RESEARCH ARTICLE

AMERICAN JOURNAL OF BOTANY

INVITED PAPER

For the Special Issue: Evolutionary Insights from Studies of Geographic Variation

Landscape genomic analysis of candidate genes for climate adaptation in a California endemic oak, *Quercus lobata*[1]

Victoria L. Sork[2,3,6], Kevin Squire[4], Paul F. Gugger[2], Stephanie E. Steele[2], Eric D. Levy[2], and Andrew J. Eckert[5]

PREMISE OF THE STUDY: The ability of California tree populations to survive anthropogenic climate change will be shaped by the geographic structure of adaptive genetic variation. Our goal is to test whether climate-associated candidate genes show evidence of spatially divergent selection in natural populations of valley oak, *Quercus lobata*, as preliminary indication of local adaptation.

METHODS: Using DNA from 45 individuals from 13 localities across the species' range, we sequenced portions of 40 candidate genes related to budburst/flowering, growth, osmotic stress, and temperature stress. Using 195 single nucleotide polymorphisms (SNPs), we estimated genetic differentiation across populations and correlated allele frequencies with climate gradients using single-locus and multivariate models.

RESULTS: The top 5% of F_{ST} estimates ranged from 0.25 to 0.68, yielding loci potentially under spatially divergent selection. Environmental analyses of SNP frequencies with climate gradients revealed three significantly correlated SNPs within budburst/flowering genes and two SNPs within temperature stress genes with mean annual precipitation, after controlling for multiple testing. A redundancy model showed a significant association between SNPs and climate variables and revealed a similar set of SNPs with high loadings on the first axis. In the RDA, climate accounted for 67% of the explained variation, when holding climate constant, in contrast to a putatively neutral SSR data set where climate accounted for only 33%.

CONCLUSIONS: Population differentiation and geographic gradients of allele frequencies in climate-associated functional genes in *Q. lobata* provide initial evidence of adaptive genetic variation and background for predicting population response to climate change.

KEY WORDS climate change; environmental gradients; Fagaceae; landscape genetics; local adaptation; *Quercus*; single nucleotide polymorphisms; spatially divergent selection

Rapid climate change is altering the distribution of many species and threatening the survival of others (Hughes, 2000; Parmesan, 2006; Parry et al., 2007). Its impact is of particular concern for tree species because their failure to survive would not only be a loss of a single species but the loss of an entire ecosystem and its biodiversity (Hughes et al., 2008; Kremer et al., 2012; Sork et al., 2013). A critical question is whether tree populations will tolerate predicted climate changes locally, shift in distribution to track favorable climates, rapidly evolve in response to climate change, or go extinct (Aitken et al., 2008). To understand the capacity of tree populations to evolve adaptively, it is important to understand how much genetic variation underlies adaptive traits within natural populations and to what extent that variation will permit survival under predicted future climate scenarios (Sork et al., 2013). The analysis of spatial patterns of genetic variation in natural populations, especially locally adaptive genetic variation, known as landscape genomics, helps indicate which genes are under selection due to environment by identifying clines in allele frequencies for specific genes that are associated with climate gradients (Sork and Waits, 2010). Such information helps identify which genes are candidates for local adaptation to climate and the potential of local populations to respond to climate change (Holderegger et al., 2006; Manel et al., 2010; Sork and Waits, 2010; Sork et al., 2013). This evidence from natural

[1] Manuscript received 9 April 2015; revision accepted 26 August 2015.

[2] Department of Ecology and Evolutionary Biology, University of California, 4140 Terasaki Life Sciences Building, 610 Charles E. Young Drive East, Los Angeles, California, USA 90095-7239;

[3] Institute of Environment and Sustainability, University of California, Box 951767, Los Angeles, California, USA;

[4] Center for High Throughput Biology, David Geffen School of Medicine at UCLA, University of California, Los Angeles, California, USA; and

[5] Department of Biology, Virginia Commonwealth University, Richmond, Virginia 23284 USA

[6] Author for correspondence (e-mail: vlsork@ucla.edu)

doi:10.3732/ajb.1500162

populations lays a foundation for the emerging studies of "adaptation genomics" (Stapley et al., 2010) from which we can start to address questions about the extent to which pre-existing genetic variation will allow survival of tree populations under future conditions (Aitken et al., 2008).

Predicted increases in atmospheric concentrations of greenhouse gases have already had measurable effects on the earth's climate, causing increased warming and changes in patterns of precipitation in many regions (Hughes, 2000; Parry et al., 2007). Such changes will likely require a physiological response by trees with respect to drought tolerance, heat stress, and growth under new climate conditions (Hanson and Weltzin, 2000; Breda et al., 2006; McDowell et al., 2008). While it might be possible for species with short generation times to evolve rapidly in response to climate change (e.g., Frankset al, 2007), it is less clear whether long-lived organisms will be able to adapt to change that is on a shorter timeframe than their generation time (Kremer et al., 2012; Sork et al., 2013). Physiological responses of trees to increases in CO_2 and temperature, moreover, will affect populations at a regional scale (Chmura et al., 2011). For plant developmental processes, such as leaf or flowering phenology, that are dependent on temperature cues or photoperiod cues tied to climate due to correlated latitudinal gradients (e.g., Chuine and Cour, 1999; Cleland et al., 2007; Hall et al., 2007; Ingvarsson et al., 2008; Luquez et al., 2008; Morin et al., 2009; Alberto et al., 2011; Alberto et al., 2013), new conditions may create asynchrony in these processes (Parmesan, 2006; Blois et al., 2013). Tree species that are locally adapted across a range of phenotypic traits that affect growth and survival may exhibit a regional, negative response to climate change (Adams et al., 2009; Chmura et al., 2011), thus calling for adaptive management of tree populations using approaches that explicitly acknowledge these patterns, such as assisted migration (Aitken and Whitlock, 2013).

To identify climate-associated candidate genes in natural populations, a first line of evidence is genetic variation that correlates with climate gradients (Endler, 1986). Recent studies of tree populations have used genome-wide scans to find individual single nucleotide polymorphisms (SNPs) that are extremely correlated with climatic gradients, thus identifying gene regions putatively affected by spatially divergent selection (Coop et al., 2010; Eckert et al., 2010a, b; Bashalkhanov et al., 2013). The advantage of genome-wide scans is that historical impacts of gene flow and genetic drift on adaptive genetic patterns can be examined simultaneously because of the large number of markers, most of which are neutral, used to detect patterns. For species with large genomes or little linkage disequilibrium such as long-lived, outcrossing trees, one limitation would be that a large portion of the genome would need to be sequenced to find significantly correlated SNPs (Neale and Savolainen, 2004). An alternative approach would be to focus on particular sequences of genes thought to be involved in adaptation to climate (e.g., González-Martínez et al., 2006a; Eckert et al., 2010b; Holliday et al., 2010; Alberto et al., 2013). An advantage of this approach is that these functional genes and their alleles can be directly implicated in controlling a portion of the observed adaptive phenotypic variation (Neale and Savolainen, 2004; González-Martínez et al., 2006b). As a first step for studies of species with limited genomic resources, this focused approach would efficiently identify the presence of putatively adaptive genetic variation warranting further study.

Because the response by tree populations to climate change will be shaped by the geographic structure of adaptive genetic variation, the overall goal of this study is to search for evidence of spatially divergent selection in natural populations of valley oak, *Quercus lobata* Née (Fagaceae), a California endemic tree species. *Quercus* species are excellent taxa for studies of spatially divergent selection in response to variable environments because their wide geographical range is climatically and edaphically diverse (Gailing et al., 2009), and in particular, populations of valley oak in California have had relatively stable populations for several hundred thousand years that are likely to reflect the impact of adaptive processes rather than recent expansion from glacial refugia (Gugger et al., 2013). Valley oak is a foundational species that contributes to oak savannah, oak woodland, and riparian oak forests throughout California, but has suffered severe loss of habitat since the arrival of Europeans due to the creation of pastures, farmland, vineyards, and housing developments. To efficiently survey for climatically associated genetic variation, we selected functional genes likely to be important in response to four types of response to climate: bud burst and flowering phenology, growth, osmotic stress, and temperature stress. We had three specific objectives. First, we assessed the extent of population differentiation across all SNPs to identify those with particularly high values of genetic differentiation (F_{ST}). For genes under spatially divergent selection, the allele frequencies should differ across populations. Second, we correlated allele frequencies of the same SNPs with climate variables to find those SNPs that are overly correlated to climate gradients. The expectation is that genes under spatially divergent selection due to climate will vary with climate gradients. Third, we conducted a multivariate analysis of suites of those SNPs that are associated with different functional categories of genes to detect the presence of complex relationships between gradients of SNP frequencies with climatic variables. This approach can be effective at identifying sets of SNPs under selection by climate (Sork et al., 2013), as demonstrated for a study of air pollution impacts on red spruce populations (Bashalkhanov et al., 2013). Finally, we addressed the role of geography and climate in shaping the distribution of SNP allele frequencies in each of the four functional groups of genes.

MATERIALS AND METHODS

Study species—*Quercus lobata* Née (section *Quercus*) is a dominant or codominant species in oak savannas, oak woodlands, and riparian forests across the foothills of the Sierra Nevada, Coastal Ranges, and Transverse Ranges that surround the Central Valley of California (Griffin and Critchfield, 1972). *Quercus lobata* populations are spread across diverse climatic and geographical zones from sea level to 1700 m a.s.l. In California, tree oaks are foundational species for the maintenance of biodiversity (Pavlik et al., 1991). This species has two major eastern and western subpopulations partially separated by the Central Valley that diverged during the Late Pleistocene (about 104 kya, CI = 28–1622 kya, Gugger et al., 2013). Prior studies have shown significant genetic structure among sites based on both chloroplast and nuclear microsatellite loci (Grivet et al., 2006; Grivet et al., 2008; Sork et al., 2009), with significant association with climate variables (Sork et al., 2010; Gugger et al., 2013), suggesting that climate has affected demographic history, migration, and selection.

Sampling—The goal of our sampling design was to achieve a spatial distribution of localities that would minimize kinship among individuals from different populations. We accomplished this design by

sampling localities that were beyond the range of spatial autocorrelation for nuclear or chloroplast microsatellite loci (V. L. Sork, unpublished analyses). Our second criterion was to select sites that maximize variation along environmental gradients so that we could detect population differences due to spatially divergent selection by climate variables. Thus, we sampled 3–4 *Q. lobata* individuals 50–300 m apart within locality and 13 localities distributed throughout the species' range and representing a range of elevations and climates, yielding a total sample size of 45 individuals (Fig. 1). We avoided any individuals with phenotypic or genotypic evidence of introgression. Hybridization in *Q. lobata* with other species is occasional, but not extensive (V. L. Sork, unpublished data; Craft et al., 2002).

Candidate gene selection—To identify genetic variation that might be associated with climate, we selected an initial list of nearly 150 gene fragments thought to play a role in adaptation to climate in other species and that were available at the time of our survey from publicly available sources. First, we searched expressed sequence tag (EST) libraries for *Quercus alba* and *Q. rubra* listed in the Fagaceae Genomics Web (www.fagaceae.org) with the following key words: cold, stress, heat, drought, osmotic stress, flowering, and growth. We further searched this database for specific genes that have previously been shown to be under local selection in other species (Krutovsky and Neale, 2005; González-Martínez et al., 2006a; Soler et al., 2008). For each of the matching ESTs, we designed primer pairs in conserved regions using the program Primer3 (Rozen and Skaletsky, 2000). Second, we supplemented the above primers with published primers for bud burst (Casasoli et al., 2006; Derory et al., 2006, 2010), osmotic stress (Porth et al., 2005a, b), and stress and metabolism (Soler et al., 2008). All primers amplified candidate gene fragments between 280 and 850 bp long.

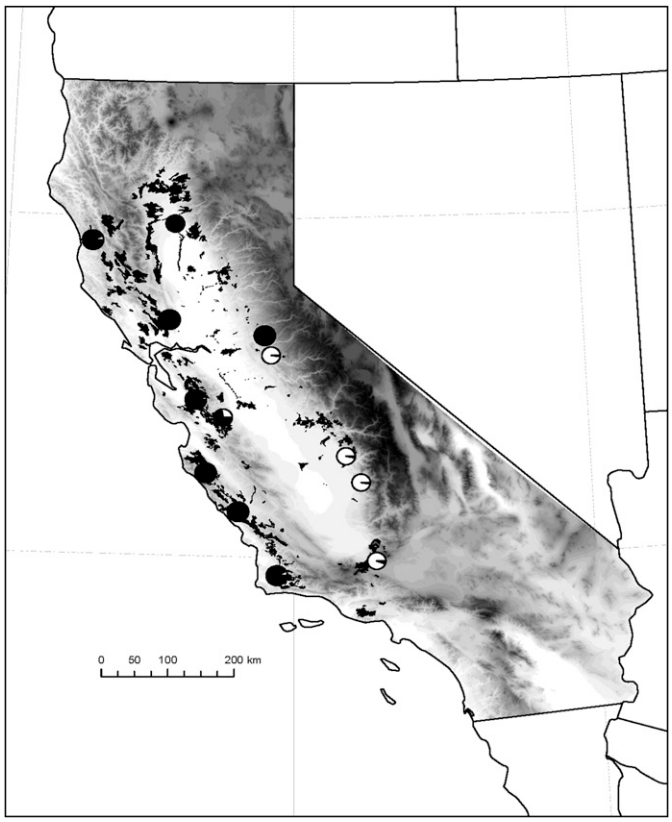

FIGURE 1 Map of sampling localities in California for *Quercus lobata*, with elevations shown by shadings. Based on Bayesian cluster analysis of microsatellite nuclear genetic markers, individuals can be assigned to two main genetic clusters as indicated by black and white, respectively.

DNA preparation—Total genomic DNA was extracted from leaf tissue using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA [CA]) according to the manufacturer's protocol. All primer pairs were initially tested in simplex using various PCR conditions and thermal cycler profiles (Appendix S1, see Supplemental Data with the online version of this article) and were visually checked on an agarose gel. In the end, we were able to optimize primers for 71 gene fragments. Primers were pooled into 16 mixes, each containing 3–5 primer pairs that amplified fragments of similar size under matching conditions (Appendix S1). Using the Qiagen Multiplex PCR kit, we then amplified each of our 45 samples separately in 16 multiplex panels each. PCR products were cleaned with ExoSAP-IT (USB Affymetrix, Santa Clara, CA) and quantified using a Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, Life Technologies, Grand Island, New York, USA). PCR products were pooled in equimolar ratios per sample into 45 libraries of 1.5 μg DNA each. By creating multiplex panels that amplified similarly sized fragments, we were able to ensure that each amplicon was approximately equally represented per sample library. Libraries were condensed using a Qiagen MinElute PCR Purification kit.

We prepared amplicon libraries for sequencing according to Roche 454 protocols (April 2009) for low molecular weight DNA samples. We used 1 μg starting DNA per sample library as determined by Nanodrop quantifications. Adapters in the ligation step were substituted with unique Multiplex Identifier (MID) tags (Roche 454) to barcode individual libraries. The expected fragment size distribution was verified for seven sample libraries on an Agilent 2100 Bioanalyzer using an RNA 6000 Pico Series II assay (UCLA Genotyping and Sequencing Core). The 45 single-stranded libraries were quantified with a Quant-iT RiboGreen RNA Assay kit (Invitrogen, Life Technologies) and pooled in equimolar ratios into a single library. Emulsion PCR and sequencing on a quarter lane of a 454 GS FLX Titanium instrument were implemented at Research and Testing Laboratories (Lubbock, TX).

Contig assembly and SNP identification—Contigs were assembled in two stages. The first step was to generate a candidate assembly. For one gene, elongation factor 1-α (Gene 59), a complete Sanger sequence was available as the reference (Gugger et al., 2013). For the remaining genes, FASTA files were extracted from the 454 SFF read files and sorted by MID tags and primer sequences. Candidate reads were identified using gene primer sequences, and a single long read was chosen in the forward and reverse direction to be used as an initial reference sequence for each gene.

For later analysis, we desired haplotype-phased contigs. We attempted an initial assembly with Roche's de novo backbone assembly software using the initial reference sequence above, but found it insufficient for determining haplotypes, so we pursued a mapping-based approach. The original 454 SFF reads were converted to fastq files and split by MID tag using PyroBayes (Quinlan et al., 2008) and custom scripts. These were aligned to the initial reference with ssaha2 (Ning et al., 2001), with the resulting alignments stored in bam format (Li et al., 2009). SNPs were called with the Genome Analysis Toolkit (GATK) (McKenna et al., 2010) and stored in vcf

format (www.1000genomes.org/node/101). We conducted the alignment and SNP results visually with the Integrated Genomics Viewer (igv) (Robinson et al., 2011).

This initial mapping identified a significant number of paralogs and pseudogenes. We observed that these fragments tended to differ significantly from the presumed primary gene, so we filtered out all reads with more than 5% mismatched bases from the initial reference, ignoring insertion/deletion (indel) mismatches.

The results from this round of alignment allowed us to refine our initial reference sequences derived from long reads, which were edited to better match our reads. A second round of alignment with ssha2 was run against these updated contigs, followed again by filtering reads with more than 3% mismatches and variant calling with GATK. Finally, GATK includes a command, ReadBackedPhasing, which we ran on our variants to produce the desired phased haplotypes.

To further visualize and annotate SNPs, we rechecked our reference amino acid sequences and reading frames by aligning our gene consensus sequences using BLASTX, and then visualized and annotated SNPs using these references in Mesquite and MacClade.

Diversity measures and linkage disequilibrium—Diversity statistics were calculated using the C++ software library libsequence (Thornton, 2002). These included the number of segregating sites (S), number of haplotypes (h), haplotype diversity (H_d), expected heterozygosity for each SNP (H_E), nucleotide diversity based on the number of pairwise differences (θ_π) and segregating sites (θ_W), and D (Tajima, 1989). We estimated levels of linkage disequilibrium (LD) using the squared allelic correlation coefficient (r^2) and the absolute value of D' ($|D'|$) among all pairs of SNPs using the rsq function in libsequence (Thornton, 2002). We used Fisher's exact tests to calculate P values under the null hypothesis that LD estimates at least as extreme as those observed could have arisen under linkage equilibrium. The decay of LD, as measured using r^2, with distance in base pairs between sites within candidate genes was evaluated with nonlinear regression using the Gauss–Newton algorithm as implemented in the nls function of the program R (Hill and Weir, 1988; Remington et al., 2001). Among-gene LD was used to investigate whether SNPs from different genes within the same functional category were correlated.

Population genetic differentiation—Hierarchical F statistics (Yang, 1998) were calculated for candidate gene SNPs using the hierfstat package version 0.04-14 (Goudet, 2005) in R. We ranked the SNPs according to F_{ST} values to identify which genes may be most likely to be under selection. Using previously published nuclear microsatellites (nSSRs) (Grivet et al., 2008; Sork et al., 2010), we calculated hierarchical F statistics for these same individuals at the same 13 localities to use as a benchmark for the genetic structure at putatively neutral loci. Recognizing that SSRs have higher mutation rates than SNPs, the comparison of genetic structure should be interpreted cautiously. Nonetheless, the SSR data give us a reference that is comparable with numerous population genetic studies conducted over that last 25 yr.

Single-locus SNP–climate environmental associations—We used five climatic variables as defined by Sork et al. (2010) measured for each locality (Table 1): mean annual precipitation (MAP), growing-degree days above 5°C (GDD5), maximum temperature (T_{max}), minimum temperature (T_{min}), and temperature seasonality (T_{seas}). Climate data were obtained by downscaling monthly 4 km PRISM climate data (Daly et al., 2008) for the period 1971–2000 with 90 m digital elevation grids using a modified gradient–inverse-distance square interpolation method (Flint and Flint, 2007). The downscaled data are preferable when looking at climate variation because local microclimate could influence local genetic composition dramatically. The reliability analysis of downscaling from the 4 km PRISM cells to 90 m for California regions showed no bias among geographic regions (Flint and Flint, 2007).

SNPs associated with climate were identified using an association mapping approach with climate as the phenotype (Eckert et al., 2010c). We employed the approach of Price et al. (2006), but without corrections for neutral population history given that our sample of genes was not a random sample of background genetic variation, but functional genes likely to be under some kind of selection. We modified the test statistic from the method of Price et al. (2006) from $R = (N - k - 1)r^2$ to $R = (N - 1)r^2$, where k is the number of principal components retained to describe neutral population structure, N is the sample size, and r^2 is the squared correlation coefficient for the correlation of the centered and standardized genotypes at a SNP with the centered and standardized values for a particular climate variable. All other aspects of the method remained the same, so that P values were obtained by assuming the modified R followed a χ^2 distribution with one degree of freedom. Multiple tests were accounted for using a simple Bonferroni correction, with the nominal α value set at 0.05 which then corresponds to a corrected significance threshold of $\alpha = 5.128 \times 10^{-05}$ [i.e., $\alpha = 0.05/(195 \text{ SNPs} \times 5 \text{ climate variables})$].

TABLE 1. Spatial and climate data for sampling localities for *Quercus lobata*. (See Materials and Methods for description of climate variables.)

Site	Latitude	Longitude	Elevation	GDD5	Climate	T_{min}	MAP	T_{seas}
4	36.099	−118.867	245	4351	355.7	16.3	405.3	46.3
5	36.486	−119.121	181	4528.3	363	23	358.7	47
11	37.979	−120.388	548	3525.7	341.7	2	786.7	42
42	39.910	−122.090	52	4198	346	21	573	43
52	34.942	−118.632	1455	2813	289	0	446	41
67	39.644	−123.531	609	2714	292	0	1777	26
71	38.488	−122.149	304	3834	333	23	690	37
80	37.063	−121.198	354	4082	324	30	350	42
88	36.236	−121.478	358	3508	341	0	460	27
90	35.669	−120.931	287	3782	338	10	410	26
91	34.734	−120.280	243	3665	267	36	427	8
98	37.325	−121.668	671	3968	292	58	601	36
101	38.267	−120.503	731	3463	326	13	945	42

Multivariate SNP–climate associations—To test the multivariate relationships between genetic variation with geography and climate, we conducted redundancy (RDA) and partial redundancy analyses (pRDAs) using the vegan library in R (Oksanen et al., 2015) for both the SNP and SSR data. For these analyses, SNP data were coded as counts of the minor allele for each tree (i.e., 0, 1 or 2 copies in each tree), which were then centered and standardized following Patterson et al. (2006). SSR data were coded in a similar fashion, which is standard practice for multivariate analyses on genotypic data (Smouse and Williams, 1982), where each allele at each locus was coded as if it were a biallelic locus (i.e., 0, 1, or 2 copies in each tree). In both cases, missing data were imputed as the mean value for each locus (i.e., 0). Given that one of our main objectives was to assess signals of spatially divergent selection apparent in the SNP data, we focused primarily on the pRDA models where the effects of climate were conditioned on the effects of geography. Geography in these models was assessed using latitude and longitude only because higher order terms (e.g., squares and cubes) and their cross products created too strong a collinearity between geographical and climatic variables. Statistical significance of RDA and pRDA models, as well as of each multivariate axis in each model, was assessed using a permutation-based analysis of variance (ANOVA) procedure with 9999 permutations. All hypothesis testing assumed $\alpha = 0.05$.

For comparison to the single-locus analyses, we correlated loadings of each SNP on the statistically significant pRDA axes from the model of climate conditional on geography to the $-\log_{10}$ of the *P* value derived from the method of Price et al. (2006). If both methods give similar results, a significant correlation (either positive or negative) between these two quantities should be observed. For these analyses, correlation was assessed using Pearson correlation coefficients and its statistical significance was tested using asymptotic, two-tailed *t* tests with $\alpha = 0.05$.

Variance partitioning of geographical and climatic effects—We further used results from the RDA and pRDA models to assess the relative contributions of climate and geography to multilocus genetic structure. We partitioned the explainable variance in both the SNP and SSR data using a standard variance partitioning approach as implemented in the varpart function in the vegan library of R. In this approach, the pure effects of climate and of geography are functions of the adjusted R^2 from the pRDA models of either climate conditioned on geography (pure climate effect) or geography conditioned on climate (pure geography effect). To obtain the shared (or joint) effect of climate and geography, the pure effects of climate and geography were subtracted from the adjusted R^2 of the RDA model with both climate and geography as predictors. Lastly, the relative contributions of each effect (pure effect of climate, pure effect of geography and their shared effect) were calculated as a fraction of the total explainable variance in the RDA model with both climate and geography as predictors (i.e., the adjusted R^2).

RESULTS

Diversity measures and linkage disequilibrium—The quarter lane run on a Roche 454 Titanium FLX Instrument yielded 121,979 reads totaling 25,675,647 bp. The mean read length was 210 bp and ranged from 12 to 573 bp. In some cases, we identified separate nonoverlapping contigs based on forward and backward reads for the same gene sequence; these contigs are labeled with an "f" or "r" following the gene label. After removing sizing on the extreme ends, this procedure resulted in 195 SNPs from 48 contigs of 51–736 bp (mean = 226 bp) from 40 candidate genes, which we grouped into the following four functional categories: osmotic stress, temperature stress, growth, and bud burst or flowering genes (Appendix S2, see online Supplemental Data). A summary of diversity measures (online Appendix S3) shows that Tajima's *D* was negative on average (−0.44), which could have resulted from population expansions and/or some form of linked selection. LD decayed rapidly within 100 bp (online Appendix S4), consistent with other highly outcrossing trees (e.g., Brown et al., 2004; Eckert et al., 2009). Inspection of a heatmap for r^2 and $|D'|$ indicated little intragenic and virtually no intergenic linkage disequilibrium (online Appendix S5), even though some intergenic estimates of r^2 were statistically significant ($P < 0.05$).

Population genetic differentiation—Using 38 genes that contained SNPs, we calculated hierarchical *F* statistics of all categories of SNPs, which yielded relatively low genetic structure, especially in comparison with estimates for neutral SSR loci (Table 2. The signature of vicariance between the western and eastern regional populations based on SSR loci ($F_{RT} = 0.054$) that has been reported elsewhere (Gugger et al., 2013) was not at all apparent for SNPs where average genetic differentiation was negligible ($F_{RT} = 0.008$). When we look at the type of SNP, we found that *F* statistic estimates were lowest for nonsynonymous SNPs ($F_{ST} = 0.007$), intermediate for synonymous SNPs ($F_{ST} = 0.025$), and highest for noncoding SNPs ($F_{ST} = 0.057$). All these values are much less than the average $F_{ST} = 0.12$ observed at nSSR loci.

The multilocus values of genetic differentiation differed across categories of functional genes, with osmotic stress and temperature stress SNPs having the lowest average values of F_{ST} and budburst/flowering genes having the highest average values. The 10 highest estimates (top 5%) of *F* statistics for individual SNPs ranged from $F_{ST} = 0.281$ to $F_{ST} = 0.628$ (Fig. 2). These values are 10 to 20 times larger than the multilocus estimate for the entire set of 195 SNPs. Within those top 10 SNPs, budburst/flowering genes contained six of the top 10 values, including two SNPs with $F_{ST} = 0.628$ that were found within genes Aux-Rep and Ras1 (see Appendix S2 for functions and Appendix S6 for full set of values). Temperature stress Genes 42 (heat shock protein 17.4) and 57 (elongation factor 1-α; see Appendix S2) had one SNP each within the top-10 values of F_{ST} (0.628 and 0.311, respectively, Appendix S6). Finally, we found two top-10 SNP estimates within growth genes 65 and 94 (see

TABLE 2. Summary of *F* statistics across all 195 SNPs (one general stress SNP is not included), for subsets of SNPs associated with functional gene categories, for SNP categories, and for putatively neutral nuclear SSR markers. (Number of genes is given in parentheses).

Category	F_{RT}	F_{SR}	F_{ST}
All SNPs	0.010	0.022	0.031
Bud burst/flowering (7)	−0.010	0.115	0.106
Growth (5)	0.004	0.035	0.039
Osmotic stress (16)	0.019	0.000	0.019
Temperature stress (10)	0.008	0.011	0.018
Noncoding	0.014	0.044	0.057
Nonsynonymous	0.009	0.016	0.007
Synonymous	0.006	0.030	0.025
nSSRs	0.054	0.094	0.122

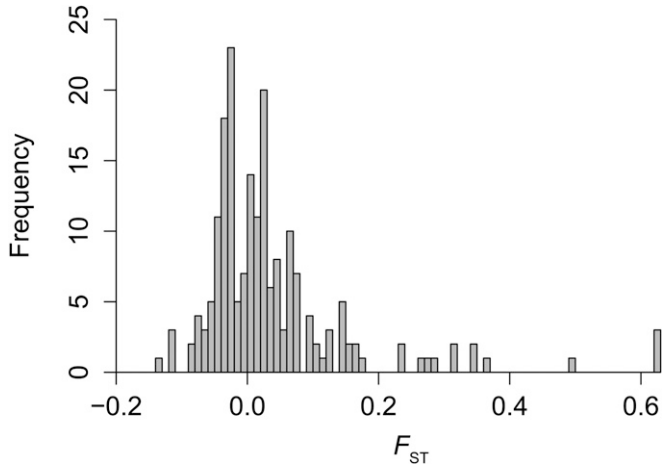

FIGURE 2 Frequency of F_{ST} values among 13 sampling localities for *Quercus lobata* for 195 single nucleotide polymorphisms (SNPs).

Appendix S6). Thus, the functional gene categories included different numbers of SNPs that identify genes that are strong candidates for differential selection across populations.

Single locus SNP-climate environmental associations—We conducted outlier analyses for the 195 SNPs examining the correlation in allele frequencies with five environmental variables (Fig. 3). Even though the threshold for significance of $\alpha = 0.05$ lowered the threshold to $P = 5.128 \times 10^{-05}$ to correct for multiple tests, we still observed several highly significant associations between candidate SNPs and mean annual precipitation (MAP; Fig. 3E): five SNPs from two budburst genes (Gene Aux and Gene Ras1) and three SNPs from two temperature stress genes (Genes 42, heat shock protein 17.4, and Gene 59, elongation factor 1-α; see Appendix S2). SNPs associated with osmotic stress genes did not have outliers across any of the climate variables (Fig. 3). The gradient of MAP was driven by a unique population in the northwest part of the distribution (Pop 67, Table 1), which greatly increased the range of precipitation values. The minimum and maximum temperature variables had the fewest number of outliers. Minimum and maximum temperature were the climate variables that had the fewest number of outliers. In sum, the four functional categories of genes differed in their associations across climate variables (Fig. 3).

Multivariate SNP–climate environmental associations—We used a pRDA model of climate conditioned on geography to assess the role of climate in structuring genetic diversity for SNPs. This model was highly, statistically significant ($F_{5, 36} = 1.3516$, $P = 0.0004$; Table 3A). This model explained 14.78% (R^2) of the overall variance, with pRDA axes 1 and 2 accounting for 34.81% and 19.12% of this effect. The percentage of overall variance (14.78%) was more than double the variance explained by the conditioning variables of latitude and longitude ($R^2 = 0.0649$). When adjusting the explained variance for the high dimensionality of the data, the R^2_{adj} for climate dropped to 0.0403. Axis 1 was highly statistically significant ($F_{1, 36} = 2.3525$, $P = 0.0001$), whereas Axis 2 was marginally significant ($F_{1, 36} = 1.2921$, $P = 0.0567$). Inspection of the loadings of environmental variables on pRDA Axis 1 revealed that MAP and GDD5 dominated it, whereas Axis 2 was dominated by T_{seas} (Table 3B).

The loadings of 195 SNPs on the first pRDA axis varied across the functional gene categories (Fig. 4). Most of the variation among categories, however, was in the tails of the distributions, with bud burst, osmotic stress, and temperature stress related gene categories skewed leftward (i.e., toward negative values). Given the negative loading of MAP onto pRDA Axis 1, these negative values represent a positive association of minor allele counts for single trees with MAP for these SNPs. This leftward skew was composed of three outliers for bud burst (GeneAuxF_79, GeneAuxF_245, GeneAuxF_264), two outliers for osmotic stress (Gene118R_47, Gene116F_47), and six outliers for the temperature stress categories (Gene42F_40, Gene59_125, Gene59_185, Gene59_329, Gene59_659, Gene59_701; Fig. 4). Outliers in this case were simply those SNPs that were greater than 1.5 times the interquartile range away from the median of the distribution of loadings for on the pRDA Axis 1 by functional gene category. Of these 11 outliers, five were significant after corrections for multiple tests in the single-locus analyses for environmental associations with MAP (Fig. 3E), and nine had P values < 0.005. The two SNPs that differed between methods were those in the leftward tail of the distribution of loadings for osmotic stress. These two SNPs, however, still had P values <0.05 for an association with MAP.

Comparison of loadings for individual SNPs on pRDA Axis 1 and the P values from the single-locus tests revealed a strong consistency in the detection of important SNPs (Fig. 5). The loadings on the pRDA Axis 1 were highly correlated with the $-\log_{10}$ of the P values from single-locus tests associating genetic variation for SNPs to MAP ($r = -0.8519$) and GDD5 ($r = -0.7136$). Both of these correlation coefficients were statistically significant ($P < 2.2e\text{-}16$). Thus, the signal of outliers was consistent across methods, with both methods identifying strongly overlapping sets of SNPs as putatively non-neutral.

Variance partitioning of geographical and climatic effects—Climate accounted for most of the explained genetic variation for SNPs (67.6%, Table 4), while geography alone explained only 12.8% (Table 4). The pRDA model of geography conditioned on climate variables, moreover, was not statistically significant for SNPs ($F_{2, 36} = 1.1546$, $P = 0.098$). To benchmark these patterns, we also partitioned the geographical and climatic effects using putatively neutral SSRs. In contrast to the SNPs, climate alone accounted for 32.1% of the explained variation and geography alone explained 40.4% (Table 4). The collinearity of climate and geography, moreover, was much lower in the SNP model than the SSR model (19.6% vs. 27.5%, Table 4). Thus, SNPs located within functional gene loci had much more of the partitioned variance explained by the pure effects of climate relative to the putatively neutral SSR loci.

DISCUSSION

Our analysis of 40 functional genes, chosen because of their potential relationship to plant responses to climate, reveals several patterns consistent with spatially divergent selection. First, we identified 10 SNPs from six genes (i.e., the top 5%) with values of F_{ST} that were 10- to 20-fold larger than the multilocus value, which makes them good candidates for diversifying selection (Fig. 2 and Table 5): bud burst genes Aux-repressed and Ras1; growth genes 65 and 94; temperature stress genes 42 (heat shock protein 17.4) and 57 (elongation factor 1α). Next, an environmental association analysis

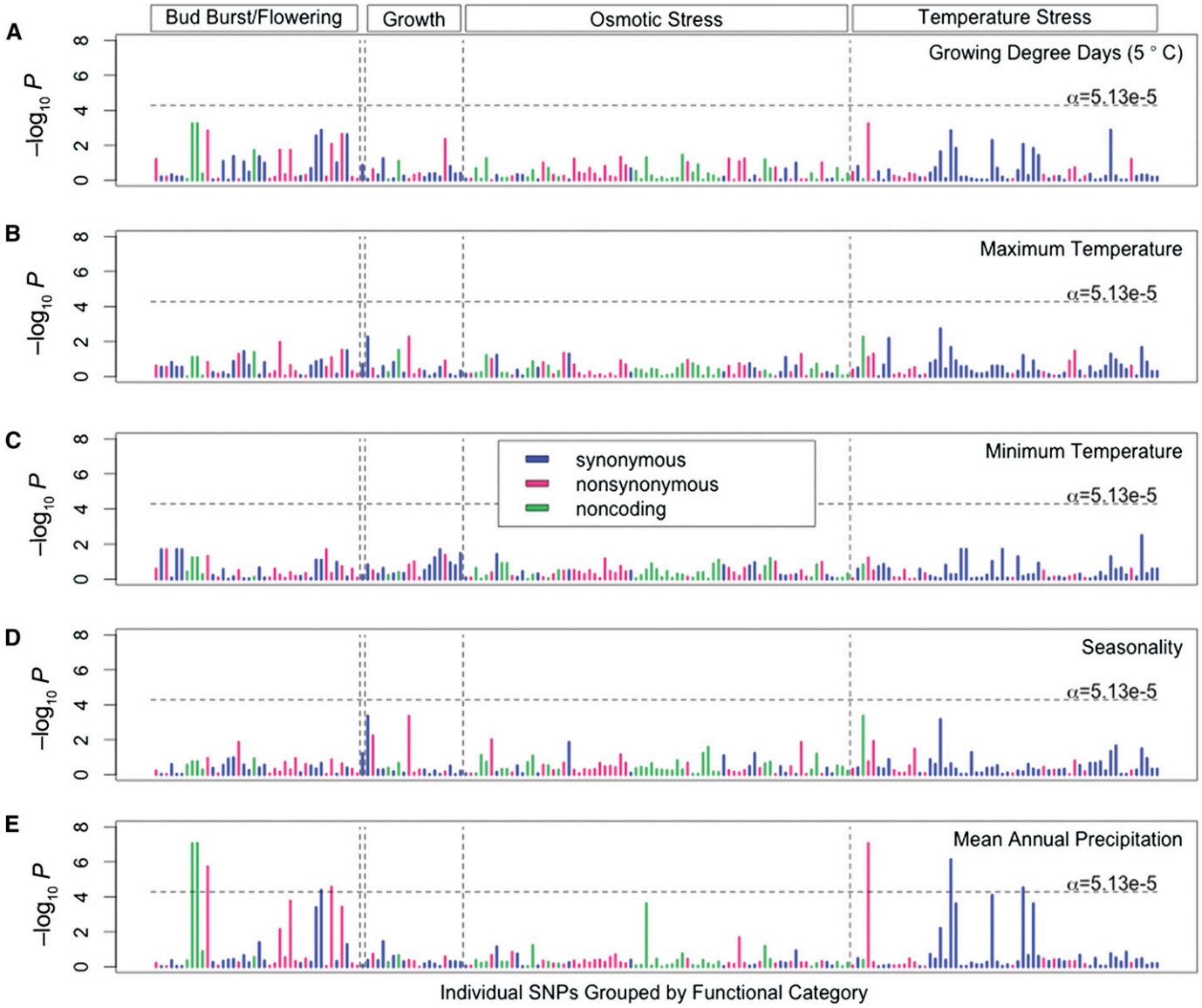

FIGURE 3 Manhattan plots showing the environmental association analysis based on *P* values ($-\log_{10}P$) of the correlation tests of SNP frequencies with climate gradients for five climate variables (A–E). Dashed horizontal lines give significance thresholds. The SNPs are organized within four functional gene categories (see online Appendix S2 for list of genes).

of the same 195 SNPs identified four of the six genes as containing SNPs significantly correlated with climate gradients. Six of eight outlier SNPs also had F_{ST} values ranked in the top 5% rank (Table 5) and a seventh SNP was ranked in the top 6%. Finally, our multivariate environmental association analysis using RDA found that the same three budburst/flowering genes and two temperature stress genes had SNPs with loading values on the first redundancy axis in the top 5%. This strong congruence between genetic differentiation and single and multivariate environmental associations in the same genes suggests that auxin-repressed gene, Ras1 gene, and heat shock protein 17.4 may be involved in local adaptation to climate. Here, we will explore in more detail the information revealed by this first search for evidence of climatically adaptive genetic variation, recognizing that our sample design calls for cautious interpretation.

Genetic differentiation among localities and across genes—Using 48 contigs derived from 40 candidate genes associated with response to climate, we found 195 SNPs from populations sampled throughout the species range. The patterns of population differentiation we observed for these SNPs differed markedly from those found for putatively neutral SSR markers. For SSR loci, we found significant population differentiation across the 13 localities between eastern and western regions (F_{RT} = 0.05) and among subpopulations across the entire range (F_{ST} = 0.12), which are similar to our other studies with much larger samples and numbers of localities (Grivet et al., 2008; Sork et al., 2010; Gugger et al., 2013). In general, *Q. lobata* has more genetic structure than the European white oak, *Q. petraea*, based on the reported mean nSSR estimate of F_{ST} = 0.013 for nine populations sampled by Derory et al. (2010). The high genetic structure in valley oak may have been shaped by

its long evolutionary history in a region of high topographic heterogeneity of California that could have influenced both migration and selection. The finding that SNPs from different functional genes differ from each other and from SSRs in their patterns of differentiation would imply that neutral processes alone cannot account for population differentiation. This finding persists even if we adjust for heterozygosity differences. For example, SNPs, on average, exhibited essentially no differentiation between populations on both sides of the Central Valley of California unlike the SSRs. Thus, the geographic genetic footprint of historical migration and population bottlenecks over the evolutionary history of these populations has not affected the geographic distribution of SNP allele frequencies in a similar manner to that of SSRs.

Our data provide two types of initial evidence for spatially divergent selection. First, we found an unusually large number (42%) of the 195 SNPs with essentially zero differentiation across localities. These SNPs may be located in genes under strong purifying selection for a single global optimum. This interpretation coincides with the negative overall average of Tajima's *D*. The second and more relevant type is spatially divergent selection due to variable climate conditions. Six genes contained SNPs with high differentiation across sites that thus merit further scrutiny as candidate genes under spatially divergent selection. Two genes were associated with budburst (Aux-Rep and Ras1, aka ASI), which were also investigated in a study of nine European oak populations grown in a common garden with different budburst timing (Derory et al., 2010). Aux-rep is an auxin-repressed protein, which Derory et al. (2006) reported was downregulated from quiescent to developing buds. In this same study, Ras1 was highly expressed in buds at the quiescent stage, which indicates that hydrolysis of storage starch is repressed in the quiescent bud. In contrast to our findings, Derory et al. (2010) reported that SNPs in these two genes show evidence of purifying selection toward a global optimum. In contrast, Alberto et al. (2013) found the ASI gene to be a significant F_{ST} outlier in a set of populations along an altitudinal gradient. They did not report any significant outlier SNPs in Aux-Rep. Thus, this comparison of the two studies with ours leads to a preliminary conclusion that the same genes may not be involved in local adaptation across species.

Overall, the magnitude of genetic differentiation of SNPs for *Q. lobata* was comparable to the estimates across bud burst genes in *Q. petraea* (Alberto et al., 2013). We observed the overall average value of F_{ST} across the 195 SNPs to be 0.03, while they report a mean F_{ST} = 0.02 for 161 SNPs across 21 populations from a latitudinal gradient. The two studies differ, however, in the magnitude of the differentiation for outliers. In our study, the top 5% of SNPs had values ranging from 0.281 to 0.628. In *Q. petraea*, using Bayescan, they identified 19 SNPs as significant outliers with estimates of differentiation ranging from 0.019 to 0.085. Thus, it appears that valley oak in California has much greater genetic differentiation at specific loci than found in the European white oak. We do not know whether the difference is due to greater climatic differences among localities that would allow the evolution of greater local adaptation, the greater ages of divergence among California subpopulations, which are more than 150,000 yr old (Gugger et al., 2013) vs. the younger European oak populations that have expanded in the last ~20,000 yr since the last glacial maximum (Petit et al., 2002). It could also be differences in methodology, but our neutral genetic structure is also greater than that found in Europe with the same markers.

We found genes with high estimates of genetic differentiation in other types of functional genes than bud burst. SNPs in two temperature stress genes 42 and 57 showed high differentiation across populations (F_{ST} = 0.629, 0.311, respectively), as did SNPs within two growth genes 65 and 94 (F_{ST} = 0.365, 0.351, respectively). Gene 42 is heat shock protein 17.4, which is induced by heat shock in *Arabidopsis thaliana* and *Oryza sativa* (Waters, 1995). Gene 57 codes for elongation factor 1-α, which is involved in protein synthesis and described as related to temperature stress found in the Fagaceae Genomics website in 2014 (www.fagaceae.org). In sum, our focus on candidate genes revealed many SNPs that appear to be under strong purifying selection toward a global optimum and several others that are good candidates for comprising some of the basis for adaption of *Q. lobata* populations.

Evidence for selection by climate variables—Our environmental association analysis provides another source of evidence that these genes may have experienced spatially divergent selection related to climate (Fig. 3). After controlling for multiple tests, the only climate variable that was significantly correlated with outlier SNPs was MAP, which was the climate variable with the greatest range of values, including an upper values represented by a single locality. Our environmental association results can be compared with those of Alberto et al. (2013) who tested for correlations of climate with allele frequencies at SNPs across a set of bud burst/flowering genes for *Q. petraea* populations. They reported seven genes of 105 as containing SNPs with significant correlations to climate variables. One of those significant SNPs was correlated with climate in multiple transects, which they proposed is an important candidate gene affecting local adaption for bud burst. Unlike our study, they found that temperature variables were more likely to be significant than precipitation variables, which may reflect a greater influence of temperature than precipitation on bud burst and growth in their

TABLE 3. Summary of redundancy analysis (RDA) of 195 SNPs with climate and geographic variables. The overall RDA was highly significant ($F_{5, 36}$ = 1.3516, P = 0.0004). (A) Summary statistics for five of eight partial redundancy axes. (B) Loadings of climate with top two redundancy axes, holding geography constant. (C) Loadings for each SNP with first partial RDA (pRDA) axis. PVE = percentage constrained variance explained.

(A) Summary of pRDA statistics.

pRDA axis	Eigenvalue	PVE Unconstrained	PVE Constrained %	*F*	Df num, den	*P*
Axis 1	19.2659	0.0550	34.8	2.3525	1, 36	0.0001
Axis 2	10.5812	0.0302	19.1	1.2921	1, 36	0.0567
Axis 3	9.8394	0.0281	17.8	1.2015	1, 36	0.1395
Axis 4	8.7357	0.0249	15.8	1.0667	1, 36	0.3712
Axis 5	6.9229	0.0198	12.5	0.8454	1, 36	0.8837

(B) Loadings of climate variables for first two axes, holding geography constant.

Climate variable	RDA1	RDA2
GDD_5	0.641	−0.177
T_{max}	0.341	−0.206
T_{min}	0.476	0.248
T_{seas}	0.174	0.403
MAP	−0.642	0.016
P value	0.001	0.057
PVE	34.8	19.1

continued

TABLE 3, *continued*

(C) Summary of loadings of 194 SNPs with two first redundancy axes, holding geography constant. The SNPs are grouped by functional category.

Bud burst and flowering			Growth			Osmotic stress			Temperature stress genes		
SNP	**RDA-1**	**RDA-2**	**SNP**	**RDA-1**	**RDA-2**	**SNP**	**RDA-1**	**RDA-2**	**SNP**	**RDA-1**	**RDA-2**
76R_265	−0.125	0.205	51F_21	−0.036	−0.292	100R_177	−0.060	0.109	10R_90	0.156	−0.158
79F_145	0.120	0.244	51F_104	−0.190	−0.259	100R_191	0.091	−0.13	10R_193	0.213	0.160
79F_167	0.120	0.244	51R_10	0.020	0.135	100R_220	0.130	−0.03	19F_27	−0.036	−0.292
79F_238	−0.030	0.060	51R_46	−0.302	−0.039	100R_33	0.110	0.018	42F_40	−0.767	−0.010
79F_259	0.120	0.244	64F_47	0.040	−0.052	100R_5	0.110	0.018	56R_23	−0.025	−0.201
79F_316	0.120	0.244	65R_101	0.017	0.046	106F_24	0.021	−0.158	56R_65	−0.006	−0.022
AuxF_126	−0.059	−0.011	65R_189	−0.096	−0.082	106F_29	−0.080	0.282	56R_80	−0.005	−0.049
AuxF_245	−0.672	−0.033	70F_35	0.015	−0.029	106R_49	0.179	0.204	56R_83	0.102	−0.129
AuxF_264	0.040	−0.052	70F_42	−0.036	−0.292	106R_61	0.179	0.204	56R_297	0.064	−0.064
AuxF_59	0.133	0.045	70F_279	−0.093	−0.039	108F_32	0.048	0.181	56R_300	0.075	0.118
AuxF_79	−0.767	−0.010	70F_282	0.190	−0.100	108F_46	0.050	0.037	56R_312	0.147	0.090
AuxF_82	−0.767	−0.010	70F_398	0.006	−0.060	108F_52	−0.089	−0.036	56R_345	−0.032	−0.029
AuxR_64	0.110	0.018	94F_41	0.148	0.175	109F_53	−0.014	−0.189	56R_375	0.011	−0.117
Dag2R_76	−0.081	0.122	94F_93	0.146	0.232	111F_103	0.065	0.085	56R_386	−0.165	−0.064
GalaF_30	0.016	0.170	94F_161	0.120	0.244	112R_180	−0.083	−0.036	56R_387	−0.184	−0.035
GalaF_35	0.125	−0.114	94F_212	−0.170	0.287	112R_51	0.107	−0.105	57F_67	0.165	0.232
GalaF_60	−0.025	−0.206	94F_236	0.083	0.180	112R_56	0.020	−0.041	57F_97	0.096	0.084
GalaF_71	0.057	0.010	94R_15	0.148	0.175	113F_40	0.093	0.109	57F_211	0.171	0.090
GalaF_92	−0.035	−0.020	94R_39	0.100	0.269	114R_41	0.006	−0.06	59_101	0.043	0.296
H3R_44	0.025	−0.067	BadhR_78	−0.038	0.121	116F_187	0.221	0.05	59_125	−0.514	0.125
Ras1F_129	−0.386	0.070	Dhn3F_27	−0.087	−0.042	116F_212	0.045	0.077	59_185	−0.379	0.134
Ras1F_130	−0.228	−0.155	Dhn3F_29	0.009	0.097	116F_228	0.154	−0.159	59_236	0.116	0.246
Ras1F_138	−0.025	−0.004	Dhn3R_46	−0.036	0.147	116F_230	0.047	−0.114	59_257	0.116	0.246
Ras1F_157	0.097	−0.031	100R_177	−0.060	0.109	116F_233	−0.284	0.042	59_272	0.165	0.195
Ras1F_191	−0.087	−0.042	100R_191	0.091	−0.13	116F_242	0.105	−0.058	59_294	0.026	0.057
Ras1F_227	−0.543	0.099	100R_220	0.130	−0.03	116F_244	−0.057	0	59_299	0.026	0.057
Ras1F_251	−0.541	0.060	100R_33	0.110	0.018	116F_261	0.026	−0.043	59_317	0.037	−0.047
Ras1F_256	0.120	0.244	100R_5	0.110	0.018	116F_27	0.125	−0.134	59_329	−0.455	0.041
Ras1F_259	−0.444	0.081	106F_24	0.021	−0.158	116F_278	0.090	0.121	59_350	−0.089	−0.037
Ras1F_26	−0.094	0.136	106F_29	−0.080	0.282	116F_286	0.032	−0.128	59_389	0.116	0.246
Ras1F_266	−0.038	0.121	106R_49	0.179	0.204	116F_47	−0.023	−0.206	59_407	0.026	0.057
Ras1F_282	−0.564	0.051	106R_61	0.179	0.204	116F_61	0.149	−0.041	59_520	0.106	0.023
Ras1F_290	−0.041	−0.190				116F_63	0.059	0.013	59_590	0.106	0.014
Ras1F_304	0.071	−0.035				116F_76	0.021	0.127	59_659	−0.422	0.164
Ras1F_306	0.110	0.018				116R_30	0.001	−0.086	59_689	−0.027	0.253
Ras1F_65	0.018	−0.047				116R_7	0.062	0.05	59_701	−0.379	0.134
Ras1F_69	0.110	0.018				117F_104	0.023	−0.111	5R_76	−0.134	0.026
Ras1F_70	0.071	−0.035				117F_123	−0.041	0.166	5R_153	−0.001	−0.042
Ras1F_87	−0.292	0.313				117F_24	0.018	−0.046	5R_190	0.016	0.072
Ras1F_97	−0.042	−0.175				118R_127	0.229	0.132	5R_201	−0.007	0.002
						118R_136	0.001	−0.251	5R_235	−0.007	0.002
						118R_204	0.007	−0.192	5R_262	0.052	0.035
						118R_232	−0.114	0.189	73F_51	−0.026	0.258
						118R_245	0.136	0.133	73F_196	0.227	−0.156
						118R_277	−0.302	0.095	73F_201	0.149	0.022
						118R_290	−0.114	0.189	73F_203	−0.074	−0.003
						118R_53	0.051	0.081	73F_264	0.007	−0.033
						34F_145	0.038	−0.053	73F_297	0.155	0.006
						34F_60	0.055	−0.014	73R_64	0.042	−0.018
						34F_78	0.156	−0.085	85F_68	0.029	−0.038
						34R_197	−0.043	0.043	85F_158	0.209	0.072
						34R_199	0.041	−0.057	85F_179	−0.080	−0.265
						34R_292	0.018	−0.09	85F_188	0.021	−0.110
						34R_321	0.041	−0.057	85R_205	−0.065	−0.029
						34R_328	0.073	0.027	91F_7	−0.178	0.292
						34R_35	−0.137	−0.138	91F_50	−0.007	0.002
						34R_39	−0.105	−0.165	91F_53	0.146	−0.006
						61F_98	−0.036	−0.292	91F_86	0.035	−0.132
						62R_293	0.135	−0.073	91F_110	0.071	−0.035
						62R_328	0.190	−0.1	91F_167	0.071	−0.035
						62R_342	0.039	0.001			
						BadhR_104	0.040	−0.052			
						BadhR_110	0.110	0.018			

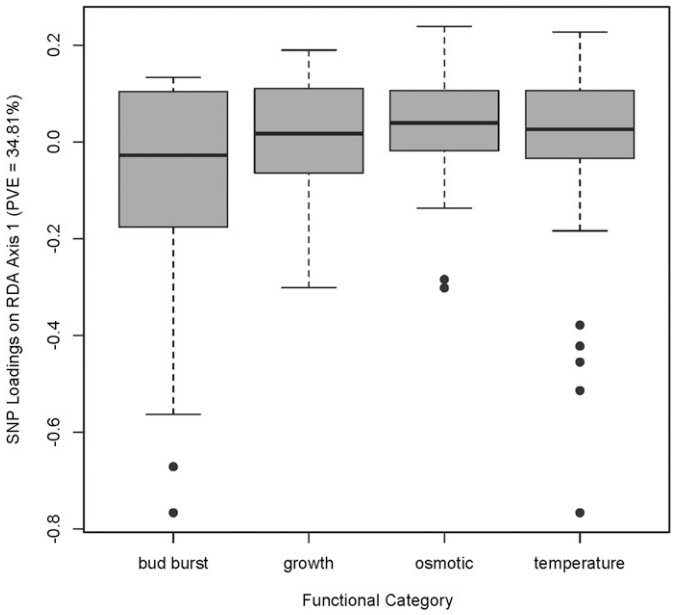

FIGURE 4 Box plot of the loadings of individual SNPs, by functional gene category, with the first partial redundancy analysis (pRDA) axis. Outliers fall outside the 95% distribution.

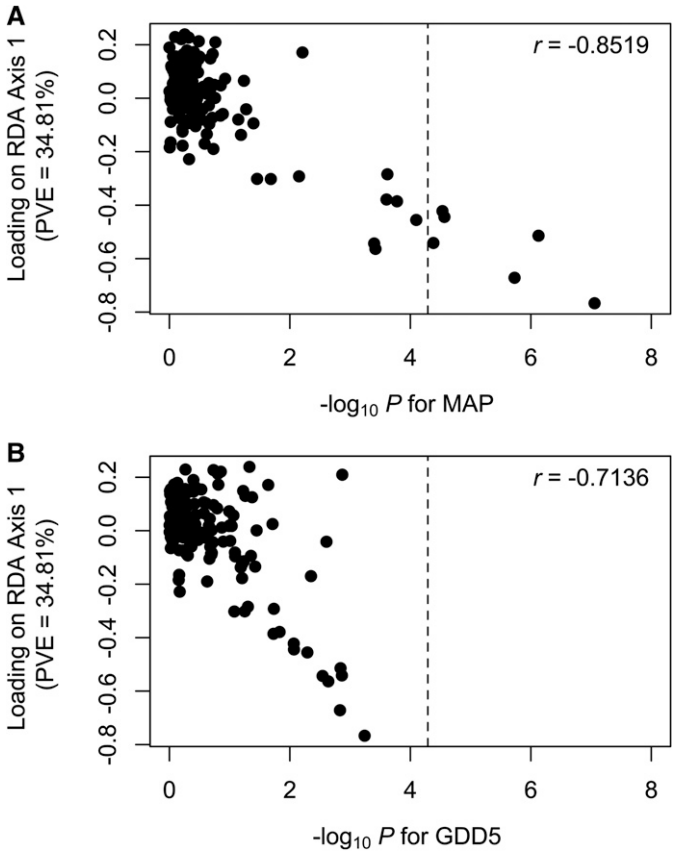

FIGURE 5 The relationship between loadings on the first pRDA axis and $-\log_{10} P$ values for (A) mean annual precipitation (MAP) and (B) growing degree-days above 5°C (GDD5). The vertical dashed line in each plot represents the multiple test corrected significance level. PVE = percentage constrained variance explained.

study region. Of the genes that overlap between the two studies, no loci that were significant for their study were significant in ours, and vice versa.

Our study also used a multivariate approach because polygenetic traits are unlikely to have strong single-locus outliers, and this method allows us to test the environmental association with genetic variation for all SNPs simultaneously (Le Corre and Kremer, 2003, 2012; Berg and Coop, 2014). We found significant correlations between genotype and climate variables for the RDA based on the 195 SNPs, conditioned on geography (Table 3). As we pointed out already, we found high congruence between SNPs with high loadings and those with high F_{ST} values and significantly high climate associations (see Fig. 5). It is reassuring that the same SNPs indicate similar levels of associations between single-locus and multivariate methods. However, the fact that several SNPs had loadings above 0.40 when associated with GDD5 were not significant outliers in the single-locus analysis raises the question of how many SNPs go undetected because the adjusted *P* values are very low after multiple tests (Fig. 5B).

The multivariate analysis points to other potential cases of false negatives. For example, in the osmotic stress induced genes, we found that no SNPs were significant outliers in the single-locus test nor were there any top 5% loadings with the first pRDA. However, when we examined the distribution of SNP loadings within the osmotic stress induced genes (Fig. 4), two genes had one SNP each that were outside the 95% distribution: Genes 116 and 118. Moreover, in each of these genes, we found two SNPs with loadings on RDA-1 axis that were in the top 10% rankings of the 195 SNPs. Both genes were experimentally identified as osmotic stress genes in oaks (Porth et al., 2005b). Gene 116 codes for glutathione *S*-transferase (GST), which Porth et al. (2005b) indicated has a role in environmental adaptation. Alberto et al. (2013) found that this same gene was significantly associated with bud phenological traits along an environmental gradient. The other osmotic stress induced gene was an oxylase-like protein in the oxylase/oxygenase gene family (Porth et al., 2005a) and had one noncoding SNP and one nonsynonymous SNP with high loadings. Future studies of valley oak should pay attention to the osmotic stress genes as candidates for climate-associated candidate genes.

Geography vs. climate—The redundancy analysis results in Table 3 indicated that all five climate variables contributed to the RDA axes 1 and 2, with precipitation (MAP) and growing degree-days above 5°C (GDD5) loading most and temperature seasonality loading least. This analysis also partitioned the variation in our genetic data sets according to the percentage of explained variance, PVE, due to climate alone, geography alone, or both (Table 4). For the SNPs, 67% of the explained variance was due to climate alone, which may reflect the fact that the genes were selected as potentially involved in response to climate. Geography alone accounted for only 13% of explained variance, when conditioned on climate. In contrast, the analysis of putatively neutral SSRs yield 32% due to climate alone and 40% due to geography alone. Recognizing that SNPs and SSRs have different mutation rates, the comparison of the variance partitioning provides a useful illustration of the differences between the climate association of genes that include adaptive variation and those that include mostly neutral variation. Our patterns contrast with a similar RDA analysis of lodgepole pine (*Pinus contorta*)

TABLE 4. Partitioning the variance associated with climate variables alone, latitude and longitude spatial location alone, and share variance between climate and geography, based on a redundancy analysis (RDA) for 195 SNPs and 9 SSRs for 13 localities. Adjusted R^2, its significance level, and percentage of explained variance (PVE) are reported.

	SNPs			SSRs		
Effect	**Adjusted R^2 (%)**	***P***	**PVE (%)**	**Adjusted R^2 (%)**	***P***	**PVE (%)**
Climate	4.0	0.0001	67.6	4.4	0.0011	32.1
Climate + shared	5.2	0.0001	87.2	8.1	0.0001	59.6
Geography	0.8	0.1022	12.8	5.5	0.0001	40.4
Geography + shared	1.9	0.0004	32.4	9.2	0.0001	67.9
Shared	1.2	—	19.6	3.7	—	27.5
Residual	94.0	—	—	86.4	—	—

based on 17 localities, 368 trees, and 438 SNPs for that species (Cullingham et al., 2014). In this study, their model explained a higher percentage variation than ours (27.3% in lodgepole pine vs. 6% in valley oak), but it also had a much lower percentage of the PVE explained by climate than we found in valley oak (15% due to environment alone in lodgepole pine vs. 67% in valley oak). Consequently, the lodgepole pine and our valley oak studies had about 4% adjusted R^2 due to the environment conditioned on geography. Sample design, choice of markers, and environmental cline apparently will influence any assessment of geography vs. climate.

Limitations and future directions—This study conveys promising findings of the ability to detect the geographic patterns of putatively adaptive genetic variation using selected candidate genes in natural populations of *Q. lobata*. This study is limited because we only used SNPs from functional genes, which included many with evidence of purifying selection that confounds genome-wide comparison for outliers (Maruki et al., 2012). Future studies should include random loci that estimate the genome-wide genetic structure shaped by demographic history to detect outlier SNPs and candidate genes against an appropriate background (Sork et al., 2013). To gain more power to identify the genes that are potentially under selection and the climate gradients that are important agents of selection, it will be necessary to include a larger number of sample localities and a greater range of climate gradients. The increase in sample size will not only yield better geographic maps of adaptive genetic variation, but it will also improve the statistical power of the tests.

This study was designed to test whether specific genes associated with response to climate in other studies show evidence of spatially divergent selection and environmental association. Other methods are available that will allow the identification of additional genes. For example, methods that sample a larger proportion of the genome, such as restriction-site-associated DNA sequencing (e.g., RADseq, Baird et al., 2008) or genotyping by sequencing (GBS, Elshire et al., 2011), are feasible for nonmodel systems (Sork et al., 2013). Studies are underway to use this approach (P. F. Gugger and V. L. Sork, unpublished data). However, these methods sample only a small percentage of the genome and are not effective at finding genes underlying specific phenotypes (V. L. Sork, personal observation). Moreover, they also generate more SNPs, if the study has at least a rough draft genome (V. L. Sork, personal observation). So a hybrid approach, which uses targeted sequencing of candidate genes and random genome-wide subsampling, will provide a robust way to find SNPs and genes that are selection by climate. As sequencing costs decline, whole-transcriptome or whole-genome sequencing will improve the detection of adaptive variation underlying local adaptation. Eventually, to understand whether genes showing spatially divergent selection enhance local adaptation in plant populations, we will need to associate that variation with phenotypes in common gardens (such studies are underway, J. W. Wright and V. L. Sork, unpublished data) and to tie those phenotypes with fitness.

Future studies should include the use of single-locus and multilocus analyses to detect climate-associated SNPs. We found that these two methods agreed with respect to detection of outliers, which lends credence to the use of multilocus methods before, or even in place of, applications of single-locus tests (Sork et al., 2013; Rellstab et al., 2015). This two-stage approach, which mirrors the practicality of standard hypothesis testing in statistics (e.g., ANOVA followed by post hoc testing) should aid in decreasing the number of unnecessary statistical tests, which is a major source in the loss of power in correlative studies. This effect within the context of landscape genomics, however, needs to be quantified with simulations. The use of multilocus methods, moreover, can better highlight the high dimensionality of data sets aimed at the detection of spatially divergent selection. For example, the single pRDA

TABLE 5. Comparison of top contigs from specific genes and SNPs (after hyphen) with evidence from at least one statistical analysis indicating spatially divergent selection. SNPs within gene contigs are ranked by F_{ST} value and then by multivariate correlation from first axis of canonical correlation model. (See Materials and Methods for description of F_{ST}, single-locus environmental association analyses, and RDA. See Appendix S2 for genes.)

Gene contig-SNP	**Polymorphism type** [a]	F_{ST} [b]	**Single-locus *P*** [c]	**Loading with RDA-1** [d]
Budburst and flowering (2 genes)				
AuxF-79	NC	0.628	8.74375E-08	–0.767
AuxF-82	NC	0.628	8.74375E-08	–0.767
AuxF-245	NS	0.492	1.86239E-06	–0.672
Ras1F-227	SY	0.315	3.3991E-07	–0.543
Ras1F-251	SY	0.346	4.14601E-05	–0.541
Ras1F-259	NS	*0.278		–0.444
Ras1F-282	NS	0.281		–0.564
Growth (2 genes)				
65R-189	NC	0.365		
94F-212	NS	0.350		
Temperature stress (3 genes)				
Gene42F-40	NS	0.628	8.74375E-08	–0.767
Gene57F-97	SY	0.311		
Gene59-125	SY	*0.236	7.45124E-07	–0.514
Gene59-329	SY		4.09813E-07	–0.455

[a] Synonymous (SY), nonsynonymous (NS) mutation, or within noncoding (NC) regions.
[b] $F_{ST} > 0.281$ represents the top 5% of the 195 SNPs.
[c] $P < 5.128 \times 10^{-5}$ represent $\alpha < 0.05$, after correction for multiple tests.
[d] Top 5% of SNPSs in RDA-1.
* Within top 10% of ranked values.

model used here captured all of the salient features of the 975 single-locus tests without the loss of power due to multiple testing, as well as additional information about the correlation structure of the climate data.

CONCLUSIONS

Evidence of spatially divergent selection in climate-associated functional genes provides an efficient first step for studying the extent of local adaptation to climate in natural tree populations. We discovered that the majority of SNPs and genes showed evidence of directional selection toward zero differentiation, but we also identified three genes that are both highly differentiated among populations and were correlated with climate gradients in both single-locus, single-climate-variable models and multivariate models. The demonstration of which genes and which phenotypes are responsible for the ability of tree populations to respond to climate change will require much more investigation, but these findings reveal that geographic analysis of adaptive genetic variation in natural populations will provide valuable initial evidence.

ACKNOWLEDGEMENTS

V.L.S. thanks David Neale and Stan Nelson for inspiration and ideas. The authors thank K. Gaddis and P. Thompson for comments on this manuscript. K.S. was supported by research funding to S. Nelson, Center for High Throughput Biology, UCLA. V.L.S. received UCLA research seed funds for molecular laboratory work, sequencing, and salaries for P.F.G. and S.E.S.

LITERATURE CITED

Adams, H. D., M. Guardiola-Claramonte, G. A. Barron-Gafford, J. C. Villegas, D. D. Breshears, C. B. Zou, P. A. Troch, and T. E. Huxman. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences, USA* 106: 7063–7066.

Aitken, S. N., and M. C. Whitlock. 2013. Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution and Systematics* 44: 367–388.

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

Alberto, F., L. Bouffier, J. M. Louvet, J. B. Lamy, S. Delzon, and A. Kremer. 2011. Adaptive responses for seed and leaf phenology in natural populations of sessile oak along an altitudinal gradient. *Journal of Evolutionary Biology* 24: 1442–1454.

Alberto, F. J., J. Derory, C. Boury, J. M. Frigerio, N. E. Zimmermann, and A. Kremer. 2013. Imprints of natural selection along environmental gradients in phenology-related genes of *Quercus petraea*. *Genetics* 195: 495–512.

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. A. Lewis, E. U. Selker, et al. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One* 3: e3376.

Bashalkhanov, S., A. J. Eckert, and O. P. Rajora. 2013. Genetic signatures of natural selection in response to air pollution in red spruce (*Picea rubens*, Pinaceae). *Molecular Ecology* 22: 5877–5889.

Berg, J. J., and G. Coop. 2014. A population genetic signal of polygenic adaptation. *PLOS Genetics* 10: E1004412.

Blois, J. L., P. L. Zarnetske, M. C. Fitzpatrick, and S. Finnegan. 2013. Climate change and the past, present, and future of biotic interactions. *Science* 341: 499–504.

Breda, N., R. Huc, A. Granier, and E. Dreyer. 2006. Temperate forest trees and stands under severe drought: A review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* 63: 625–644.

Brown, G. R., G. P. Gill, R. J. Kuntz, C. H. Langley, and D. B. Neale. 2004. Nucleotide diversity and linkage disequilibrium in loblolly pine. *Proceedings of the National Academy of Sciences, USA* 101: 15255–15260.

Casasoli, M., J. Derory, C. Morera-Dutrey, O. Brendel, I. Porth, J. M. Guehl, F. Villani, and A. Kremer. 2006. Comparison of quantitative trait loci for adaptive traits between oak and chestnut based on an expressed sequence tag consensus map. *Genetics* 172: 533–546.

Chmura, D. J., P. D. Anderson, G. T. Howe, C. A. Harrington, J. E. Halofsky, D. L. Peterson, D. C. Shaw, and J. B. St Clair. 2011. Forest responses to climate change in the northwestern United States: Ecophysiological foundations for adaptive management. *Forest Ecology and Management* 261: 1121–1142.

Chuine, I., and P. Cour. 1999. Climatic determinants of budburst seasonality in four temperate-zone tree species. *The New Phytologist* 143: 339–349.

Cleland, E. E., I. Chuine, A. Menzel, H. A. Mooney, and M. D. Schwartz. 2007. Shifting plant phenology in response to global change. *Trends in Ecology & Evolution* 22: 357–365.

Coop, G., D. Witonsky, A. Di Rienzo, and J. K. Pritchard. 2010. Using environmental correlations to identify loci underlying local adaptation. *Genetics* 185: 1411–1423.

Craft, K. J., M. V. Ashley, and W. D. Koenig. 2002. Limited hybridization between *Quercus lobata* and *Quercus douglasii* (Fagaceae) in a mixed stand in central coastal California. *American Journal of Botany* 89: 1792–1798.

Cullingham, C. I., J. E. K. Cooke, and D. W. Coltman. 2014. Cross-species outlier detection reveals different evolutionary pressures between sister species. *New Phytologist* 204: 215–229.

Daly, C., M. Halbleib, J. I. Smith, W. P. Gibson, M. K. Doggett, G. H. Taylor, J. Curtis, and P. P. Pasteris. 2008. Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States. *International Journal of Climatology* 28: 2031–2064.

Derory, J., P. Leger, V. Garcia, J. Schaeffer, M. T. Hauser, F. Salin, C. Luschnig, et al. 2006. Transcriptome analysis of bud burst in sessile oak (*Quercus petraea*). *New Phytologist* 170: 723–738.

Derory, J., C. Scotti-Saintagne, E. Bertocchi, L. Le Dantec, N. Graignic, A. Jauffres, M. Casasoli, et al. 2010. Contrasting relationships between the diversity of candidate genes and variation of bud burst in natural and segregating populations of European oaks. *Heredity* 104: 438–448.

Eckert, A., A. Bower, S. González-Martínez, J. Wegrzyn, G. Coop, and D. Neale. 2010a. Back to nature: Ecological genomics of loblolly pine (*Pinus taeda*, Pinaceae). *Molecular Ecology* 19: 3789–3805.

Eckert, A. J., J. van Heerwaarden, and J. L. Wegrzyn. 2010b. Patterns of population structure and environmental associations to aridity across the range of loblolly pine (*Pinus taeda* L., Pinaceae). *Genetics* 185: 969–982.

Eckert, A. J., J. D. Liechty, B. R. Tearse, B. Pande, and D. B. Neale. 2010c. DnaSAM: Software to perform neutrality testing for large datasets with complex null models. *Molecular Ecology Resources* 10: 542–545.

Eckert, A. J., J. L. Wegrzyn, B. Pande, K. D. Jermstad, J. M. Lee, J. D. Liechty, B. R. Tearse, et al. 2009. Multilocus patterns of nucleotide diversity and divergence reveal positive selection at candidate genes related to cold hardiness in coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*). *Genetics* 183: 289–298.

Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler, and S. E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One* 6: e19379.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, New Jersey, USA.

Flint, A. L., and L. E. Flint. 2007. Application of the basin characterization model to estimate in-place recharge and runoff potential in the Basin and Range carbonate-rock aquifer system, White Pine County, Nevada, and adjacent areas in Nevada and Utah. Scientific Investigations Report 2007-5099, U. S. Geological Survey, Department of the Interior, Washington, D.C., USA. Available at http://pubs.water.usgs.gov/sir20075099.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences, USA* 104: 1278–1282.

Gailing, O., B. Vornam, L. Leinemann, and R. Finkeldey. 2009. Genetic and genomic approaches to assess adaptive genetic variation in plants: Forest trees as a model. *Physiologia Plantarum* 137: 509–519.

González-Martínez, S. C., E. Ersoz, G. R. Brown, N. C. Wheeler, and D. B. Neale. 2006a. DNA sequence variation and selection of tag single-nucleotide polymorphisms at candidate genes for drought-stress response in *Pinus taeda* L. *Genetics* 172: 1915–1926.

González-Martínez, S. C., K. V. Krutovsky, and D. B. Neale. 2006b. Forest-tree population genomics and adaptive evolution. *New Phytologist* 170: 227–238.

Goudet, J. 2005. HIERFSTAT, a package for R to compute and test hierarchical *F*-statistics. *Molecular Ecology Notes* 5: 184–186.

Griffin, J. R., and W. B. Critchfield. 1972. The distribution of the forest trees in California. Pacific SW Forest and Range Experiment Station, U.S. Department of Agriculture Forest Service, Berkeley, California, USA.

Grivet, D., M.-F. Deguilloux, R. J. Petit, and V. L. Sork. 2006. Contrasting patterns of historical colonization in white oaks (*Quercus* spp.) in California and Europe. *Molecular Ecology* 15: 4085–4093.

Grivet, D., V. L. Sork, R. D. Westfall, and F. W. Davis. 2008. Conserving the evolutionary potential of California valley oak (*Quercus lobata* Née): A multivariate genetic approach to conservation planning. *Molecular Ecology* 17: 139–156.

Gugger, P. F., M. Ikegami, and V. L. Sork. 2013. Influence of late Quaternary climate change on present patterns of genetic variation in valley oak, *Quercus lobata* Née. *Molecular Ecology* 22: 3598–3612.

Hall, D., V. Luquez, V. M. Garcia, K. R. St Onge, S. Jansson, and P. K. Ingvarsson. 2007. Adaptive population differentiation in phenology across a latitudinal gradient in European Aspen (*Populus tremula* L.): A comparison of neutral markers, candidate genes and phenotypic traits. *Evolution* 61: 2849–2860.

Hanson, P. J., and J. F. Weltzin. 2000. Drought disturbance from climate change: Response of United States forests. *Science of the Total Environment* 262: 205–220.

Hill, W., and B. Weir. 1988. Variances and covariances of squared linkage disequilibria in finite populations. *Theoretical Population Biology* 33: 54–78.

Holderegger, R., U. Kamm, and F. Gugerli. 2006. Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landscape Ecology* 21: 797–807.

Holliday, J. A., K. Ritland, and S. N. Aitken. 2010. Widespread, ecologically relevant genetic markers developed from association mapping of climate-related traits in Sitka spruce (*Picea sitchensis*). *New Phytologist* 188: 501–514.

Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11: 609–623.

Hughes, L. 2000. Biological consequences of global warming: Is the signal already apparent? *Trends in Ecology & Evolution* 15: 56–61.

Ingvarsson, P. K., M. V. Garcia, V. Luquez, D. Hall, and S. Jansson. 2008. Nucleotide polymorphism and phenotypic associations within and around the *phytochrome B2* locus in European aspen (*Populus tremula*, Salicaceae). *Genetics* 178: 2217–2226.

Kremer, A., O. Ronce, J. J. Robledo-Arnuncio, F. Guillaume, G. Bohrer, R. Nathan, J. R. Bridle, et al. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters* 15: 378–392.

Krutovsky, K. V., and D. B. Neale. 2005. Nucleotide diversity and linkage disequilibrium in cold-hardiness- and wood quality-related candidate genes in Douglas fir. *Genetics* 171: 2029–2041.

Le Corre, V., and A. Kremer. 2003. Genetic variability at neutral markers, quantitative trait loci and trait in a subdivided population under selection. *Genetics* 164: 1205–1219.

Le Corre, V., and A. Kremer. 2012. The genetic differentiation at quantitative trait loci under local adaptation. *Molecular Ecology* 21: 1548–1566.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, et al. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25: 2078–2079.

Luquez, V., D. Hall, B. R. Albrectsen, J. Karlsson, P. Ingvarsson, and S. Jansson. 2008. Natural phenological variation in aspen (*Populus tremula*): The SwAsp collection. *Tree Genetics & Genomes* 4: 279–292.

Manel, S., S. Joost, B. Epperson, R. Holderegger, A. Storfer, M. Rosenberg, K. Scribner, et al. 2010. Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology* 19: 3760–3772.

Maruki, T., S. Kumar, and Y. Kim. 2012. Purifying selection modulates the estimates of population differentiation and confounds genome-wide comparisons across single nucleotide polymorphisms. *Molecular Biology and Evolution* 29: 3617–3623.

McDowell, N., W. T. Pockman, C. D. Allen, D. D. Breshears, N. Cobb, T. Kolb, J. Plaut, et al. 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist* 178: 719–739.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, et al. 2010. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* 20: 1297–1303.

Morin, X., M. J. Lechowicz, C. Augspurger, J. O'Keefe, D. Viner, and I. Chuine. 2009. Leaf phenology in 22 North American tree species during the 21st century. *Global Change Biology* 15: 961–975.

Neale, D. B., and O. Savolainen. 2004. Association genetics of complex traits in conifers. *Trends in Plant Science* 9: 325–330.

Ning, Z., A. J. Cox, and J. C. Mullikin. 2001. SSAHA: A fast search method for large DNA databases. *Genome Research* 11: 1725–1729.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, et al. 2015. vegan: Community Ecology Package, version 2.2-1. Website http://CRAN.R-project.org/package=vegan.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics* 37: 637–669.

Parry, M. L., O. F. Canziani, J. P. Palutikof, P. J. d. Linden, and C. E. Hanson. 2007. Climate Change 2007: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Patterson, N., A. L. Price, and D. Reich. 2006. Population structure and eigenanalysis. *PLOS Genetics* 2: 2074–2093.

Pavlik, B. M., P. C. Muick, S. G. Johnson, and M. Popper. 1991. Oaks of California. Cachuma Press, Los Olivos, California, USA.

Petit, R. J., S. Brewer, S. Bordács, K. Burg, R. Cheddadi, E. Coart, J. Cottrell, et al. 2002. Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *Forest Ecology and Management* 156: 49–74.

Porth, I., M. Koch, M. Berenyi, A. Burg, and K. Burg. 2005b. Identification of adaptation-specific differences in mRNA expression of sessile and pedunculate oak based on osmotic-stress-induced genes. *Tree Physiology* 25: 1317–1329.

Porth, I., C. Scotti-Saintagne, T. Barreneche, A. Kremer, and K. Burg. 2005a. Linkage mapping of osmotic stress induced genes of oak. *Tree Genetics & Genomes* 1: 31–40.

Price, A. L., N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick, and D. Reich. 2006. Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics* 38: 904–909.

Quinlan, A. R., D. A. Stewart, M. P. Stromberg, and G. T. Marth. 2008. Pyrobayes: An improved base caller for SNP discovery in pyrosequences. *Nature Methods* 5: 179–181.

Rellstab, C., F. Gugerli, A. J. Eckert, A. M. Hancock, and R. Holderegger. 2015. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology* 24: 4348–4370.

Remington, D. L., M. J. Thornsberry, Y. Matsuoka, L. M. Wilson, S. R. Whitt, J. Doebley, S. Kresovich, et al. 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proceedings of the National Academy of Sciences, USA* 98: 11479–11484.

Robinson, J. T., H. Thorvaldsdóttir, W. Winckler, M. Guttman, E. S. Lander, G. Getz, and J. P. Mesirov. 2011. Integrative genomics viewer. *Nature Biotechnology* 29: 24–26.

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology* 132: 365–386.

Smouse, P. E., and R. C. Williams. 1982. Multivariate analysis of HLA-disease associations. *Biometrics* 38: 757–768.

Soler, M., O. Serra, M. Molinas, E. Garcia-Berthou, A. Caritat, and M. Figueras. 2008. Seasonal variation in transcript abundance in cork tissue analyzed by real time RT-PCR. *Tree Physiology* 28: 743–751.

Sork, V. L., S. N. Aitken, R. J. Dyer, A. J. Eckert, P. Legendre, and D. B. Neale. 2013. Putting the landscape into the genomics of trees: Approaches for understanding local adaptation and population responses to changing climate. *Tree Genetics & Genomes* 9: 901–911.

Sork, V. L., F. Davis, and D. Grivet. 2009. Incorporating genetic information into conservation planning for California valley oak. *In* A. Merenlender, D. McCreary, and K. L. Purcell [technical eds.], Proceedings of the Sixth California Oak Symposium: Today's challenges, tomorrow's opportunities, 497–509, Albany, California, USA. U. S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, California.

Sork, V. L., F. W. Davis, R. Westfall, A. Flint, M. Ikegami, H. F. Wang, and D. Grivet. 2010. Gene movement and genetic association with regional climate gradients in California valley oak (*Quercus lobata* Née) in the face of climate change. *Molecular Ecology* 19: 3806–3823.

Sork, V. L., and L. P. Waits. 2010. Landscape genetic contributions to molecular ecology—Approaches, insights, and future potential. *Molecular Ecology* 19: 3489–3495.

Stapley, J., J. Reger, P. G. D. Feulner, C. Smadja, J. Galindo, R. Ekblom, C. Bennison, et al. 2010. Adaptation genomics: the next generation. *Trends in Ecology & Evolution* 25: 705–712.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.

Thornton, K. 2002. libsequence. Website: http://molpopgen.github.io/libsequence/.

Waters, E. R. 1995. The molecular evolution of the small heat-shock proteins in plants. *Genetics* 141: 785–795.

Yang, R. C. 1998. Estimating hierarchical *F*-statistics. *Evolution* 52: 950–956.