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# EFFECTS OF HISTORICAL DEMOGRAPHY AND ECOLOGICAL CONTEXT ON SPATIAL PATTERNS OF GENETIC DIVERSITY WITHIN FOXTAIL PINE (*PINUS BALFOURIANA*; PINACEAE) STANDS LOCATED IN THE KLAMATH MOUNTAINS, CALIFORNIA[1]

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The density and dispersion of individuals, nonequilibrium demographics, and habitat fragmentation all affect the magnitude and extent of spatial genetic structure within forest tree populations. Here, we investigate the link between historical demography and spatial genetic structure within ecologically contrasting stands of foxtail pine (*Pinus balfouriana*) in the Klamath Mountains of northern California. We defined two stand types a priori, based largely on differences in foxtail pine density and basal area, and for each type we sampled two stands. Population expansions, likely from Pleistocene bottlenecks, were detected in three of the four stands. The magnitude and extent of spatial autocorrelation among genotypes at five nuclear microsatellites differed dramatically among stands, with those having lower foxtail pine density exhibiting strong patterns of isolation by distance. Moran's *I* statistics were 7-fold higher for the first distance class (<25 m) in these stands relative to those observed in stands with higher foxtail pine density ($I_{25}$ = 0.14 vs. 0.02). We conclude that differences in spatial genetic structure between stand types are due to differences in ecological attributes that affected expansion from inferred bottlenecks.

**Key words:** foxtail pine; historical demography; Klamath Mountains; Moran's *I*; *Pinus balfouriana*; Pinaceae; spatial autocorrelation; spatial genetic structure.

Spatial genetic structure arises within natural plant populations due to several genetic and ecological processes. It is the combined effects of localized genetic drift, gene flow, natural selection, and the dispersion of individuals that largely determine the magnitude and spatial scale of this structure (Wright, 1943; Malécot, 1969; Epperson, 2003; Rousset, 2004). Ecological processes that alter those effects, ranging from competition to dispersal limitations, can in theory produce effects on localized spatial genetic structure, especially if stand structure is affected (Dyer and Sork, 2001). Additional effects of historical demography on these patterns acting through both genetic and ecological processes are also important (Ibrahim et al., 1996; Le Corre et al., 1997; Epperson, 2000).

Species with wide-ranging dispersal of both pollen and seed are expected to have approximately random distribution of genotypes. This type of distribution has been found for many forest tree populations (Epperson and Allard, 1989; Knowles, 1991; Xie and Knowles, 1991; reviewed by Vekemens and Hardy, 2004; Epperson, 2007). Extensive patterns of spatial autocorrelation of genotypes, however, have been identified across several wind-dispersed species where human activity has altered the distribution of individuals (Fore et al., 1992; Aldrich and Hamrick, 1998; Baucom et al., 2005; Ally and Ritland, 2007). For example, disturbance history affected the spatial genetic structure within two stands of eastern white pine (*Pinus strobus* L.; Epperson and Chung, 2001; Marquardt and Epperson, 2004; Walter and Epperson, 2004). Disturbance also affected fine-scale spatial genetic structure within two stands of tamarack [*Larix laricina* (Du Roi) K. Koch], with logging producing differing patterns (Knowles et al., 1992). Logging left individual founder trees from which the stand regenerated, thus creating spatial genetic structure due to localized inbreeding.

Similarly, historical demography can also be an important driver of fine-scale genetic structure within populations undisturbed by human activity (Ibrahim et al., 1996; Le Corre et al., 1997; Epperson, 2000; Troupin et al., 2006). Historical demographic processes resulting in decreased densities, increased mating of relatives, and nonrandom dispersion of individuals will increase fine-scale spatial genetic structure (Vekemens and Hardy, 2004). Comparisons of fragmented vs. continuous natural populations of plant species (e.g., De-Lucas et al., in press) have found increases in spatial genetic structure due to the aforementioned processes. Exceptions to this pattern are those where habitat fragmentation resulted in increased gene flow through creation of a highly porous environmental matrix (Williams et al., 2007; Born et al., 2008). In western North America, demographic changes within forest tree populations are likely to be the result of historical climate fluctuations (Westfall and Millar, 2004; Millar et al., 2004). The confounding of these events (i.e., historical population size change as a function of climate changes) with ecological interactions among coexisting species, moreover, is important given the influence

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of dispersion on generation of spatial autocorrelation of genotypes (Doligaz et al., 1998; Lee and Hastings, 2006).

Here, we analyze patterns of spatial genetic structure within ecologically contrasting stands of northern foxtail pine (*Pinus balfouriana* Grev. & Balf. subsp. *balfouriana*) in an effort to understand the effects of historical demography and ecological context on patterns of fine-scale spatial genetic structure. Within the Klamath Mountains, foxtail pine is distributed in small stands isolated along high elevation ridges and mountaintops, with census sizes in the hundreds to thousands of trees per stand (Eckert and Sawyer, 2002; Eckert, 2006a). Differing strongly among these stands is the diversity of associated subalpine conifer species, with the abundance, density, and basal area of foxtail pine being inversely related to the density of shade-tolerant competitors (Eckert and Sawyer, 2002). The persistence of foxtail pine in this region is hypothesized to be a function of localized habitat heterogeneity, which is defined as the cover of boulders, coupled with inhabitance of ultramafic substrates, that isolate foxtail pines from shade-tolerant competitors (Eckert, 2006a). Habitat heterogeneity is also linked to down-slope expansion for foxtail pine in the Klamath Mountains (Eckert and Eckert, 2007). Specifically, all sampled foxtail pine stands show signs of down-slope expansion, with stands having the highest habitat heterogeneity or those with lower importance of shade-tolerant competitors in the down-slope vegetation having the greatest disparity between age class distributions at low and high local elevations.

The ecological patterns observed in the Klamath Mountains, especially the relationship between habitat heterogeneity and the persistence of foxtail pine populations, as well as patterns of demographic expansion set the stage for an investigation into the effects of historical demography and ecological context on patterns of fine-scale spatial genetic structure. Our aims are to (1) test for the presence of signatures of population expansion among ecologically contrasting stand types using organellar DNA sequence data, (2) quantify and test the significance of local spatial genetic structure within and across stand types, and (3) relate signatures of population expansion to inferences of fine-scale spatial genetic structure. In doing so, we highlight the need for further analyses of spatial genetic and demographic structure in the subalpine forests of the Klamath region.

## MATERIALS AND METHODS

*Study area*—The Klamath Mountains are a geologically complex mosaic of mountain ranges characterized by diverse substrates, climates, and vegetation types (Major, 1988; Sawyer and Thornburgh, 1988). In northern California, the Klamath Mountains ecoregion covers approximately 30 300 km$^2$. Substrate types range from igneous to ultramafic, with most ultramafic substrates being located in the eastern portion of the region. Vegetation types range from lowland chaparral to subalpine conifer forests with high levels of plant endemism and diversity (Jepson, 1925; Stebbins and Major, 1965; Sawyer and Thornburgh, 1988; Raven and Axelrod, 1995). A suite of endemic and disjunct subalpine conifers contributes to this diversity. Foxtail pine is one of those conifers with a distribution that is disjunct between the Klamath Mountains of northern California and the Sierra Nevada of southern California. These regional populations likely diverged from one another during the Pleistocene (Eckert et al., 2008) and have since developed several quantitative differences in secondary chemistry and morphology (Bailey, 1970; Mastrogiuseppe and Mastrogiuseppe, 1980).

*Field sampling*—We identified four foxtail pine stands in the Klamath Mountains that differ for several environmental and ecological attributes (Table 1; Fig. 1). These stands represent different ends of environmental and ecological gradients documented previously within the subalpine forests of this region (Eckert and Sawyer, 2002; Eckert, 2006a; Eckert and Eckert, 2007). Specifically, sampled stands differ with respect to species composition, richness and diversity, foxtail pine importance, total tree density and basal area, and a measure of local environmental heterogeneity (i.e., boulder cover [%], cf. Eckert, 2006a). We designated stands that have high levels of species diversity, total tree density, total tree basal area and local environmental heterogeneity as low foxtail importance (LFI) stands (Russian Peak [RP], Crater Lake [CL]) because foxtail pine importance is inversely correlated with those quantities. Conversely, stands with low levels of those quantities are designated as stands of high foxtail pine importance (HFI) (Scott Mountains [SM], Seven-Up Peak [SP]). Thus, the four stands form a set of replicated types at opposite ends of ecological structure gradients. These gradients primarily reflect ecological context (e.g., density and diversity of competitors and substrate types), as opposed to stand structure in a strict sense because the latter is likely to have changed through time if individual species comprising extant stands experienced population size changes.

We established a single 250 m by 250 m plot within each stand from which we sampled 100 foxtail pine trees. These trees were used solely for spatial analyses within each stand. The plot was located in the center of each stand to remove effects of spatial expansion around the lower elevation margins (Eckert and Eckert, 2007). To ensure that this area was covered completely, we stratified our sampling along four linear transects spaced ~60 m apart. Trees were sampled at random along each transect by generating random stopping points and sampling the closest adult foxtail pine tree. The diameter at breast height (DBH) was measured for each tree, and only trees with a DBH greater than 35 cm were sampled. Ten fascicles of needle tissue (~50 needles) were gathered from each of those trees, placed in indicator-type desiccant (Qiagen, Germantown, Maryland, USA), and stored at −80°C until being processed for DNA extraction. We also collected needle tissue from an additional 20 individuals, not located within the 6.25-ha sampling unit, as previously described for estimation of genetic diversity and tests of demographic equilibrium within each stand.

*Generation of molecular data*—Total genomic DNA was extracted from each sample using the DNeasy Plant Mini Kit (Qiagen). The success of each extraction was quantified using UV spectrophotometry and by electrophoresis on 1.5% agarose gels. The resulting DNA was standardized to a concentration of 25 ng/μL for polymerase chain reaction (PCR) assays.

We surveyed the literature for PCR primer sets yielding polymorphic markers in other conifer species. Optimization of PCR conditions and verification that selected markers were polymorphic within foxtail pine were addressed using a 24 individual panel randomly selected from the 500 individuals sampled by Eckert (2006b). This validation procedure resulted in a set of four chloroplast DNA (cpDNA) sequence markers and five nuclear microsatellite markers (nSSRs) able to be reliably amplified and successfully sequenced or genotyped for foxtail pine (Appendix 1). All subsequent PCR assays were carried out using standard conditions (30 μL reaction volume: 25–50 ng genomic DNA, 2 mM Tris-HCl, 5 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer, and 1.5 units *Taq* DNA polymerase) and amplification protocols (5 min denaturation at 95°C; 30 cycles of 94°C for 45 s, 52°C for 30 s, and 72°C for 1 min; 5 min final extension at 72°C).

Amplified PCR products were either genotyped or directly sequenced using an ABI 3100 analyzer (PE Applied Biosystems, Foster City, California, USA). For microsatellite genotyping, all primer sets were labeled with 6FAM florescent dye. Alleles at each microsatellite locus were defined to be those that had repeatable peak sizes (± 0.5 bp) across three replicated PCR assays per individual in the validation panel using the GeneScan ROX 400HD size standard (PE Applied Biosystems). Genotypes for the remaining samples were manually called using the GENEMAPPER version 3.0 software (PE Applied Biosystems), with the set of documented alleles at each locus used as a reference. When a new allele was found, we verified it using three replicated runs on the sample in which that new allele was first documented. New alleles that could not be replicated three times were discarded. The presence of null alleles was checked for using the MicroChecker software prior to further analysis (van Oosterhout et al., 2004).

Sequence data were obtained by direct sequencing in both directions using the BigDye Terminator version 3.1 chemistry (PE Applied Biosystems). Sequence traces were edited using the program Sequencher version 4.2 (Gene Codes Corp., Ann Arbor, Michigan, USA), assembled into contigs, and manually aligned using the program Se-Al version 2.0 to maximize homology (Rambaut, 1996).

*Statistical hypothesis testing*—Levels of polymorphism within each stand were described using standard summary statistics. These were calculated with the set of 20 additional sampled trees (cf. *Field sampling*). For microsatellite data, these included the number of alleles ($A$), genic diversity ($H_d$), and an estimator of $\Theta = 4N_e u$, where $N_e$ is the effective population size and $u$ is the locus-specific

TABLE 1. Location and ecological attributes of sampled stands of *Pinus balfouriana*. All values are from Eckert and Sawyer (2002), Eckert (2006a) or Eckert and Eckert (2007). Local heterogeneity is defined as the cover of boulders. The first row gives the stand classification with respect to the attributes listed in the table (cf. Materials and Methods).

| | Low FI | | High FI | |
|---|---|---|---|---|
| Stand attribute | Russian Peak | Crater Lake | Scott Mountains | Seven-Up Peak |
| Latitude (°N) | 41.302 | 41.384 | 41.214 | 40.958 |
| Longitude (°E) | −122.955 | −122.582 | −122.786 | −122.878 |
| Substrate | granodiorite | granodiorite | ultramafic | ultramafic |
| Local heterogeneity | 74.33 | 59.47 | 23.99 | 10.90 |
| Density (trees/ha) | 315 | 381 | 161 | 133 |
| PIBF density | 69 | 68 | 99 | 109 |
| Basal area ($m^2$/ha) | 18.80 | 8.67 | 23.64 | 16.43 |
| PIBF basal area | 4.14 | 1.56 | 14.65 | 13.47 |
| Species richness | 8 | 6 | 4 | 3 |
| Species diversity ($H'$) | 1.926 | 1.596 | 0.944 | 0.586 |

*Notes:* Low FI, low foxtail pine importance; H', species diversity using Shannon's index; High FI, high foxtail pine importance; PIBF, *Pinus balfouriana*.

mutation rate, from sample homozygosity ($\theta_{hom}$; Chakraborty and Weiss, 1991). For DNA sequence data, we calculated the number of segregating sites ($S$) and an estimator of $\Theta$, which for cpDNA is $2N_e u$, from the number of pairwise differences ($\theta_\pi$). All analyses were performed using the program ARLEQUIN version 3.1 (Excoffier et al., 2005).

Fixation indices were calculated within and among all stands. These indices were calculated with the set of 20 additional sampled trees as done for the diversity indices. Deviations from Hardy–Weinberg equilibrium (HWE) were assessed using the inbreeding coefficient ($F_{IS} = 1 - H_o/H_e$). Significance of this measure was determined by permuting alleles among individuals within each stand ($N = 10000$). Differentiation among stands was measured with Weir and Cockerham's (1984) estimator of $F_{ST}$, with its significance being assessed with bootstrapping over loci ($N = 10000$). We also performed a hierarchical analysis of $F$-statistics, where individuals were grouped into stands that were nested into stand type (LFI vs. HFI). We tested the significance of $F_{CT}$, the effect of stand type, by permuting stands between stand type categories 10000 times. Analyses were performed using the FSTAT ver. 2.9.3 software package (Goudet, 1995), as well as the hierfstat (Goudet, 2005) package as part of the R computing environment (R Core Development Team, 2007).

The extent of demographic disequilibrium within each stand was assessed with cpDNA sequence data ($N = 20$ sequences/stand) using Fu's (1997) $F_S$ statistic and mismatch distributions. Significantly negative values of $F_S$ are consistent with population expansions (Fu, 1997). We also fit expected mismatch distributions under a sudden expansion model to those observed within each stand. Under a demographic model of sudden expansion, the mismatch distribution is expected to be smooth and unimodal (Slatkin and Hudson, 1991). The raggedness ($r_g$) statistic was used to assess the smoothness of the observed mismatch distribution (Harpending, 1994). The observed moments of the mismatch distribution can also be used to estimate parameters of the sudden expansion model assuming that the current population size tends to infinity (Rogers and Harpending, 1992). Here, we report the time at which the sudden expansion occurred ($\tau = 2ut$, where $u$ is the mutation rate and $t$ is the time in generations). The significance of $F_S$ and $r_g$ were evaluated with 10000 coalescent simulations under the null model of genetic drift within a population remaining at a constant size. All simulations were conditioned on the observed value of $\theta_\pi$ using the program DnaSP version 4.50.3 (Rozas et al., 2003).

Spatial autocorrelation analyses were conducted for each allele of each nSSR locus using Moran's $I$ statistic, with the caveats that only one allele from biallelic markers was used, that alleles observed in five individuals or less were assumed to be uninformative and that nSSR were completely unlinked (Sokal and Oden, 1978; Epperson and Li, 1996). We followed the methods of Epperson (2003), which produce correlations among genotypes that are converted into gene frequencies. These analyses were conducted on 100 sampled trees per stand sampled within the 6.25-ha plot.

For each allele, frequency values were assigned to genotype classes as follows: 1.0 for homozygous present, 0.5 for heterozygous, and 0.0 for homozygous absent. Individuals were paired as joins and classified into one of 10 non-overlapping distance classes. We used a distance-based connection matrix among individuals with intervals specified to capture most of the nearest neighbors in the first distance class (Epperson, 2003). This system creates a set of binary weights in the calculation of Moran's $I$, so that the weight is one when a pair is within a specified distance class and zero otherwise. The set of distance classes maximizing the number of nearest neighbors in the first class was: 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 m.

For each distance class, Moran's $I$ was calculated for each allele of each locus and then each value of $I$ was tested using its normal approximation at a significance threshold of $P = 0.01$ (Cliff and Ord, 1981). The set of $I$ values for a given allele across all 10 distance classes forms a correlogram, the overall significance of which was tested using a Bonferroni procedure (Oden, 1984). All analyses were conducted using the program PASSAGE version 1.0 (Rosenberg, 2001).

Average correlograms for each sampled stand were constructed from the unweighted averages of Moran's $I$ values across all alleles and loci for each distance class. The significance of the average Moran's $I$ for each distance class was tested by transforming them into standard normal deviates (SNDs) following Walter and Epperson (2004) and Epperson (2004). We also calculated SNDs for the first distance class for each locus within each stand using the method

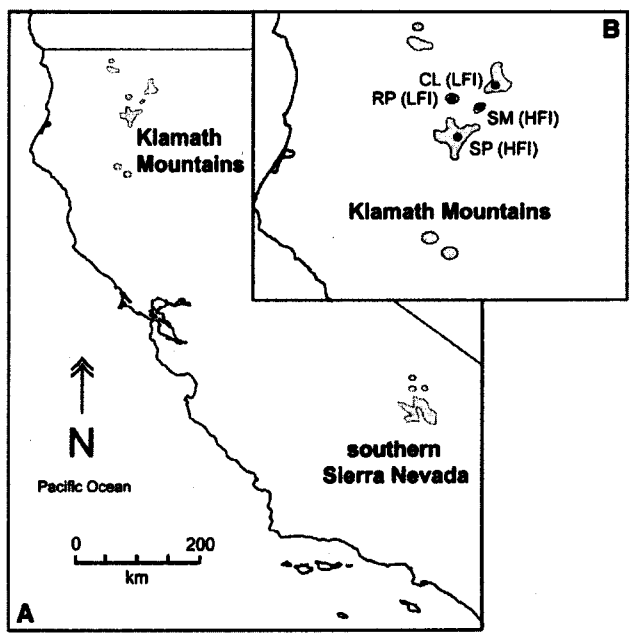


Fig. 1. Distribution of *Pinus balfouriana* in California. (A) The distribution is disjunct within California, with a 500 km gap isolating populations in the Klamath Mountains from those in the southern Sierra Nevada. (B) Distribution in Klamath Mountains of northern California. Filled circles are the four sampling localities used for this study. CL, Crater Lake; HFI, high foxtail pine importance; LFI, low foxtail pine importance; RP, Russian Peak; SM, Scott Mountains; SP, Seven-Up Peak.

described previously. This was done because this was the distance class with the greatest number of significant values and it is a powerful indication of overall spatial autocorrelation among genotypes under isolation by distance (Oden, 1984; Epperson, 2005).

## RESULTS

***Data summary***—Patterns of genetic diversity differed moderately among stands and between stand types (Table 2). The total number of nSSR alleles observed across loci varied from 16 to 18, with LFI stands having 16 alleles and HFI stands having 18 alleles. The population mutation rate estimate ($\theta_{hom}$) differed among stand types, with smaller average estimates for LFI stands and much larger estimates for HFI stands. A similar trend was apparent for cpDNA nucleotide diversity ($\theta_\pi$), which was based on a total of 3228 bp sequenced per tree per stand. Insertion-deletion (indel) polymorphisms were not observed in the cpDNA sequence data.

Deviations from HWE were apparent in all stands, with LFI stands having a strong deficit of heterozygotes for most nSSR loci (Table 3). The average value of $F_{IS}$ differed among stands, with LFI stands having values of ~0.15 and HFI stands having much smaller values of ~0.04 or less. Patterns of nuclear genetic diversity were also structured among stands (Table 3). Values of Weir and Cockerham's (1984) estimator for $F_{ST}$ ($\theta$) varied across loci, ranging from 0.004 to 0.164, with a global average over loci of 0.080.

Low to moderate frequency (0.05–0.15) null alleles were detected in the LFI stands for the three nSSR loci deviating strongly from HWE (Table 3). We retained all five SSR loci for further analysis, however, because all stands had complete data for the diversity panel ($N = 20$), as well as the samples used for spatial analyses ($N = 100$). A hierarchical analysis of $F$ statistics, where individuals were classified into stands and stands were nested into stand types, had a nonsignificant estimate of $F_{CT}$ ($F_{CT} = 0.021$, $P = 0.421$). Large allele frequency differences between stand types would be required for only null alleles, therefore, if the observed patterns of $F_{IS}$ within LFI stands were due solely to their occurrence.

***Historical demography***—All stands had negative values of Fu's $F_S$. These values represented significant departures from those expected under a null model of demographic equilibrium and genetic drift for three of the four stands (Table 2). The exception was the Russian Peak stand, which also had the lowest observed chloroplast nucleotide diversity.

We explored that pattern further using mismatch distributions. In all cases, the mismatch distribution expected under a sudden expansion model fit those observed within each stand fairly well (Fig. 2). The $r_g$ statistic confirmed this pattern, with all but one stand having $r_g$ values too extreme to occur under demographic equilibrium (Table 2, Fig. 2). The time at which the sudden expansion occurred ($\tau$) was estimated from each distribution. Three of the four stands had similar estimates on the order of $\tau = 2.0$. This translates into ~15 000–30 000 yr ago by using estimates of per-site mutation rates published previously (Willyard et al., 2007) and assuming a generation time of 50 yr.

***Spatial autocorrelation***—Stands differed in the extent and magnitude of spatial autocorrelation among genotypes (Fig. 3). LFI stands exhibited strong spatial patterns in autocorrelation among genotypes. Individuals less than 25 m apart within these stands had significantly positive values of Moran's $I$ (range: 0.044–0.319). This trend continued up to distance class three. By distance class eight, Moran's $I$ values were significantly negative. Those patterns translated into 78% and 89% of the correlograms being significant after a Bonferroni adjustment in the Crater Lake and Russian Peak stands, respectively.

As opposed to those patterns, HFI stands exhibited weak to nonexistent spatial patterns of autocorrelation. Individuals less than 25 m apart within these stands had largely nonsignificant values of Moran's $I$ (range: –0.065 to 0.130). Trends across distance classes were weak to nonexistent. Those patterns translated into 6% and 11% of the correlograms being significant after a Bonferroni adjustment in the Scott Mountains and Seven-Up Peak stands, respectively.

Average correlograms illustrated the same patterns across loci (Fig. 3). For example, values of Moran's $I$ across loci for the first distance class varied an order of magnitude between stand types, with most SNDs for LFI stands being highly improbable under a random distribution of genotypes (Table 4). Most of the SNDs were significant ($P < 0.01$) with those from the first few distance classes being significantly positive and those from the last few distance classes being significantly negative (Fig. 3). Only the closest distance class was significant in both HFI stands.

## DISCUSSION

***Historical demography***—The mismatch distributions in three of the four stands were similar to that expected under a sudden expansion model and are consistent with the down-slope

TABLE 2. Summary of molecular genetic data for each stand of *Pinus balfouriana*. Values of $\theta_\pi$ for the cpDNA are standardized by the amount of sequenced data (3228 bp). Values for the nuclear microsatellites are averages (standard deviations) across loci.

| Statistic | RP | CL | SM | SP |
|---|---|---|---|---|
| Chloroplast | | | | |
| $S$ | 4 | 7 | 5 | 6 |
| $\theta_\pi$ | 0.00028 | 0.00070 | 0.00061 | 0.00069 |
| $F_s$ | −1.571 | −5.321*** | −3.372** | −5.475*** |
| $r_g$ | 0.057 | 0.038* | 0.038** | 0.037** |
| Nuclear | | | | |
| $A$ | 3.2 (0.8) | 3.2 (1.3) | 3.6 (1.1) | 3.4 (1.1) |
| $H_d$ | 0.445 (0.104) | 0.402 (0.081) | 0.559 (0.150) | 0.474 (0.129) |
| $\theta_{hom}$ | 0.690 (0.258) | 0.569 (0.210) | 1.296 (0.745) | 1.233 (0.919) |

*Notes:* $A$, number of alleles per locus; CL, Crater Lake; cp, chloroplast; $F_S$, Fu's (1997) $F_S$ statistic; $H_d$, genic diversity; $\theta_{hom}$, estimate of $\theta = 4N_e u$ from sample homozygosity; $\theta_\pi$, estimate of $\theta = 2N_e u$ from the average number of pairwise sequence differences; $r_g$, raggedness index; RP, Russian Peak; $S$, the number of segregating sites, SM, Scott Mountains; SP, Seven-Up Peak. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

TABLE 3. Fixation indices measured for each stand of foxtail pine (*Pinus balfouriana*) based on nuclear markers. Statistical significance was assessed through randomization of alleles relative to individuals within populations for $F_{IS}$ and through bootstrapping ($N = 10000$ replicates) over loci for $F_{ST}$ (θ, cf. Weir and Cockerham, 1984).

| Locus | $F_{IS}$ | | | | $F_{ST}$ |
|---|---|---|---|---|---|
| | RP | CL | SM | SP | |
| *PtTX3025* | 0.239* | 0.228* | 0.026 | −0.010 | 0.115 |
| *PtTX3020* | 0.280** | 0.323** | −0.016 | −0.019 | 0.004 |
| *Rps12* | 0.070 | 0.042 | 0.020 | 0.026 | 0.164 |
| *Rps34b* | 0.048 | 0.092 | −0.060 | −0.052 | 0.049 |
| *Rps84* | 0.183* | 0.120 | 0.037 | −0.007 | 0.038 |
| Average | 0.164 | 0.161 | 0.001 | −0.012 | 0.080** |
| SD | 0.102 | 0.113 | 0.040 | 0.028 | 0.029 |

*Notes:* $F_{IS}$, inbreeding coefficient ($1 - H_{obs}/H_{exp}$); RP, Russian Peak; SD, standard deviation; SM, Scott Mountains; SP, Seven-Up Peak; θ, Weir and Cockerham's (1984) estimator of $F_{ST}$; * $P < 0.05$ or ** $P < 0.01$.

expansion observed within stands located in this region (Eckert and Eckert, 2007). Here, both stands with low foxtail pine importance and high species diversity had no to only moderate deviations from demographic equilibrium. Both of these stands had high importance of shade-tolerant competitors in downslope stands and exhibited a lower disparity between age class distributions at low and high local elevations (Eckert and Eckert, 2007). This result, therefore, is not surprising. The only stand without significant values of $F_S$ or $r_g$, moreover, was the Russian Peak stand. This stand also had the lowest level of cpDNA diversity, thus limiting the power of the statistics used to test demographic equilibrium (cf. Fu, 1997). These expansions were dated to 15000–30000 yr ago using these distributions and per site mutation rates published previously for conifer chloroplast genomes (Willyard et al., 2007). The estimated dates fall within the range of major glacial maximums for the Klamath region (Sharp, 1960), thus supporting circumstantially that these events may represent growth from population bottlenecks. Fossil evidence suggests that the distribution of foxtail pine at larger spatial scales has been more extensive in this

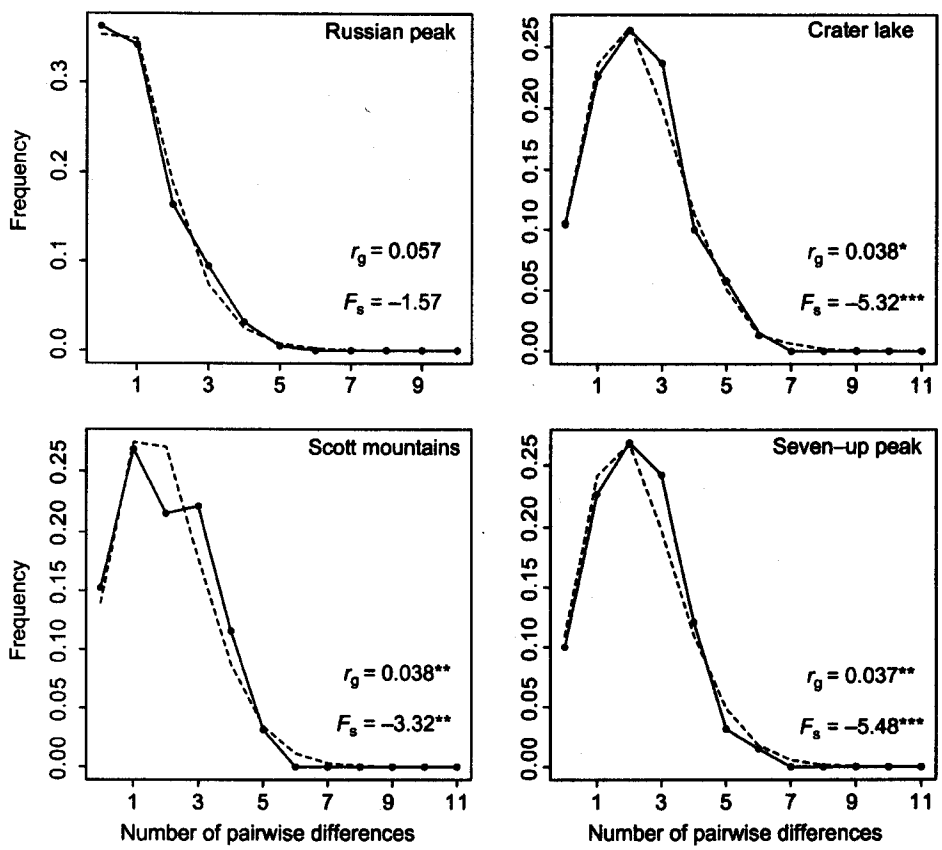


Fig. 2. Mismatch distributions for each stand of *Pinus balfouriana*. The expected distribution under a sudden expansion model is given as a dashed line in each case. Lower right of each plot: raggedness statistic ($r_g$), Fu's $F_S$. Asterisks denote values that are significant (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.005$) for a null model of genetic drift within a constant population size.

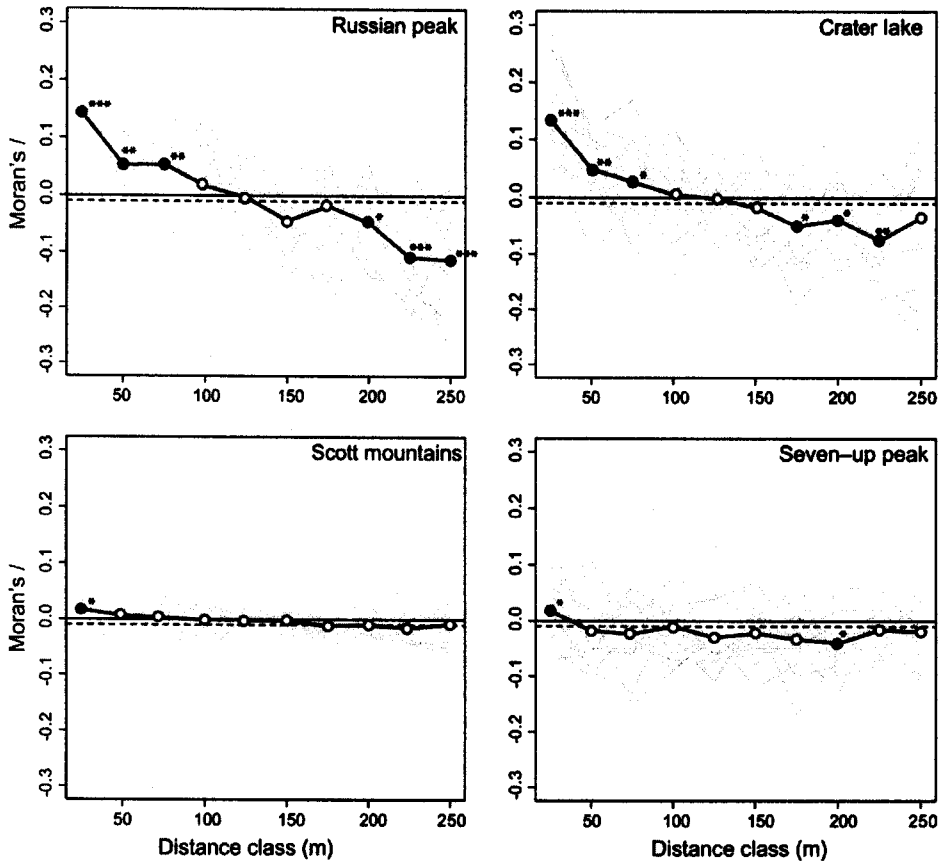


Fig. 3. Correlograms for each stand of *Pinus balfouriana*. Light gray lines are the correlograms for each allele sampled across the five microsatellite loci ($N$ = 16–18). The average (unweighted) correlogram is given as a bold black line. Points denote the value for Moran's $I$ at each of the 10 distance classes, with filled points denoting values significant (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.005$).

region during the Quaternary (Critchfield, 1977), again consistent with a bottleneck or range retraction scenario.

***Spatial genetic structure***—The shape of the correlograms observed within LFI stands support the hypothesis of restricted gene flow. In particular, the large values of Moran's $I$ in the first distance class (<25 m; $I$ = 0.13–0.14) suggest limited gene dispersal within these stands. These values are ~1.5–2.5 times larger than those reported in other wind pollinated and dispersed species. Contrasting with that pattern are the correlograms observed in the HFI stands. The shapes of these, as well as the magnitudes of Moran's $I$ statistics, are consistent with widespread gene dispersal in these stands. Values for the average $I$ statistics in the first distance class for these stands ($I$ = 0.02–0.03) are similar to those reported elsewhere.

Estimates of Wright's neighborhood sizes ($N_e = 4\pi D_e\sigma^2_t$, where $D_e$ is the effective density of individuals and $\sigma^2_t$ is the total axial dispersal variance) differ strongly among stands. For LFI stands, $N_e$ was estimated to be ~40, while for HFI stands it was ~120. These estimates were obtained from the log-linear relationship between $N_e$ and Moran's $I$ for adjacent individuals, here defined to be joins in the first distance class (Epperson, 2007; $I(1) = 0.571 - 0.113\ln N_e$). The values of Moran's $I$ were adjusted relative to the expected value under a random distribution of genotypes [$E(I) = -1/(n - 1) = -0.0101$] prior to the calculation of $N_e$. Previous studies in pines at similar spatial scales have produced estimates of $N_e$ on the order of 100 to 200 (Marquardt and Epperson, 2004), values similar to those observed in HFI stands.

The estimates of $N_e$ correspond to total axial dispersal distances ($\sigma_t$) of ~30–38 m for LFI stands and ~39-53 m for HFI stands. For those calculations, we estimated $D_e$ from $D_e = D[4/(2 + V)][1/(1 + F_{IS})]$, where $D$ is the observed density (Table 1), $V$ is the variance of the lifetime reproductive success among individuals and $F_{IS}$ is Wright's inbreeding coefficient (Crawford, 1984). Assuming that $V$ is in the range of 5 to 10, this give values for $D_e$ of 20–34 trees/ha for LFI stands and 33–62 trees/ha for HFI stands. Of course, larger values of $V$ will reduce these estimates, and parentage analyses in conifer seed orchards have shown much heterogeneity in reproductive success among individuals (Erickson and Adams, 1989).

Seed dispersal relative to pollen movement is more likely to be limited in foxtail pine stands (Ennos, 1994; Ally and Ritland, 2007). It is difficult, however, to differentiate limited pollen vs. seed dispersal directly from biparental data (Epperson, 2000; Heuertz et al., 2003). This is because $\sigma^2_t$ at equilibrium is a linear function of both seed ($\sigma^2_s$) and pollen ($\sigma^2_p$) dispersal variances (Crawford, 1984). If we assume that $\sigma^2_p$ is within the range of standard conifer pollen dispersal kernels ($\sigma_p$ = 25–55 m; cf. Wright, 1952; Wang et al., 1960), $\sigma_s$ would be on the order of 10–30 m in LFI stands and 35–55 m in HFI stands. These estimates rely on mutation–drift equilibrium, which is not likely to hold within most of our sampled stands.

TABLE 4. Average values of Moran's *I* for the first distance class listed by locus. Standard errors for each average were calculated as described in Epperson (2004).

| Locus | *I* | *I* − E(*I*) | SE | SND |
|---|---|---|---|---|
| *PtTX3025* | | | | |
| RP | 0.145 | 0.155 | 0.039 | 3.97*** |
| CL | 0.135 | 0.145 | 0.047 | 3.09*** |
| SM | 0.005 | 0.015 | 0.029 | 0.52 |
| SP | 0.010 | 0.020 | 0.014 | 1.43 |
| *PtTX3020* | | | | |
| RP | 0.137 | 0.147 | 0.037 | 3.97*** |
| CL | 0.123 | 0.133 | 0.039 | 3.41*** |
| SM | 0.043 | 0.053 | 0.056 | 0.95 |
| SP | 0.003 | 0.013 | 0.040 | 0.33 |
| *Rps12* | | | | |
| RP | 0.123 | 0.133 | 0.031 | 4.29*** |
| CL | 0.100 | 0.110 | 0.037 | 2.98*** |
| SM | 0.030 | 0.040 | 0.029 | 1.38 |
| SP | 0.015 | 0.025 | 0.016 | 1.56 |
| *Rps34b* | | | | |
| RP | 0.152 | 0.162 | 0.041 | 3.95*** |
| CL | 0.158 | 0.168 | 0.056 | 3.00*** |
| SM | −0.010 | 0.000 | 0.032 | 0.00 |
| SP | 0.018 | 0.028 | 0.018 | 1.56 |
| *Rps84* | | | | |
| RP | 0.180 | 0.190 | 0.056 | 3.39*** |
| CL | 0.143 | 0.153 | 0.052 | 2.94** |
| SM | 0.028 | 0.038 | 0.014 | 2.71* |
| SP | 0.048 | 0.058 | 0.023 | 2.52* |
| Average | | | | |
| RP | 0.144 | 0.154 | 0.029 | 5.31*** |
| CL | 0.134 | 0.144 | 0.035 | 4.11*** |
| SM | 0.018 | 0.028 | 0.011 | 2.54* |
| SP | 0.022 | 0.032 | 0.014 | 2.29* |

*Notes:* CL, Crater Lake; E(*I*), expectation of Moran's *I* statistic; *I*, Moran's *I* statistic; RP, Russian Peak; SM, Scott Mountains; SP, Seven-Up Peak; SE, standard error; SND, standard normal deviate. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.005$

The estimated values for $\sigma_s$ and $\sigma_t$ in LFI stands are lower than that expected for a conifer with wind-dispersed seeds. Parentage and seedling neighborhood analyses often document much longer seed movements for pines. For example, 45% of the seedlings in a Scots pine (*P. sylvestris* L.) stand located in Poland were derived from seed dispersals >40 m (Burczyk et al., 2006). Considerable heterogeneity, however, also characterizes estimates of seed dispersal dependent upon the site that was surveyed. For example, Ally and Ritland (2007) estimated dispersal distributions for seeds that significantly differ between old growth and clear-cut sites for mountain hemlock [*Tsuga mertensiana* (Bong.) Carr.], with the mean seed dispersal distance being 30 m less in the old-growth site. Thus, the differences observed here are consistent with patterns in other conifer species as a function of disturbance and stand structure.

Pollen donors may also be limited within the sampled stands (Koenig and Ashley, 2003; Robledo-Arnuncio and Austerlitz, 2006). This phenomenon has been documented in several wind-pollinated taxa (Knapp et al., 2001; Sork et al., 2002; Davis et al., 2004). Significant effects of distances between pollen donors and mother trees have also been documented in conifer seed orchards (Erickson and Adams, 1989). The underlying commonality of these patterns at small spatial scales, however, is one of low density, high range fragmentation or nonrandom distribution of trees within the sampling locality relative to the axial dispersal of pollen. Here, the observed densities of foxtail pine are on the order of 50–100 trees/ha. The distribution of individual trees, however, is unknown. Under our hypothesis and that put forward by Eckert (2006a), competitive interactions coupled with limited seed dispersal may create patterns of extreme aggregation in LFI stands. Future research into spatial patterns of tree distribution at local scales in the subalpine forests of the Klamath region thus seems warranted.

***Historical demography and ecological context***—The striking differences between stand types are suggestive of limited gene dispersal within stands characterized by low foxtail pine density. These patterns are also correlated with levels of inbreeding, with LFI stands exhibiting a strong deficit of heterozygotes across most microsatellite loci. The observed patterns are analogous to those observed in other conifer species where logged sites have been compared to those that are undisturbed (Knowles et al., 1992; Marquardt and Epperson, 2004; Walter and Epperson, 2004; Ally and Ritland, 2007) and to those comparing continuous to fragmented populations (De-Lucas et al., in press). Effects of canopy position and density on spatial genetic structure have also been documented clearly across a number of forest tree species, with vegetation structure often decreasing effective gene dispersal (cf. Dyer and Sork, 2001; Vekemens and Hardy, 2004; De-Lucas et al., 2008). In the sampled foxtail pine stands, total tree density differed by 2-fold between LFI and HFI stands, with the ratio of foxtail to total tree density differing 3- to 4-fold between stand types (Table 1).

We conclude that it was not the historical demographic event per se that affected differences in spatial genetic structure between stand types, but that the expansion from bottlenecks resulted in different outcomes within each stand type that depended upon ecological context (e.g., the distribution and importance of shade-tolerant competitors). A similar effect was detected within an expanding stand of Aleppo pine (*Pinus halepensis* Miller), where development of spatial genetic structure was highly dependent upon the initial distribution of founding trees (Troupin et al., 2006). Analogously, the dispersion of the foxtail pine trees likely differed between stand types prior to the inferred expansions and thus resulted in increased spatial genetic structure for the HFI stands. This explanation is in agreement with simulation studies of populations that are expanding spatially (Ibrahim et al., 1996; Le Corre et al., 1997, Davies et al., 2004), as well as the significantly positive values of $F_{IS}$, the lower densities and higher spatial genetic structure in LFI stands, and the correlations between foxtail pine density, the abundance of shade-tolerant competitors and habitat heterogeneity (Eckert, 2006a).

***Limitations***—Our conclusions should be tempered by several limitations. First, our sample size of four stands does not represent fully the environmental and ecological diversity of foxtail pine stands located in the Klamath Mountains. Our samples, however, are replicates of the extreme tails for gradients of foxtail pine importance and species diversity. Second, null alleles and variable mutation rates affect patterns of spatial genetic structure. The number of alleles, however, did not differ greatly among loci, all stands had complete data (i.e., no missing data), and the locus with the largest number of alleles (*Rps84*) tended to have the highest signal of spatial genetic structure (Tables 2, 4). Third, significant differentiation was detected among stands (see also Oline et al., 2000). Levels of differentiation, however, were not correlated to the differences among *I* correlograms. The Russian Peak stand (LFI) was as

equally differentiated from the Crater Lake (LFI) stand as it was to the Seven-Up Peak stand (HFI). Third, we assumed dispersal–drift equilibrium for the calculations relating *I* statistics to neighborhood sizes. The scale and design of our sampling could have affected estimates of *I* statistics (cf. Vekemans and Hardy, 2004), but this does not limit the comparison among stands because the scale and type of sampling were the same for each stand. Pulsations in reproductive output that differ strongly between stands could also account for these patterns, and future work would benefit greatly from quantifying this phenomenon. Last, a temporal Wahlund effect could have inflated estimates of inbreeding due to our lumping of several cohorts together into the adult class from which we sampled. If our sampled trees are stratified into five additional classes, estimated inbreeding coefficients decrease but remain positive for each class within LFI stands (data not shown).

***Conclusions and implications***—Spatial genetic structure within foxtail pine stands is significant and correlated with ecological context. The implications of these patterns are that single species investigations at broad spatial scales may often miss important ecological correlates to observed patterns. Such information could change interpretations of pollen and seed movement, as well as conservation efforts to bolster such processes for rare species in the face of climate change. As Westfall and Millar (2004) noted, most forest tree populations in western North America are likely to be far from genetic and demographic equilibrium. Efforts to describe the effects of ecological and climatic variables on these patterns of diversity, therefore, need to include multiple spatial scales.

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APPENDIX 1. Primer information and GenBank accession numbers for each locus and genome.

| Locus | Primers | Source | GenBank accessions |
|---|---|---|---|
| Chloroplast | | | |
| *matK* | F: 5′-GAACTCGTCGGATGGAGTG-3′ | Wang et al. (1999) | EF032507, EF032509–EF032511 |
| | R: 5′-GAGAAATCTTTTTCATTACTAC-3′ | | |
| | F: 5′-CGTACTTTTATGTTTACAGGCTAA-3′ | | |
| | R: 5′-TAAACGATCCTCTCATTCACGA-3′ | | |
| *psaA-trnS* | F: 5′-GGAGCCATCGGGATTATTC-3′ | Demesure et al. (1995) | EF044137, EF044140 |
| | R: 5′-CGGAAAGACGAAAGGACATT-3′ | | |
| *rpl16* | F: 5′-TTTGGAACCGTGCTATGCTT-3′ | Gernandt et al. (2003) | EF033671, EF033683 |
| | R: 5′-TCTAGCGACGGTTTCGGATA-3′ | | |
| *rpl20-rps18* | F: 5′-CTTCGTCGTTTGTGGATTAC-3′ | Wang et al. (1999) | EF033686, EF033693, EF033694 |
| | R: 5′-AGTCGATTTATTAGTGAGCA-3′ | | |
| Nucleus | | | |
| *PtTX3025* | F: 5′-CACGCTGTATAATAACAATCTA-3′ | Elsik et al. (2000) | AF143970 |
| | R: 5′-TTCTATATTCGCTTTTAGTTTC-3′ | | |
| *PtTX3020* | F: 5′-GTCGGGAAGTGAAAGTA-3′ | Elsik et al. (2000) | AF143969 |
| | R: 5′-CTAGGTGCAAGAAAAGAGTAT-3′ | | |
| *RPS12* | F: 5′-TCAATGTGGAGATGGTGATT-3′ | Echt et al. (1996) | U60242 |
| | R: 5′-ACTTCTGACCTAACCAGAAACC-3′ | | |
| *RPS34B* | F: 5′-CAGTGTTCTCTTATCACAGCGCA-3′ | Echt et al. (1996) | U60246 |
| | R: 5′-GCACTATAATGAAATAGCGCA-3′ | | |
| *RPS84* | F: 5′-CCTTTGGTCATTGTATTTTTGGAC-3′ | Echt et al. (1996) | U60250 |
| | R: 5′-CTTCCTTTTCCTTCTTGCTCCAC-3′ | | |

*Notes:* GenBank accession numbers for the chloroplast data refer to the distinct haplotypes observed at each locus. Accession numbers for nuclear microsatellite loci refer to the DNA sequence submission by the listed source to NCBI. Those markers were developed for either loblolly pine (*Pinus taeda* L., *PtTX3025* and *PtTX3020*) or for eastern white pine (*P. strobus* L., *RPS12*, *RPS34B* and *RPS84*).