functions observed to be associated with climate in the study cited above and to one conducted on a larger sample of ponderosa pine suggests that many of these associations are likely real for the geographic area represented (Appendix S4; Shu & Moran, 2023). This includes the prevalence of SNPs with annotations indicating function in signal transduction, stress response, the abscisic acid pathway, and ubiquitination, as well as April snowpack being the primary associated climate variable for both *P. lambertiana* and *P. ponderosa* in our LFMM analyses. In pines, abscisic acid (ABA) is increased as a signal for trees to keep stomata closed (Brodribb et al., 2014) during dry conditions, and has clear links to drought tolerance in tree species (Moran, Lauder, et al., 2017). Ubiquitination also has potential links to drought stress through its involvement in ABA signaling (Ryu et al., 2010). Genetic association studies in black spruce (*Picea mariana*) and ponderosa pine have also uncovered relationships between genes involved in the ubiquitin pathway and environmental variables with links to water availability (Prunier et al., 2011; Shu & Moran, 2023). In addition, in this study we found multiple loci involved in abiotic stress response, including functions related to osmotic stress (loci 62,255, 132,219 and 285,530) and drought tolerance (loci 6142, 6152, and 122,233) (Appendix S4).

Our results indicate that this type of small-scale opportunistic GEA study should not be used for seed source selection in most cases; source elevation and/or SZ performed better as a predictor of performance. Range-wide association studies or those focusing on the area from which seed sources will be drawn will probably yield more useful predictions. Moreover, when testing the predictive ability of GEAs, it would be better if plantings could be sampled early (in the first or second year) so that a higher number of seedlings can be sampled, and more years of survival and growth observations included in the analysis.

Previous studies indicate that genotype-environment or GPA does have strong potential for improving seed selection (Beaulieu et al., 2014; Brawner et al., 2012; Grattapaglia & Resende, 2011; Isik et al., 2016; Jaramillo-Correa et al., 2015; Resende et al., 2012). Elevation bands of 500 ft (152.4 m) within SZs can encompass a wide climatic range (Figure 3). Any tools that can more precisely identify source trees whose seedlings are more likely to perform well under future conditions would be helpful complements to the rough approximation that is SZ climate matching. Since there are concerns that trying to match planting site conditions projected for more than 20 or 30 years into the future may result in seedlings being maladapted to current site conditions (Grady et al., 2015; Sáenz-Romero et al., 2021), further testing of seed sources from source locations significantly warmer than the planting site is also warranted. This will help to determine to what extent matching to future climate projections, rather than already-observed changes, is practical.

AUTHOR CONTRIBUTIONS

Jessica W. Wright and her team established the field experiment in 2017–2019, and Canning oversaw field teams during annual remeasurements. Rainbow DeSilva conducted the genetic analyses. Emily V. Moran built the statistical models, conducted the growth and survival analyses, and wrote the first draft of the paper. Emily V. Moran, Jessica W. Wright, and Rainbow DeSilva edited subsequent drafts of the paper.

ACKNOWLEDGMENTS

Genetic analyses and 2020-2021 fieldwork were funded by USDA Forest Service Grant 20-JV-11272138-050. Dana Walsh, USFS, was the silviculturist who made this study possible. Troy Shea and the Eldoardo National Forest Timber crews helped with tree planting and measurements. Later measurements (2020–2021) were collected by UC Merced undergraduate researchers Cassandra Hay, Emily Semans, Roxana Khalili, and Veronica Magana-Buie. Lucy Sullivan, Pakeeza Azizpor, and Noah Kaufman performed the DNA extractions. The sequencing was carried by the DNA Technologies and Expression Analysis Core at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 1S10OD010786-01. Genomic analysis used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation Grant number ACI-1548562. The use of trade

or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy. This work was supported in part by the U.S. Department of Agriculture, Forest Service.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Code for the full statistical models and input files for seedling data and climate (emoran5, 2024) are available from Zenodo: https://doi.org/10.5281/zenodo.11086667. Raw DNA sequencing data are available at the National Center for Biotechnology Information (NCBI) under accession number PRJNA1112904: https://www.ncbi.nlm.nih.gov/sra/PRJNA1112904. Individual SNP genotypes (Moran et al., 2022) are available from Dryad: https://doi.org/10.6078/D1MH7P.

ORCID

Emily V. Moran https://orcid.org/0000-0003-4624-1910

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Moran, Emily V., Rainbow DeSilva, Courtney Canning, and Jessica W. Wright. 2024. "Testing Source Elevation versus Genotype as Predictors of Sugar Pine Performance in a Post-Fire Restoration Planting." *Ecosphere* 15(10): e70010. https://doi.org/10.1002/ecs2.70010