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ORIGINAL ARTICLE

Patterns of seed dispersal and pollen flow in *Quercus garryana* (Fagaceae) following post-glacial climatic changes

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ABSTRACT

Aim We examined the genetic structure of *Quercus garryana* to infer post-glacial patterns of seed dispersal and pollen flow to test the hypotheses that (1) peripheral populations are genetically distinct from core populations and from one another; (2) genetic diversity declines towards the poleward edge of the species' range; and (3) genetic diversity in the chloroplast genome, a direct measure of seed dispersal patterns, declines more sharply with increasing latitude than diversity in the nuclear genome. We address our findings in the context of known historical oak distribution from pollen core data derived from previously published research.

Location Oak–savanna ecosystems from southern Oregon, USA (core populations/non-glaciated range) northward to Vancouver Island, British Columbia, Canada (peripheral populations/glaciated range).

Methods We genotyped 378 trees from 22 sites with five chloroplast and seven nuclear microsatellite loci. For both sets of markers, we estimated genetic diversity and differentiation using an analysis of molecular variance and generated Mantel correlograms to detect genetic and geographical distance correlations. For the nuclear markers, we also used a Bayesian approach to infer population substructure.

Results There was a large degree of population differentiation revealed by six chloroplast haplotypes, with little (≤ 3) or no haplotype diversity within sites. Peripheral island locations shared the same, maternally inherited chloroplast haplotype, whereas locations in mainland Washington had greater haplotype diversity. In contrast, genetic diversity of the nuclear markers was high at all locations sampled. Populations clustered into two groups and were significantly positively correlated over large spatial scales (≤ 200 km), although allele richness decreased significantly with latitude. Population substructure was observed between core and peripheral populations because rare alleles were absent in peripheral localities and common allele frequencies differed.

Main conclusions The observed pattern of chloroplast haplotype loss at the northern periphery suggests restricted seed dispersal events from mainland sites to peripheral islands. This pattern was unexpected, however, as refugial oak populations remained near the current post-glacial range even during the Last Glacial Maximum. Using nuclear markers, we found high within-population diversity and population differentiation only over large spatial scales, suggesting that pollen flow is relatively high among populations.

Keywords

Genetic differentiation, glacial refugia, latitudinal gradient, microsatellite, North America, oak, peripheral populations, post-glacial migration, *Quercus garryana*, range shift.

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INTRODUCTION

Colonization from elsewhere is the main reason that previously glaciated landscapes have floristic diversity (Hewitt, 1999). Organisms that have undergone such a post-glacial range shift usually show a genetic signature of that migration (Taberlet *et al.*, 1998). We studied *Quercus garryana* Dougl. ex Hook. (section *Quercus*, Oregon white oak or Garry oak) in the Pacific Northwest of North America in order to determine the genetic effects of colonizing peripheral island locations after the Last Glacial Maximum (LGM). In contrast to post-glacial colonization in Europe, much of the *Q. garryana* range was not glaciated at the LGM (Waitt & Thorson, 1983; Fig. 1), providing an opportunity to investigate the signature of post-glacial colonization for a taxon that did not shift large distances after the LGM.

For most angiosperm taxa, the path of post-glacial colonization has been examined using maternally inherited chloroplast markers because they provide information only on the dispersal of seed (Birky, 1976; Dumolin *et al.*, 1995). Patterns of population differentiation for oak species observed using chloroplast markers have typically been attributed to population responses during and following the period of highly variable climates of the Pleistocene (Petit *et al.*, 1993b; Dumolin-Lapègue *et al.*, 1997; Grivet *et al.*, 2006). Typically, the highest genetic diversity is found within the refugial range (Hewitt, 2000). Genetic diversity is often lost along the migratory path, permitting inference of the path and source populations through genetic analysis of the descendant populations. Diversity is initially lost because post-glacial recolonization is not from a random draw of seed (Feng *et al.*, in press). Instead, dispersing seeds are drawn most frequently from the poleward edge of the shifting distribution. Once a post-glacial region is colonized, newly established populations often limit establishment from other sources by filling the niche space (Taberlet *et al.*, 1998; Hewitt, 2000). Further, it is likely that a few colonizing individuals contribute the bulk of seed to newly established populations because colonization events are infrequent (Petit *et al.*, 1997). Under this model, one expects low genetic diversity within post-glacial populations and an overall loss of genetic diversity with increasing geographical distance from the original source (Cwynar & MacDonald, 1987; Dumolin-Lapègue *et al.*, 1997).

Nuclear markers, in contrast, provide a biparentally inherited view of biogeographical patterns, incorporating both pollen flow and seed dispersal. Wind pollination in trees can be effective over great distances, and pollen donors may

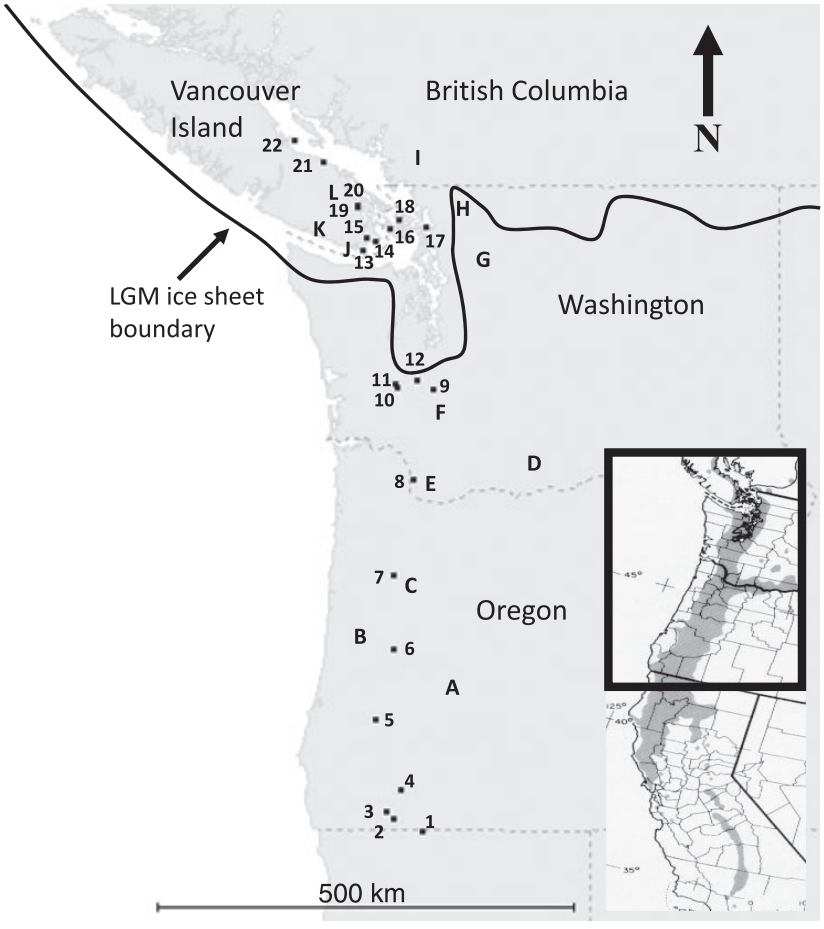

Figure 1 *Quercus garryana* species range map inset into study area in the Pacific Northwest. Numbered dots are sampling sites (n = 22). Capital letters are pollen sediment sampling sites from the literature (n = 12). Estimate of ice sheet extent at the LGM drawn from Waitt & Thorson (1983). Range map inset reprinted from Burns & Honkala (1990). Base map created using GPSVisualizer (http://www.gpsvisualizer.com).

frequently come from outside a recipient population (Liepelt *et al.*, 2002; Bacles *et al.*, 2005; Craft & Ashley, 2006, 2007). High within-site nuclear diversity is a common finding for oaks, and it is usually attributed to long-distance pollen flow (Dow & Ashley, 1996; Streiff *et al.*, 1999; Aldrich *et al.*, 2003b). For wind-pollinated species, there is also evidence that pollen flow can be enhanced in a fragmented or open landscape, as would be found in the wake of a retreating glacier (Bacles *et al.*, 2005; but see Sork *et al.*, 2002). For example, in an open, fragmented landscape, Craft & Ashley (2007) could not detect population structure for *Quercus macrocarpa* over 200-km geographical distances. In addition, Muir *et al.* (2004) found little nuclear differentiation among peripheral populations of *Quercus petraea* in sites in Ireland that were island colonizations and now in a highly fragmented landscape. This high within-population genetic diversity and low population differentiation has the effect of obscuring source populations of post-glacial locations while mitigating potentially deleterious genetic consequences of small founding populations.

In many systems, species are thought to have travelled great distances from their glacial refugia to colonize their post-glacial ranges (Brewer *et al.*, 2002). European oaks were relegated to refugia south of the Alps, a major migratory barrier (Hewitt, 1999; Petit *et al.*, 2002), and chloroplast genetic signatures reflect the loss of diversity with northward migration in distinct latitudinal lineages (Grivet *et al.*, 2006). The pattern in eastern North America was thought to be similar to that in Europe, with temperate species restricted to southern refugia, but gaps in fossil data made determining glacial distributions difficult (Jackson *et al.*, 2000). Evidence is emerging that oak and other temperate species may have maintained refugial populations relatively close to the ice sheet, potentially allowing some species to persist at low densities much further north than the pollen or macrofossil records suggest (McLachlan & Clark, 2004; Magni *et al.*, 2005; McLachlan *et al.*, 2005; Loehle, 2007; Birchenko *et al.*, in press). In the Pacific Northwest of North America, species distributions were greatly influenced by the Laurentide ice sheet much further east. By the time the Cordilleran ice lobes were reaching their greatest extent on Vancouver Island and in the Puget Trough, temperate communities were advancing towards the ice (Whitlock, 1992). Maximum ice extent clearly did not restrict taxa to distant refugia. In addition, the Cordilleran ice sheet did not reach its maximum extent in the lowlands until alpine glaciers were retreating (Waitt & Thorson, 1983). The varied topography of the Pacific Northwest, including its north-to-south-trending mountain ranges, probably allowed small populations to wait out unfavourable conditions of the LGM.

We used pollen records to complement our phylogeographical study because they provide a temporal and spatial context for interpreting results (Cruzan & Templeton, 2000; Petit *et al.*, 2002; López de Heredia *et al.*, 2007). Pollen record data indicate that oak populations were present at low densities in Oregon and Washington before and during the Fraser glaciation (25–10 kyr BP, Fig. 1; Table 1), a period when lowland ice lobes reached their maximum extent (15 kyr BP). These data suggest that during what would appear to be region-wide unfavourable conditions (15–14 kyr BP), *Q. garryana* was not extirpated from even the northern parts of its mainland geographical range (compare Fig. 1 and Table 1). For example, high pollen levels were found at Mosquito Lake Bog in northern Washington (48.77°N, 122.12°W, 198 m) from 15–9 kyr BP, providing evidence that *Q. garryana* maintained populations close to the ice sheet (Hansen & Easterbrook, 1974). The only portion of the current range of *Q. garryana* that was glaciated during the LGM is the Puget Sound region and San Juan Islands of Washington northward into British Columbia (Brubaker, 1991; Brown & Hebda, 2002). As the Juan de Fuca lobe of the Cordilleran ice sheet retreated, marked increases in oak at most peripheral sites occurred from 10–4 kyr BP due to regional warming and drying (Barnosky, 1985a; Sea & Whitlock, 1995). *Quercus garryana* probably colonized Vancouver Island at this time from nearby locations, as the oldest oak pollen record from Vancouver Island is from just under 11 kyr BP.

One previous study has examined the genetic diversity of *Q. garryana* across glaciated and unglaciated regions. This research used allozyme markers and suggested a weak but statistically significant increase in genetic divergence among populations with increasing physical distance (Ritland *et al.*, 2005). Allozyme polymorphisms indicate that populations on Vancouver Island and the Gulf Islands are more genetically similar to each other than they are to the populations from the species core, and that both allele richness and heterozygosity decline with increasing latitude.

In this study we investigated the biogeography of *Q. garryana* using microsatellite markers in the chloroplast and nuclear genomes. Microsatellites have the advantage of high levels of polymorphism at many loci, allowing for a fine-scale analysis of local and regional patterns of genetic diversity (Degen *et al.*, 1999), and maternally inherited chloroplast markers have the potential to reveal patterns of seed dispersal since the LGM. Thus, this study expands on that of Ritland *et al.* (2005) in that we include the biogeographical distribution of chloroplast haplotypes and have greater statistical power for revealing additional mechanisms for the patterns observed.

Our motivation for studying the biogeography of *Q. garryana* was to infer seed dispersal and pollen-flow patterns to understand the post-glacial colonization of peripheral regions, particularly where refugial populations were in close proximity to the post-glacial expanded range. Based on previous research and population models, we had different expectations for each marker system. We expected the genetic differentiation across sampling sites to be higher in the chloroplast markers than in the nuclear markers due to the strictly maternal inheritance of the chloroplast markers and the difference in pollen flow and seed dispersal (Petit *et al.*, 1993a; Ennos, 1994; Grivet *et al.*, 2008). Because known glacial refugia were close to the post-glacial colonization sites, we expected this difference to be less than is typically found for

Table 1 Presence of *Quercus garryana* pollen in lake sediments by 500-year interval.

	Site											
yr BP	A	B	C	D	E	F	G	H	I	J	K	L
500	+	+		+	+	+	+					
1000		+	+	+	+		+			+		
1500		+	+		+	+			+			
2000	+	+	+	+	+				+			
2500		+	+		+	+			+		+	
3000		+	+	+	+				+			+
3500		+	+	+	+		+					+
4000	+	+	+	+	+			+	+	+		
4500		+	+	+	+			+	+	+		+
5000	+		+	+	+			+	+			+
5500		+	+	+	+		+	+	+	+	+	
6000		+	+	+	+	+			+	+		+
6500	+	+	+	+	+	+		+	+	+		+
7000	+	+	+	+	+			+	+	+	+	
7500	+	+	+	+	+		+	+	+			
8000	+		+	+	+	+		+	+		+	
8500	+	+	+	+		+		+	+			
9000	+	+	+	+				+	+			
9500	+	+	+	+		+	+	+				
10,000	+	+		+		+		+			+	
10,500			+	+		+		+				
11,000		+		+		+		+		+		
11,500				+		+		+				
12,000				+	+	+		+				
12,500					+	+		+				
13,000		+										
13,500						+		+				
14,000								+				
14,500		+				+		+				
15,000				+		+		+				

Data were compiled from The National Climatic Data Center (http://www.ncdc.noaa.gov/paleo/pollen.html). Locations are marked with letters as in Fig. 1. Sites are organized from south to north on the mainland (A–I) and on Vancouver Island (J–L). +, At least one grain of oak pollen was present within the 500-year interval; empty cell, pollen was absent; grey-shaded cell, no data collected. Original sources for data: (A) Sea & Whitlock, 1995; (B) Worona & Whitlock, 1995; (C) Sea & Whitlock, 1995; (D) Barnosky, 1985b; (E) Barnosky, 1985a; (F) Barnosky, 1981; (G) Cwynar, 1987; (H) Hansen & Easterbrook, 1974; (I) Mathewes, 1973; (J) Brown & Hebda, 2002; (K) Brown & Hebda, 2002; (L) Brown & Hebda, 2003.

oak species, resulting from relatively high seed dispersal to areas that now represent the furthest reaches of the species' range. However, the unstable climatological history of the region made it difficult to predict how many recognizable regional clusters we would find.

Specifically, we sampled *Q. garryana* populations along a latitudinal gradient from the species' core to the poleward periphery, focusing on three questions: (1) Are peripheral populations genetically distinct from core populations and from one another? (2) Is genetic diversity reduced in northern peripheral populations relative to core locations? (3) Do the nuclear and chloroplast data indicate different patterns of pollen flow and seed dispersal? We examine our results in the context of the pollen sediment record for *Q. garryana* in order to understand how post-glacial colonization results in current spatial genetic patterns.

MATERIALS AND METHODS

Study species and study region

Quercus garryana is the sole oak species in oak–savanna ecosystems of the Pacific Northwest region of North America. The current distribution of *Q. garryana* ranges from isolated mountain areas in the Sierra Nevada, California, to the coastal regions and valleys of northern California and Oregon, along the Columbia River region of Oregon and Washington, the Puget Trough, Washington, and southern British Columbia (Fig. 1 inset). It is the only native oak species north of southern Oregon (Glendenning, 1944). At the northern range boundary on Vancouver Island, British Columbia, populations occur in fragmented patches surrounded by coniferous forest. At its extreme northern limit, *Q. garryana* occurs on rocky

outcrops and on southerly and westerly facing slopes where Douglas fir (*Pseudotsuga menzesii*) grows poorly. Towards the centre of the species' range, populations of *Q. garryana* are less isolated and fragmented, sometimes forming extensive woodlands.

These oak habitats harbour high botanical diversity and are threatened by coniferous encroachment, non-native species and anthropogenic disturbance (Thysell & Carey, 2001). *Quercus garryana* and its associated communities are of conservation concern, particularly in the northern portion of its geographical range (Ward *et al.*, 1998). Under current predictions of climate change, oak–savanna ecosystems are expected to expand their geographical ranges northward on Vancouver Island into areas currently occupied by coniferous forest (Hamann & Wang, 2006).

Sample collection

Twigs of *Q. garryana* were collected from 22 sampling sites throughout the northern half of the species range in May 2005 and February and April 2007 (Fig. 1). Sampling locations included Siskiyou Mountain and Rogue River regions, Willamette Valley, Puget Trough, the San Juan Islands, Vancouver Island and Hornby Island. At each sample site, twigs were collected from nine to 22 trees at least 20 m apart. Twigs collected in 2005 were shipped to the laboratory on water ice and frozen at −80°C until DNA extraction. In 2007, most twigs were cut and dried on silica gel to maintain integrity of the DNA, although a small subset of the 2007 samples were shipped fresh to the laboratory.

DNA extraction and genotyping

We ground fresh, frozen and dried twigs under liquid nitrogen using a Freezer/Mill 6850 (Spex CertiPrep Group, Metuchen, NJ, USA). Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol with the following modification: twig powder in extraction buffer was shaken in a tube with a 1/4-inch cylindrical ceramic bead (Qbiogene, Montreal, Canada) in a FastPrep FP 120 (Qbiogene) for three cycles of 15 s each to increase cell lysing.

We used five chloroplast microsatellite markers developed for *Quercus petraea* (Mattuschka) Liebl. and *Quercus robur* L. (Deguilloux *et al.*, 2003) to reveal chloroplast haplotypes. Seven nuclear microsatellite markers, five developed from *Quercus rubra* L. (Aldrich *et al.*, 2002, 2003a) and two from *Q. petraea* (Steinkellner *et al.*, 1997), were used to amplify microsatellite loci in *Q. garryana* (see Appendix S1 and Table S1 in Supporting Information). We performed the polymerase chain reaction (PCR) for both chloroplast and nuclear markers in a total volume of 25 μL containing 5 U GoTaq Flexi (Promega, Madison, WI, USA), 1× colourless GoTaq Flexi buffer, 2.5 mM $MgCl_2$, 200 nM each dNTP (Fermentas, Burlington, Canada), and 5 pM forward and reverse primer. The PCR protocol consisted of a 2-min initial denaturing step at 95°C followed by 35 cycles of 30 s at 95°C, 30 s at marker-specific annealing temperatures (Table S1), and 30 s at 72°C, then a 10-min final elongation at 72°C. Microsatellite fragment lengths were detected using forward primers labelled with WellRED dye (Sigma-Aldrich, St Louis, MO, USA) on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). For each marker, we ran samples once on plates with positive and negative controls, to determine if the fragment lengths were accurate and free of contamination. If amplified fragments were difficult to score, PCR was performed again for the given sample and marker combination. Samples that did not amplify or that remained too difficult to score accurately were discarded. Over all repeated sample and marker combinations we had a genotyping error rate of 0.5%.

Statistical analyses

We used Arlequin 3.1 (Excoffier *et al.*, 2005) to examine population substructure through an analysis of molecular variance (AMOVA) (10,000 permutations) for the chloroplast microsatellite data. Also, we determined within-population diversity (H_S), overall diversity (H_T), and population differentiation (G_{ST}) using the program cpSSR (http://www.pierroton.inra.fr/genetics/labo/Software/PermutCpSSR/index.html) following Pons & Petit (1995, 1996).

For the nuclear data, we used Arlequin 3.1 to test for departures from Hardy–Weinberg (HW) equilibrium, generate observed (H_o) and expected (H_e) heterozygosity, and examine population substructure using AMOVA (10,000 permutations). Micro-checker (van Oosterhout *et al.*, 2004) was used to investigate the possibility of null alleles at each locus for each sample location. This program randomly assigns alleles to genotypes and compares the random assignments with the observed allele frequencies. If the marker shows HW disequilibrium, and an excess of homozygotes is spread evenly across the alleles, Micro-checker makes a probabilistic determination of null alleles. In addition, we obtained null allele frequency using the Brookfield 1 estimate (Brookfield, 1996) in Micro-checker.

For the nuclear data, we used Structure 2.2 (Pritchard *et al.*, 2000) to investigate population substructure using a Bayesian approach that calculates the likelihood of the data given K groups. We evaluated K as $K = 1$ to $K = 10$, and the value of K with the highest log-likelihood probability was selected as the most likely to represent real groups. We ran a set of models in which individuals could have admixed ancestry and independent allele frequencies ($\lambda = 1$). We replicated runs for each of the ten models ($K = 1$–10) a minimum of three times to ensure consistent natural log probabilities, and ran each model with a 100,000-step burn-in phase and a 1,000,000-step Markov chain Monte Carlo data collection. We then ran AMOVA with the regional groupings defined in Structure to investigate the proportion of genetic variation explained between the discernable groups.

We generated F_{ST} values for pairwise population comparisons in Arlequin 3.1, and using the program PASSAGE (Rosenberg, 2001) we performed a Mantel test (Mantel, 1967; Smouse *et al.*, 1986) to correlate the F_{ST} values with geographical distance. To determine a more spatially refined relationship between genetic and geographical distance, we conducted Mantel correlograms for both nuclear and chloroplast data using 100-km interval geographical distance classes. We calculated a pollen seed-flow ratio based on F_{ST} values following Ennos (1994).

Table 2 Six chloroplast haplotypes of *Quercus garryana* described by the amplified fragment length of each of the five repeat loci.

Haplotype	*µcd4*	*µcd5*	*µdt1*	*µdt3*	*µdt4*
A	96	75	83	126	148
B	96	75	83	126	146
C	96	75	82	126	148
D	96	75	82	125	147
E	95	75	82	125	147
F	94	74	84	124	146

RESULTS

In five chloroplast microsatellites, we detected two to three alleles at each marker and a total of six haplotypes over 22 sampling locations (Fig. 2; Table 2). Three of the haplotypes were common and were found at multiple locations; the remaining three were found at only one sample site each (Fig. 2; Table 3). Within-population diversity (H_S) was low (0.08 ± 0.03 SE), overall diversity (H_T) was higher (0.67 ± 0.06 SE), and population differentiation (G_{ST}) was high (0.88 ± 0.05 SE; Fig. 2; Table 4). At 16 out of the 22 chloroplast sampling sites, all individuals within the population shared a single chloroplast haplotype.

The seven nuclear microsatellite loci ranged from six (*quruGA*-1M17) to 17 alleles (ssrQpZAG 9) (Table S1). For the 22 sites genotyped with nuclear microsatellite markers, the overall allelic richness ranged from 27 alleles (sites 16 and 18) to 43 alleles (site 3) (Fig. 3). The allelic richness decreased significantly with increasing latitude ($R^2 = 0.63$, $P < 0.001$) (Fig. 3). This reduction in the number of alleles at the range periphery was due to a lack of alleles that are rare in the species core. Forty-two of the 61 total alleles were found across the entire study region. Of the remaining 19 alleles, 11 were unique to southern Oregon. We found five alleles that were not represented in southern Oregon samples: two alleles further

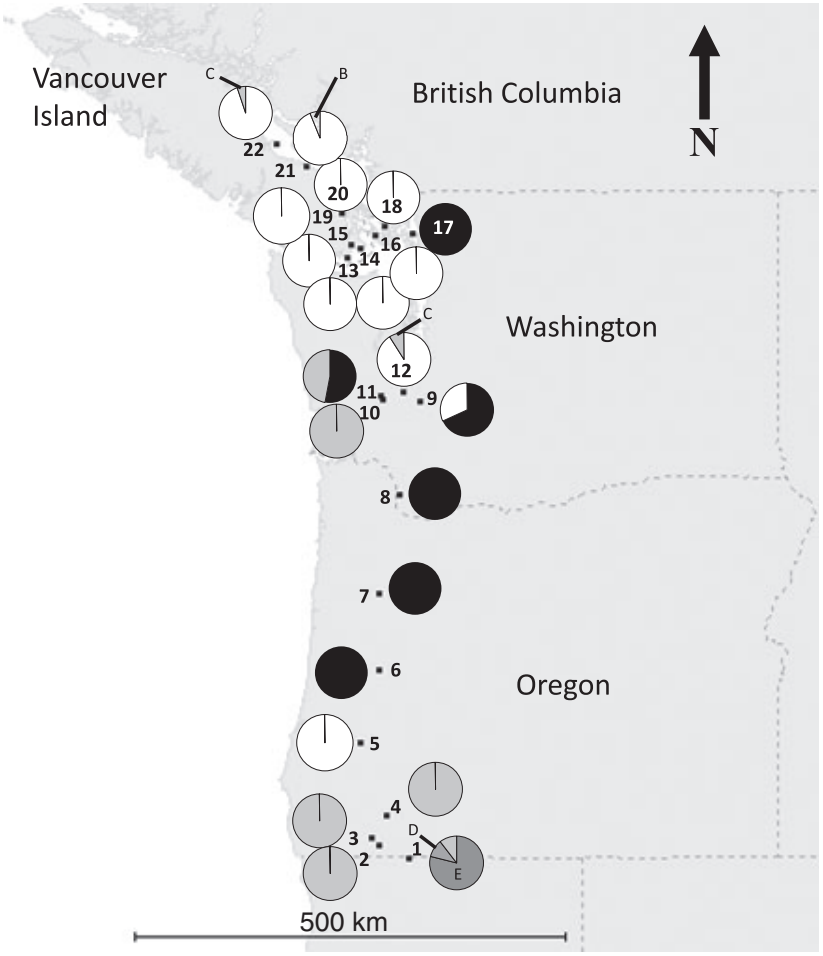

Figure 2 Chloroplast haplotypes of *Quercus garryana* from 22 sampling sites. Haplotypes A = white, C = light grey, F = black are the predominant haplotypes over the study region. Haplotypes B (one individual at site 22 only), D (two individuals at site 1 only), E (17 individuals at site 1 only) are less common and are marked on the map.

Table 3 Distribution table of the six chloroplast haplotypes found for *Quercus garryana* by sample site.

	Haplotype						
Sample site	A	B	C	D	E	F	∑
1			2	2	15		19
2			19				19
3			15				15
4			17				17
5	17						17
6						9	9
7						22	22
8						15	15
9	7					15	22
10			16				16
11			9			10	19
12	20		2				22
13	16						16
14	18						18
15	19						19
16	13						13
17						16	16
18	12						12
19	18						18
20	18						18
21	16	1					17
22	18		1				19
∑	192	1	81	2	15	87	378

Sites 1–22 correspond to those in Fig. 1.

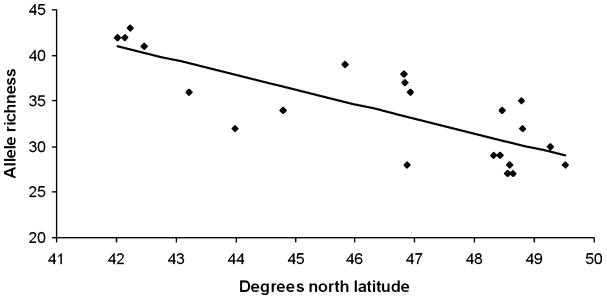

Figure 3 Correlation between latitude and allele richness combined over all seven nuclear microsatellite loci. $R^2 = 0.63$, $P < 0.001$.

north in both mainland and peripheral island locations, two alleles further north confined to the mainland, and one allele limited to the peripheral island populations. An additional three alleles were found in all regions except the peripheral islands. Pairwise F_{ST} values ranged from 0.000 to 0.184 among all 22 sampling sites, with a mean F_{ST} of 0.049 ($P < 0.0001$).

Observed heterozygosity from each sample site averaged over all loci ranged from 0.44 to 0.66 (Table 5). Significant departures from HW equilibrium ($P < 0.05$) occurred in at least one sampling site for each marker. Markers *quru*-0G12 and *quru*-1M17 showed significant departures at one sampling site each; ssrQpZAG 36 and ssrQpZAG 9 showed significant departures at two sampling sites; *quru*-1G13 showed departures at three sites; *quru*-0C19 showed departures at four sites; and *quru*-0M05 showed departures at six sites (Table S2). Departures from equilibrium caused by homozygote excess were commonly found to be due to null alleles. Micro-checker analyses showed that short allele dominance or scoring errors due to stutter are not implicated in our markers. Instead, we found that marker *quru*-0M05 exhibited evidence for null alleles at six of the 22 sampling sites, and markers *quru*-0C19 and ssrQpZAG 9 exhibited null alleles at two sites each (Table S2). At the site and marker combinations showing null alleles, frequencies ranged from 0.11 to 0.29 (Table S2). Site 8 showed evidence for null alleles in two markers, but the other eight sites with null alleles had them only in one marker each. The presence of null alleles has the potential to bias results, but because of their low number (10 of the 154 marker and site combinations show a tendency for null alleles with frequency > 0.1) and haphazard distribution across markers and sample sites in our study, we interpret our results without modification. Departures from HW equilibrium at markers and sites not exhibiting null alleles could be due to the relatively small sample number per location. It is not likely that departures from equilibrium are the result of inbreeding.

Bayesian analyses in Structure indicated the data are most likely when $K = 2$ ($P > 0.999$) (Fig. 4). In the two-cluster case, all populations north of approximately 48° north latitude grouped together. This northern cluster also contained one Oregon population (7) and one southern Washington population (12) (Fig. 4). All other populations group together in a single southern cluster. For the AMOVA given two groups,

Table 4 AMOVA for both chloroplast and nuclear microsatellite data for populations of *Quercus garryana*.

	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	P
Chloroplast	Among populations	21	104.7	0.287	86.07	0.861	< 0.0001
	Within populations	356	16.6	0.047	13.93		
	Total	377	121.3	0.334			
Nuclear	Among populations	21	113.3	0.109	4.94	0.049	< 0.0001
	Within populations	646	1352.7	2.094	95.06		
	Total	667	1466.0	2.203			

Significance of variance components assessed with 10,000 permutations.

Table 5 Summary statistics of nuclear markers at 22 *Quercus garryana* sampling sites.

Sample site	*n*	Mean H_e/H_o	Mean *A*
1	16	0.61/0.55	6.000
2	19	0.61/0.58	6.000
3	17	0.67/0.64	6.143
4	17	0.66/0.61	5.857
5	15	0.58/0.49	5.143
6	8	0.64/0.57	4.571
7	17	0.64/0.66	4.857
8	13	0.61/0.44	5.571
9	15	0.67/0.62	5.429
10	15	0.61/0.59	5.286
11	14	0.57/0.56	4.000
12	20	0.64/0.58	5.143
13	15	0.57/0.50	4.143
14	16	0.55/0.55	4.143
15	16	0.58/0.51	4.857
16	12	0.56/0.51	3.857
17	12	0.54/0.48	4.000
18	12	0.53/0.44	3.857
19	17	0.59/0.56	5.000
20	16	0.58/0.49	4.571
21	17	0.54/0.52	4.286
22	15	0.56/0.57	4.000

Sample site numbers refer to sites shown in Fig. 1. Mean *A* is the number of alleles averaged over all seven loci. Mean H_e and H_o per locus are shown in Table S2.

significant levels of variation were explained within sampling site, within regions among sampling sites, and between the two regions (Table 6). Yet over 90% of the genetic variation was captured within sampling sites, indicating that all sites had a high level of genetic diversity (Table 6). Very little (*c.* 6%) of the nuclear diversity is explained among populations. The pattern driving the differences between regions was due, in part, to lower allelic diversity at the range periphery (Fig. 3). A weaker, but similar pattern was observed for levels of heterozygosity (not shown).

Geographical distances were significantly correlated with both chloroplast (Mantel test: 10,000 permutations, rM = 0.402, $P = 0.0012$) and nuclear genetic distance (Mantel test: 10,000 permutations, rM = 0.622, $P = 0.0001$). Mantel correlograms showed a significant positive genetic and geographical relationship within the first 200 km for both chloroplast and nuclear data (Fig. 5). Likewise, for both marker systems a significant negative correlation was detected between 700 and 900 km (Fig. 5). The only discrepancy in the relationship with geographical distance between the two genomes is at the 600–700-km range. The nuclear data show a negative correlation with geographical distance, whereas the chloroplast data show a positive correlation. This positive correlation with chloroplast data is driven by a shared haplotype from a site in southern Oregon (haplotype 1, site 5) with sites on Vancouver Island. From our data we estimated pollen to flow at a rate of *c.* 120 times that of seed dispersal.

DISCUSSION

The chloroplast markers harboured very little genetic diversity within a sampling location. Genetic differentiation among sampling sites was high, as has been found for oak by other investigators (Dumolin-Lapègue *et al.*, 1997; Romero-Severson *et al.*, 2003; Deguilloux *et al.*, 2004; Grivet *et al.*, 2006). Only a single sampling location had three haplotypes represented, and the majority of sites had only a single haplotype. The regional pattern of haplotype distribution includes the lack of mainland haplotypes on the peripheral islands, providing a signal consistent with post-glacial expansion. All haplotypes are represented towards the core of the distribution, whereas populations at the northern periphery retain a subset of haplotype diversity. In addition, our data support the 'leading-edge hypothesis' of Soltis *et al.* (1997) because the single predominant haplotype found on the glaciated island locations is the one that is represented in a subset of nearby unglaciated mainland localities. The low level of haplotype diversity also plays an important role in determining distances at which populations show significant genetic correlations. Virtually all peripheral populations share a single haplotype. In addition, over the whole study region a given site usually shares the same predominant haplotype with its nearest neighbour. Therefore, sites closest to one another (even over relatively large spatial scales of up to 200 km) exhibit positive genetic correlations with geographical distance.

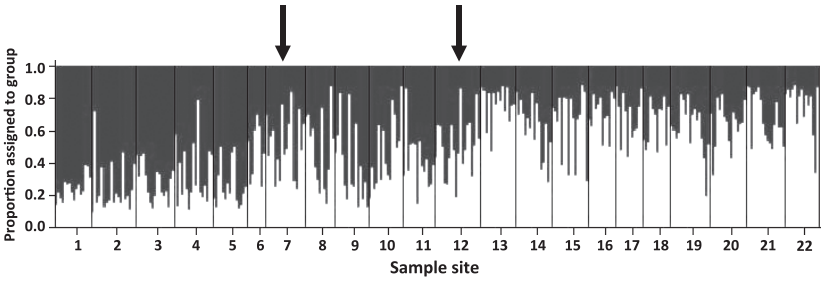

Figure 4 Degree of admixture per individual across a latitudinal gradient (site 1–22; refer to Fig. 1). Model $K = 2$ with admixture (shown as assignment probabilities for the proportion grey to white per sample). Sampling locations with black arrows (7 and 12) denote sites that are located in the species core but have a greater proportion of the individuals assigned to the northern cluster.

Table 6 AMOVA for *Quercus garryana* nuclear microsatellite data including a two-region grouping selected from groups assigned in the Bayesian cluster approach (Structure 2.2).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	P
Between regions	1	26.7	0.068	3.03	0.030	0.0001
Among populations within region	20	86.6	0.074	3.30	0.034	< 0.0001
Within populations	646	1352.7	2.094	93.67	0.063	< 0.0001
Total	667	1466.0	2.236			

Significance of variance components assessed with 10,000 permutations.

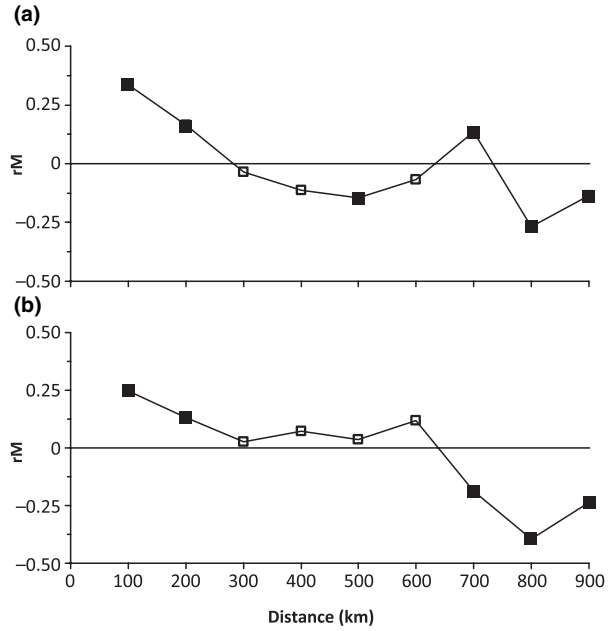

Figure 5 Mantel correlograms from chloroplast (a) and nuclear (b) data. Filled squares indicate significant correlations between genetic and geographical distance at $P < 0.05$. Points above the zero line are positively correlated; those below the line are negatively correlated.

The peripheral island samples are homogeneous for chloroplast haplotypes, with two exceptions. At the second most northern site (site 21), one individual possessed a unique haplotype. The Bayesian assignment test groups this individual with the others in its sample site, indicating that it might be an *in situ* mutation. At the most northerly site (site 22), however, another single individual had a haplotype found only in more southerly mainland populations. The nuclear assignment test for this individual groups it with the core cluster with a higher probability than with the northern cluster (Fig. 4). We postulate that this tree is a more recent colonist to this site, and could have been planted by humans since this location is managed as a community park of a rural residential development.

Unique haplotypes also occur at the most southerly site in Oregon, and we postulate that these are *Q. garryana* var. *fruticosa* (Engelm.) Govaerts. *Quercus garryana* var. *fruticosa* (formerly and occasionally called var. *breweri*) is a high-elevation, shrubby variety of *Q. garryana*, long recognized as being taxonomically separate from *Q. garryana* var. *garryana* (Watson, 1880). Because we do not have other *Q. garryana* var. *fruticosa* samples for comparison, this is only a suggestion.

Similarly to the patterns observed for the chloroplast microsatellites, we determined from nuclear microsatellite markers that allelic richness was reduced at the range periphery. We also observed latitudinal shifts in the frequency of common alleles. This reduction of alleles and allele frequency change at the range periphery is a signature of founder events during post-glacial colonization (Tomaru *et al.*, 1997). Also similarly to the chloroplast data, we found that genetic distance was positively correlated with geographical distance within the first 200 km. This provides evidence that gene flow may be counteracting the effects of genetic drift over a reasonably large spatial scale, a different interpretation from a similar pattern from the chloroplast markers. Genetic and geographical distances were negatively correlated beyond 600 km, indicating that at spatial scales far larger than the expected size of a pollination neighbourhood, genetic drift has a stronger influence than migration. Still, the greatest amount of genetic diversity remains at the population level, and all predominant alleles present on the mainland were also present at the periphery of the species' range and were dramatically different from the chloroplast data. Thus, the level of pollen flow within and across sites has been sufficiently high to blur the genetic identity of founder populations over geographical distances less than 600 km. These findings of weak differentiation in the nuclear markers over large spatial scales are in agreement with the general patterns of allozyme diversity observed by Ritland *et al.* (2005).

Populations in the periphery are recognizably distinct from core populations, but are similar to each other. Our data support the supposition that colonization to the peripheral islands came from few mainland colonists and from one or few source populations. The apparently low-level of cross-colonization by seed among glacial source populations, given the age of these populations, provides evidence that effective seed dispersal in this species is limited. High nuclear diversity and little genetic differentiation due to high levels of pollen flow within our peripheral study region, however, are not unexpected, given that oaks persisted very near the ice sheet in unglaciated peripheral sites throughout the glacial period (e.g. site H in Table 1).

Even with the palynological evidence that *Q. garryana* persisted in populations near the ice sheet during the last glacial period, we still observe the loss of haplotypes in the glaciated range and high levels of chloroplast haplotype genetic differentiation. Our pollen-seed flow ratio of *c.* 120 is slightly less than (but of the same order of magnitude as) has been found for European oaks (Ennos, 1994), but it is higher than for *Q. lobata* in California (Grivet *et al.*, 2008). We suggest that water between the mainland and island sites prevented greater seed dispersal to the peripheral islands, effectively increasing the distance between the two regions. Taking all our analyses into account, we found evidence for large pollination neighbourhoods (two clusters with considerable admixture among individuals within and across sites) and smaller seed-dispersal neighbourhoods with early, infrequent colonization of the post-glacial peripheral island sites.

It is important to recognize that low population differentiation determined from nuclear markers and high differentiation from chloroplast markers can occur even in situations of low pollen flow for tree species. Firstly, the effective population size of the chloroplast genome is one-quarter that of the nuclear genome (Muir *et al.*, 2004). In addition, founding populations may no longer be small at the time of seed reproduction. For example, Austerlitz *et al.* (2000) modelled tree colonization and found that when the time to first reproduction is taken into account (often *c.* 50 years for oaks), founder effects were reduced by the accumulation of migrants over time, forming a large founding population with much of the genetic diversity of the source. In our study system, if founding events on the islands were common, we might expect to see more mainland haplotypes represented on the islands (those found at sites 9–12 and 17 in Fig. 2). We do not. Therefore, founding events were probably rare, and pollen flow is the likely mechanism for high nuclear diversity within peripheral populations. In addition, it has been shown with stochastic modelling that in situations with minimal seed dispersal, pollen flow has a strong homogenizing effect among populations (Austerlitz & Garnier-Géré, 2003).

We have low chloroplast haplotype richness across our entire study region, even with sample sizes larger than is typical for chloroplast haplotyping. We found very little within-population diversity and high levels of differentiation (in agreement with other studies) with approximately four times the number of samples per site than in many previous studies (Dumolin-Lapègue *et al.*, 1997; Romero-Severson *et al.*, 2003; Deguilloux *et al.*, 2004; Grivet *et al.*, 2006). We observed 14 total alleles across our five loci, indicating that chloroplast mutation rates are high enough to detect population-level differences.

CONCLUSIONS

In *Q. garryana*, chloroplast haplotype and nuclear allele richness in the glaciated island periphery was lower than that found in unglaciated areas where oak persisted. Low chloroplast diversity on the mainland was reduced to a virtual monomorphism in the island periphery. Although nuclear allele richness was reduced in peripheral populations due to the loss of mainland rare alleles, nuclear diversity within populations remains high. This suggests that regionally high pollen flow counteracts the effects of drift due to founder effects. As the current climate changes, a poleward geographical range shift may be possible for *Q. garryana* if seed dispersal limitation is overcome. After successful colonization of new localities, long-distance pollen flow to the new populations is likely to restore regional genetic diversity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Primer sequences, repeat motifs, annealing temperatures, allelic size ranges and number of alleles for all nuclear and chloroplast markers used in this study, along with their original citation and GenBank accession numbers.

Table S2 Heterozygosity and null allele frequency per locus for each sample site.

Appendix S1 A description of nuclear marker screening and selection.

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BIOSKETCHES

Travis D. Marsico researches dispersal and colonization to inform predictions regarding future responses to environmental change and limitations to geographical ranges using experimental and genetic approaches. Species' peripheries are of particular interest due to the fragmented nature of the habitat and often marginal environmental conditions.

Jessica J. Hellmann studies the responses of populations and species to global change with a focus on ecological and evolutionary processes that limit range expansion. Her research considers insects, plants and their interactions, and management strategies for reducing negative effects of global change.

Jeanne Romero-Severson studies the role of genetic drift and selection in shaping the biogeographical distribution and population substructure of forest trees. Her current research interests include the effects of Pleistocene glaciations on the genetic structure of North American red oaks (Lobatae) and the effects of epidemic disease on the introgression of Japanese walnut into butternut, a related species native to eastern North America.

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