Isozyme variation and the conservation genetics of Garry oak

K. Ritland, L.D. Meagher, D.G.W. Edwards, and Y.A. El-Kassaby

Abstract: Garry oak (*Quercus garryana* Dougl. ex Hook) has a long north–south distribution along the inland Pacific coast. In British Columbia, it is a keystone species in a unique and endangered "Garry oak meadow" ecosystem. Here, we apply isozyme markers to address issues in the conservation and phylogeography of Garry oak. Among 42 populations and 23 gene loci, gene diversity (expected heterozygosity) averaged 0.17, and number of alleles per locus averaged 1.84. These values are about one-half of those found in other white oak species. Using progeny arrays, we found outcrossing rates in two Vancouver Island populations to average 0.96, with no detectable biparental inbreeding; also inbreeding coefficients of the 42 populations averaged near zero (0.025); thus inbreeding is not a significant concern. Cluster analysis of genetic distances identified two major groups of populations: southern Washington – Oregon and Vancouver Island – Gulf Islands; populations within the island region were particularly homogenous. An isolated mainland British Columbia population near Sumas, British Columbia, perhaps of anthropogenic origin, showed the least genetic variability and greatest genetic distance. Generally, geographically isolated populations were more genetically depauperate, which may place handicaps on their survival, but also more genetically distinct, providing a greater evolutionary legacy to the species.

Key words: isozymes, genetic diversity, outcrossing rates, population structure, gene conservation, Garry oak.

Résumé : Le chêne de Garry (*Quercus garryana* Dougl. ex Hook) est distribué selon un axe nord–sud, le long de la côte du Pacifique. En Colombie-Britannique (BC), c'est une espèce de référence dans l'écosystème menacé exceptionnel de « Garry oak meadow ». Les auteurs ont utilisé des marqueurs isozymiques pour étudier la phylogéographie et les problèmes de conservation du chêne de Garry. Parmi 42 populations et 23 lieux génétiques, la diversité génétique (hétérozygocitée attendue) moyenne est de 0,17, et le nombre d'allèles moyen par locus est de 1,84. Ces valeurs sont environ la moitié de celles qu'on retrouve chez d'autres espèces de pin blanc. En utilisant le réseau des progénitures, on constate que les taux de croisement externes, dans deux populations de l'Île de Vancouver, sont en moyennes de 0,96, sans apparence de consanguinité biparentale; de plus, le coefficient de consanguinité des 42 populations est près de zéro (0,025); ainsi la consanguinité ne pose pas de problème. L'analyse par regroupement des distances génétiques fait ressortir deux grands groupes de populations : Washington–Oregon méridional et Île de Vancouver – Îles du Golfe; les populations de la région des îles sont particulièrement homogènes. Une population continentale isolée en BC, près de Sumas, possiblement d'origine anthropique, montre la variabilité génétique la plus faible et la plus forte distance génétique. En général, les populations géographiquement isolées sont plus fortement appauvries génétiquement, ce qui pourrait compromettre leur survie, mais à la fois plus fortement distinctes génétiquement, ce qui confère un héritage évolutif plus important à l'espèce.

Mots clés : isozymes, diversité génétique, taux de croisement, structure des populations, conservation des gènes, chêne de Garry.

[Traduit par la Rédaction]

Introduction

Garry oak (*Quercus garryana* Dougl. ex Hook), also known as Oregon white oak, is a deciduous oak species native to western North America (Pavlik et al. 1992), and is the only oak found in Washington State and British Columbia (BC). Occurring from Vancouver Island to California, it is a persistent climax or subclimax species on dry sites, or under regimes of periodic fire. In Washington and British Columbia, Garry oak populations are typically disjunct and scattered. On southeastern Vancouver Island and the neighboring Gulf Islands, Garry oak is associated with a unique suite of plant species, comprising the "Garry oak meadow" (Fuchs 2001). Urban development, and some agricultural development, has left less than 8% of this ecosystem unmodified, and even this proportion has been degraded by fragmentation, human use, and introduced species (Ward et al. 1998).

Kremer and Petit (1993) found oaks to display levels of isozyme diversity that are among the highest in woody plants, with average expected heterozygosity of 0.186 across 36 species, a level comparable to conifers. However, among *Quercus* species, there is wide variation of heterozygosity, with a range of 0.083–0.398 for white oaks and 0.058–0.319 for red oaks. While no studies of Garry oak isozyme genetic variation have been conducted, in two other California species (*Quercus lobata* Née–valley oak; *Quercus dou-*

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K. Ritland[1] and Y.A. El-Kassaby. Department of Forest Sciences, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada.
L.D. Meagher and D.G.W. Edwards. Pacific Forestry Centre, 506 W. Burnside Road, Victoria, BC V8Z 1M5, Canada.

[1]Corresponding author (e-mail: Kermit.Ritland@ubc.ca).

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Table 1. Geographic locations, gene diversity (heterozygosity *H*, number of alleles per locus N_a, and percentage of polymorphic loci at 95% criterion PLP95) and inbreeding coefficients (*F*) for *Quercus garryana.*

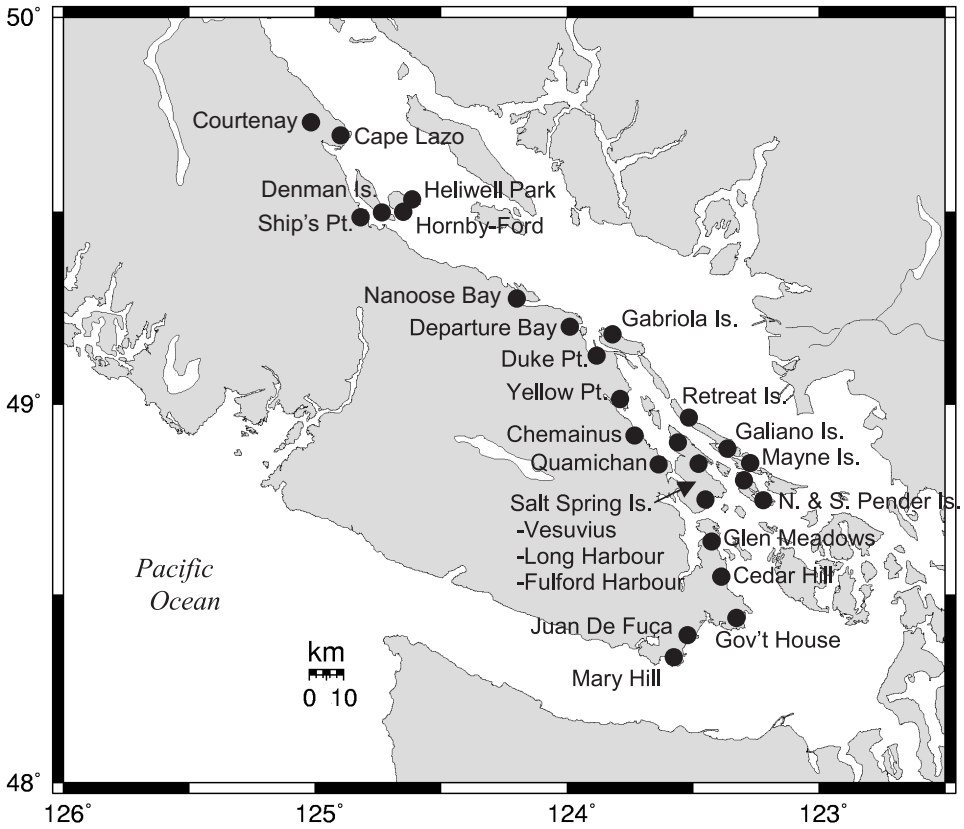
Population	Latitude	Longitude	*H*	N_a	PLP95	*F*
Courtenay	49°45′N	125°01′W	0.143	1.83	0.52	0.087
Cape Lazo	49°42′N	124°52′W	0.137	1.74	0.43	0.047
Yale	49°34′N	121°24′W	0.161	1.65	0.48	–0.163
Helliwell Pk.	49°32′N	124°36′W	0.157	1.70	0.39	0.021
Ships Pt.	49°30′N	124°48′W	0.150	1.70	0.43	0.103
Denman Is.	49°30′N	124°45′W	0.134	1.78	0.48	–0.027
Hornby-Ford	49°30′N	124°41′W	0.113	1.83	0.35	0.094
Nanoose Bay	49°17′N	124°09′W	0.157	1.78	0.48	–0.132
Gabriola Is.	49°13′N	123°46′W	0.182	1.87	0.52	0.066
Departure Bay	49°13′N	123°52′W	0.173	1.87	0.57	–0.011
Duke Pt.	49°09′N	123°53′W	0.191	1.91	0.48	0.109
Sumas Mtn.	49°06′N	122°07′W	0.137	1.74	0.43	–0.119
Yellow Pt.	49°02′N	123°45′W	0.166	1.83	0.61	0.092
Retreat Is.	48°56′N	123°30′W	0.196	1.78	0.61	0.099
Chemainus	48°55′N	123°43′W	0.219	2.00	0.57	0.081
Vesuvius Bay	48°53′N	123°34′W	0.194	1.87	0.57	0.015
Long Harbour	48°52′N	123°26′W	0.155	1.70	0.48	0.089
Galiano Is.	48°52′N	123°19′W	0.197	1.96	0.57	–0.011
Mayne Is.	48°51′N	123°15′W	0.185	1.83	0.48	0.040
Quamichan	48°47′N	123°34′W	0.173	2.04	0.52	0.001
Fulford Harbour	48°46′N	123°26′W	0.168	1.87	0.48	–0.064
N. Pender Is.	48°45′N	123°16′W	0.181	1.87	0.52	0.040
S. Pender Is.	48°44′N	123°11′W	0.174	1.87	0.52	0.059
Glen Meadows	48°38′N	123°27′W	0.155	1.74	0.52	–0.102
Juan de Fuca	48°27′N	123°28′W	0.187	2.04	0.52	0.092
Cedar Hill	48°26′N	123°21′W	0.152	1.96	0.52	0.052
Gov'ment Hse.	48°24′N	123°20′W	0.161	1.87	0.52	0.056
Mary Hill	48°20′N	123°32′W	0.168	2.00	0.48	–0.058
Bellingham	48°40′N	122°30′W	0.160	1.70	0.43	–0.013
Oak harbor	48°17′N	122°35′W	0.136	1.74	0.30	–0.091
Sequim	48°05′N	123°05′W	0.155	1.65	0.39	–0.106
Fort Lewis	47°05′N	122°33′W	0.197	1.87	0.61	0.070
Centralia	46°42′N	123°00′W	0.181	1.74	0.57	0.063
Kalama	46°08′N	122°53′W	0.154	1.70	0.57	0.033
Salem	44°45′N	123°55′W	0.163	1.78	0.52	0.201
Corvallis	44°31′N	123°20′W	0.172	1.96	0.48	0.029
Eugene	43°59′N	122°58′W	0.184	1.87	0.52	0.029
Roseburg	43°15′N	123°30′W	0.214	1.96	0.61	0.016
Medford	42°15′N	122°45′W	0.209	2.04	0.57	0.107
Yreka	41°51′N	122°35′W	0.170	1.74	0.48	–0.039
Weed	41°26′N	122°29′W	0.191	1.91	0.61	0.055
Redding	40°40′N	122°38′W	0.193	2.04	0.48	0.149
Mean			0.170	1.84	0.51	0.025
SE (mean)			0.004	0.018	0.011	0.012

glasii Hook. and Arn.–blue oak), Riggs et al. (1991) found comparatively little genetic variation. Baseline information about levels of isozyme genetic variation in Garry oak is needed.

Acorns are large and not passively dispersed; animal and possibly human dispersal (Turner and Bell 1971) determine rates of gene flow. Garry oak acorns are a favorite food of western gray squirrels, acorn woodpeckers, and black tailed deer (Coblentz 1980), and were also a significant food resource for indigenous peoples (Turner and Bell 1971; Norton 1979; Boyd 1999). A previous study of biochemical and morphological features of foliage found minor genetic differences among oak populations in Washington State (Taylor and Ross 1975). A correlation between locations of Native American communities and stands of Garry oak was found, leading these authors to speculate that these people distributed seed from a common source. Data on genetic markers might shed light on such hypotheses about anthropogenic influences upon the genetic structure of this species.

While Garry oak is monoecious, male and female flowers are borne on separate axes (Kaul 1985). Despite anomeophilous pollination, Bacilieri et al. (1996) found almost com-

Fig. 1. Collection sites on British Columbia islands for Garry oak (*Quercus garryana*).



plete outcrossing in *Quercus petraea* (Mattushka) Lieblein (sessile oak) and *Quercus robur* L. (pedunculate oak) fruit crops. However, matings between related individuals (biparental inbreeding) has been detected (Hokanson et al. 1993). Again, this component of the population genetics of Garry oak has not been previously documented. As inbreeding can have detrimental effects upon the growth and survival of plant species (Lynch and Walsh 1998), aspects of the population survival of Garry oak may depend upon its mating system.

The purpose of the present study was to (*i*) document levels of isozyme genetic variability, and compare these levels with those of other oak species; (*ii*) determine the partitioning of genetic variation among populations; (*iii*) evaluate evidence for anthropogenic factors in forming this structure; and (*iv*) infer levels of inbreeding within populations of this species. Knowledge of the genetic diversity, population structure, and mating system of Garry oak can enable more effective conservation strategies, and enhance appreciation of the genetic uniqueness of Garry oak populations.

Materials and methods

Samples from 28 populations were collected from November 1996 to February 1997. Sampled locations were chosen to cover the range of the distribution in BC (Table 1; Figs. 1 and 2); herein, non-mainland populations from BC will be termed "BC Islands" populations. Some outer BC Island populations (Saturna, Valdes) were not sampled because of access limitations. No Garry oaks were found in one previously documented location, south Surrey, despite intensive searches. In January 1998, 14 populations were also sampled in Washington, Oregon, and northern California, USA (Fig. 2). Because of the endangered status of BC populations, sampling was more intensive in BC compared with the United States. The co-authors D.G.W.E. and M.D.M. conducted these collections.

Dormant winter buds were collected from natural populations without regard to tree form or health. Using a long-handled pole pruner, at least 12 twigs per tree were collected. In most cases, at least 30 trees per population were sampled; care was taken to avoid sampling clumps of trees that might have arisen from one parent because of stump or root sprouting or animal caching (Stein 1990). Buds were placed in refrigerated storage (2–4 °C) until delivery to the isozyme lab. For mating-system analysis, acorns were nursery sown from about 25 trees collected in the fall of 1998 from each of two populations. After 1 year of growth, dormant buds from 10 progeny in each family were sampled for isozyme extraction.

Several buds from each tree, stripped of scales, were used as the tissue source. Protein extraction was conducted by crushing the tissues in liquid nitrogen. Extraction buffers and their running conditions were as described by El-Kassaby and Yanchuk (1994). The following enzyme systems and loci were assayed: phosphoglucoisomerase loci (two loci: Pgi1, Pgi2), malic enzyme (Me), esterase (three loci: Est1, Est2, Est3), phosphoglucomutase (Pgm), leucine amino-peptidase (Lap), alcohol dehydrogenase (Adh), isocitrate dehydrogenase (two loci: Idh1, Idh2), fructose bisphos-

Fig. 2. Collection sites in mainland Canada and the United States for Garry oak (*Quercus garryana*).

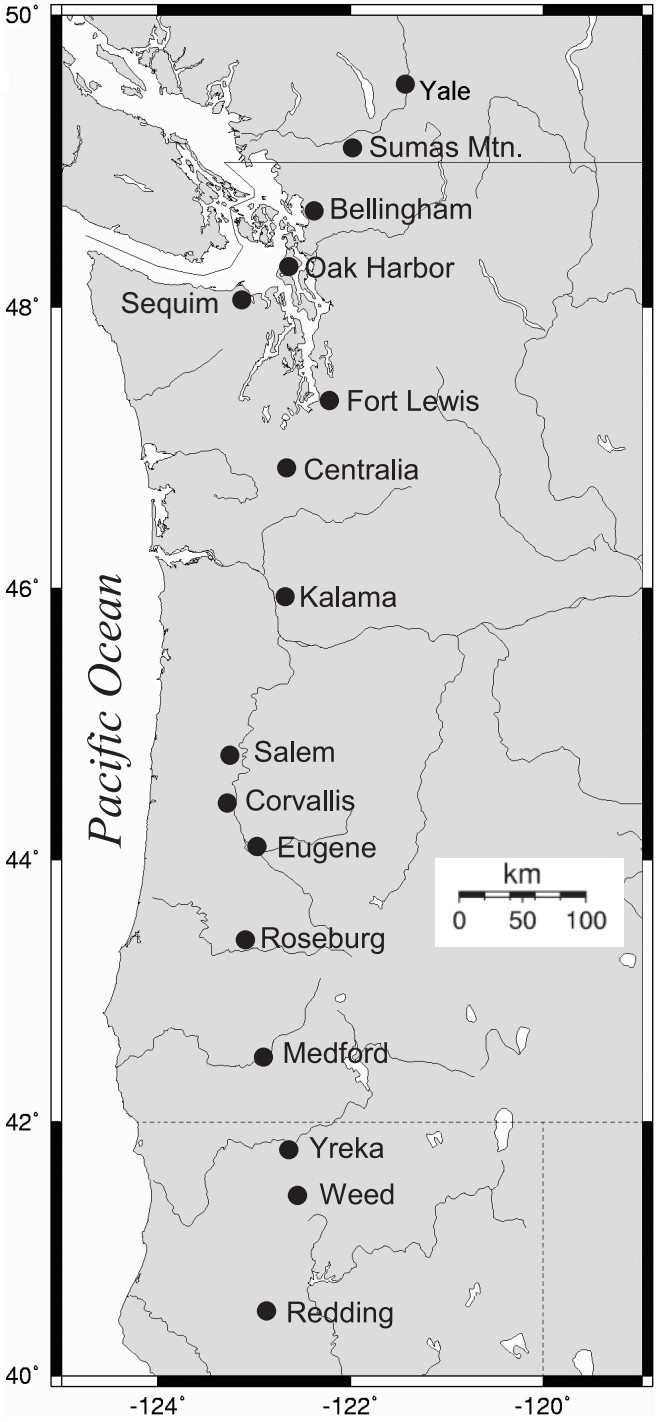
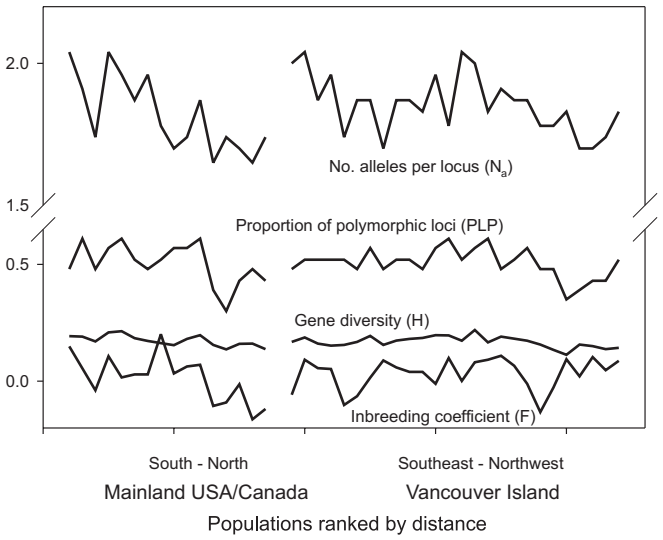


Fig. 3. Diversity trends across the range of Garry oak (*Quercus garryana*) (populations are rank ordered by distance).

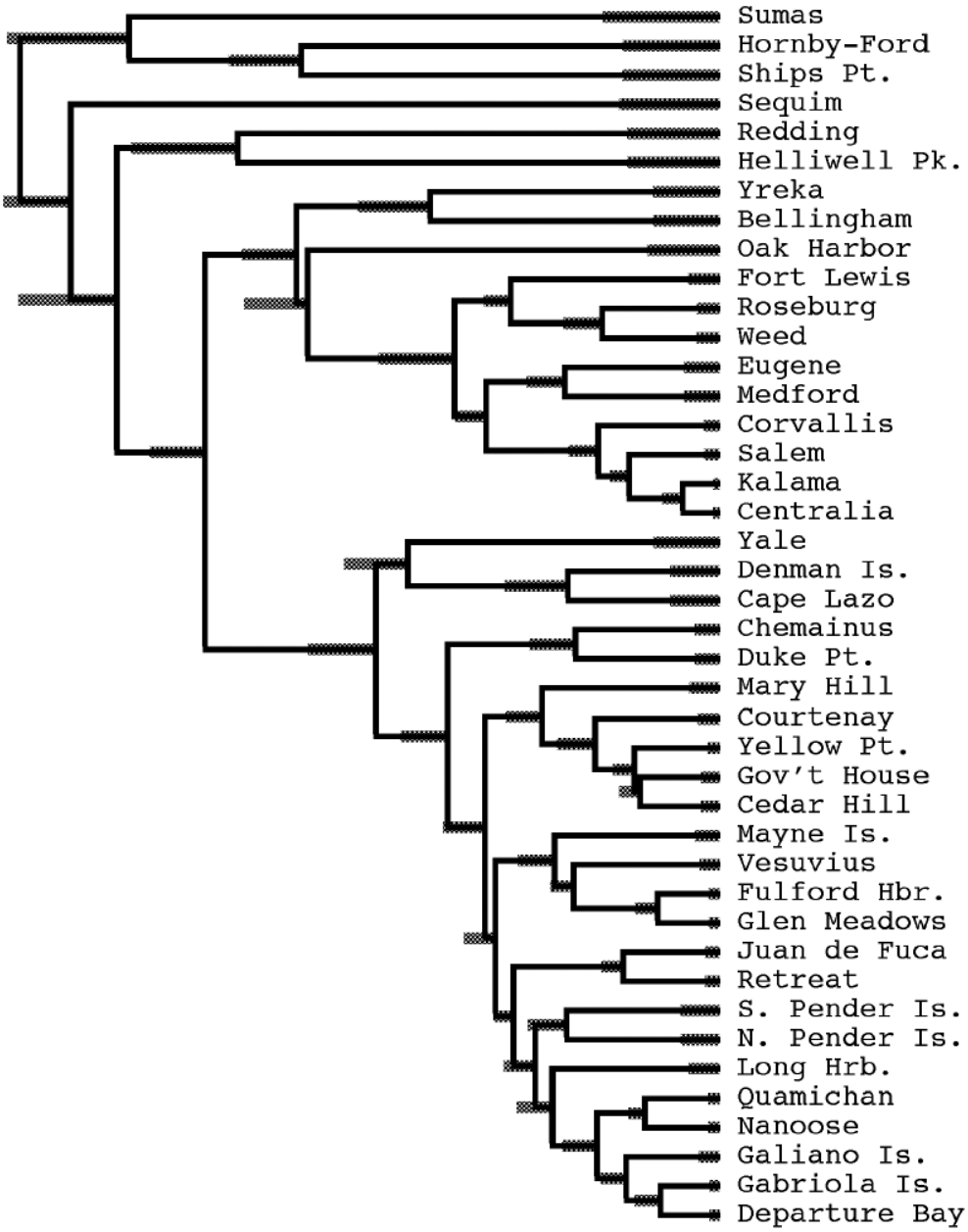


phatase (two loci: Fdp1, Fdp2), diaphorase (Dia), malate dehydrogenase (Mdh1), shikimic dehydrogenase (two loci: Skd1, Skd2), 6-phosphogluconate dehydrogenase (two loci: 6pgd1, 6pgd2), glutamate dehydrogenase (Gdh), UTP-glucose-1-phosphate uridylyltransferase (Ugpp), and aspartate amino tranferase (two loci: Aat1, Aat2). Populations and individual trees were withdrawn randomly from storage for assay. A consistent standard was included in each gel run for all materials. For the two populations assayed for outcrossing rate, the following polymorphic enzymes were assayed: Pgi2, Me, Est1, Est2, Est3, Pgm, Idh1, Mdh1, Skd1, and 6Pgd2.

FORTRAN 95 programs, written by K.R., calculated three diversity statistics: within-population expected heterozygosity (H), average number of alleles per locus (N_a), and proportion polymorphic loci (PPL, 95% criterion), separately by population. H was estimated as one minus the sum of squared gene frequencies, averaged over loci. The inbreeding coefficient (F) was estimated as $F = 1 - H_o/H$, where H_o is the observed proportion of heterozygotes (averaged over loci) and H is the expected heterozygosity. (Nei's G_{st}) (Nei 1973) and genetic distance (D) between populations (Nei 1972) were calculated, and from the genetic distances, a dendrogram was constructed using the unweighted pair group method (Sneath and Sokal 1973). To provide statistical confidence in the dendrogram groupings, the procedure described in Ritland (1989*a*) found the standard error of the branch length at the level of a cluster. In the figure generated, standard errors of branch lengths are indicated by a thicker, partially shaded bar on the right side of the thinner bar. If the length of the thicker bar is less than half that of the thinner bar, then the two groups of populations connected by a branch are significantly different (e.g., not an accidental grouping incurred by sampling few loci). Geographical distances were also computed among populations, based upon their longitude and latitude, and their relationship with genetic distance was examined via regression analysis.

To examine levels of inbreeding, mating-system parameters were estimated using the computer program MLTR (Ritland 2002). Single-locus and multilocus estimates of outcrossing were found, as well as the "correlation of paternity" (Ritland 1989*b*). To determine if the pollen pool was heterogeneous, pollen gene frequencies were estimated separately from egg (ovule) frequencies. Variances of estimates were determined by the distribution of 100 bootstrap estimates.

Fig. 4. Dendrogram showing the genetic relationships among the 42 sampled Garry oak (*Quercus garryana*) populations.



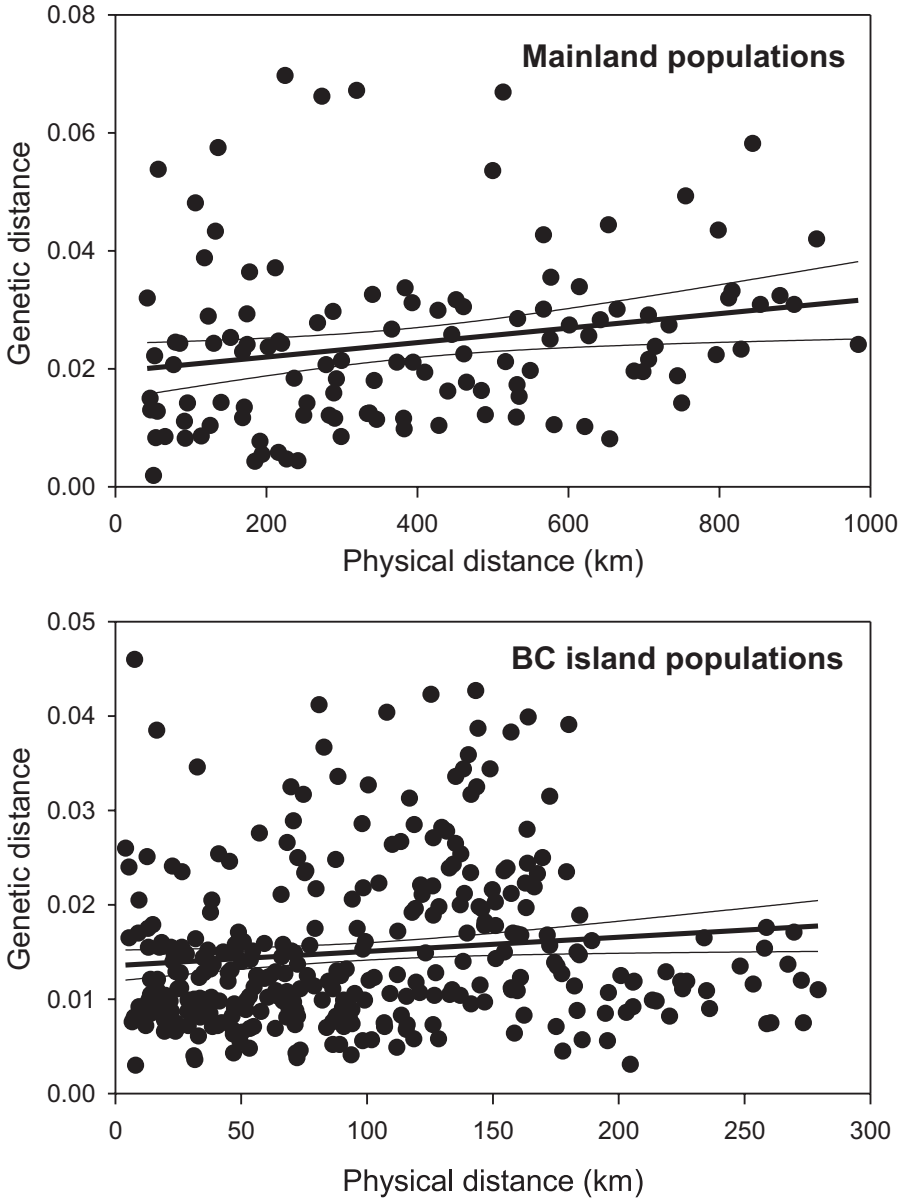
Results

Table 1 presents the diversity and inbreeding statistics for each of the 42 populations (gene frequencies underlying these statistics are available upon request from K.R.). Expected heterozygosity varied from 0.113 (Hornby-Ford) to 0.219 (Chemainus), averaging 0.170. Number of alleles per locus varied from 1.65 (Yale, Sequim) to 2.04 (Redding, Medford, Juan de Fuca, Quamichan), averaging 1.84. Proportion of polymorphic loci varied from 0.30 (Oak Harbor) to 0.61 (Yellow Point, Retreat Island, Fort Lewis, Roseburg, Weed), averaging 0.51. The measure of population differentiation, Nei's G_{st}, was 0.084. In other words, 8.4% of genetic variation is due to differences among populations, as opposed to differences within populations.

Figure 3 illustrates how diversity and inbreeding varies within the two geographic regions sampled: BC Islands versus Mainland Canada–USA (including Oak Harbor on Whidbey Island). In both regions, all three measures of diversity (H, N_a, and PLP) tended to be lower in the northern populations. The inbreeding coefficient also declined with latitude, but only in the Mainland Canada–USA populations. Regressions of these parameters on latitude were usually significantly negative; regression coefficients and their standard errors for H, N_a, PLP, and F were, for Mainland Canada–USA: –0.0258 (0.0103; $p < 0.05$), –0.1419 (0.0414; $p < 0.05$), –0.0628 (0.0278; $p < 0.05$), and 0.0261 (0.0326, ns), respectively. For BC Islands, they were –0.0054 (0.0015; $p < 0.05$), –0.0342 (0.0078; $p < 0.05$), –0.0143 (0.0070, $p \approx 0.05$), and –0.0217 (0.0069; $p < 0.05$), respectively.

Figure 4 is a dendrogram of genetic distances, which de-

Fig. 5. Relationship between genetic distance and physical distance of sampled populations of Garry oak (*Quercus garryana*) found on mainland Canada–United States (*a*) and British Columbia (BC) Islands (*b*).



picts the hierarchical structure of genetic relatedness among populations. In Fig. 4, the most prominent, statistically significant grouping is the BC islands populations plus Yale (the most easterly source on the BC mainland). Within this group, there was little further resolution of groups. The second major group consisted of most mainland populations (and Oak Harbor) in the center of the area sampled. Populations in northern Oregon and central Washington showed particular affinity. The remaining six populations (Sumas Mtn. to Helliwell Park, Fig. 4) did not group in any meaningful pattern, indicating their genetic remoteness; indeed, the standard error of branch lengths indicates that effectively these six outliers are a "star" phylogeny (populations are equally related to each other, at a rather great distance). Also, nine alleles were found only in USA populations, while eight others were found only in BC populations (both Islands and Mainland). Four alleles were unique to specific populations: two in the USA (Redding and Corvallis) and two in BC (Juan de Fuca and Fulford Harbour).

Figure 5 shows the relationship between genetic distance and physical distance. Separate plots are given for (*a*) Mainland Canada–USA and (*b*) BC Islands populations, because the scale and intensity of sampling differed from BC to US sources. In both cases, genetic distance increased significantly with physical distance ($p = 0.035$ for BC Islands and $p = 0.020$ for Mainland Canada–USA). The rate of increase for mainland populations was 0.0123 distance units per 1000 km, while that for BC Islands populations was 0.0150 distance units per 1000 km (the latter slope appears lower in the figure because the X and Y scales differ between the graphs).

The inbreeding coefficient F ranged from –0.163 (Yale) to 0.201 (Salem), averaging 0.025 (Table 1; negative values are due to random error of estimates, and possibly by bal-

Table 2. Mean (±SE) inferred pollen (male) and egg (female) gene frequencies in the two Garry oak (*Quercus garryana*) populations sampled for mating system.

	Cedar Hill		Glen Meadows	
Locus or allele	Pollen	Egg	Pollen	Egg
Pgi1 1	0.996±0.003	0.981±0.000	0.946±0.017	0.959±0.014
Pgi1 2	0.004±0.003	0.019±0.000	0.005±0.003	0.020±0.014
Pgi1 3	0.000	0.000	0.050±0.015	0.020±0.000
Pgi2 1	0.988±0.006	0.981±0.014	0.995±0.003	0.958±0.025
Pgi2 2	0.012±0.006	0.019±0.014	0.005±0.003	0.042±0.025
Me 1	0.808±0.035	0.846±0.053	0.807±0.030	0.813±0.050
Me 2	0.192±0.035	0.154±0.053	0.193±0.030	0.188±0.050
Est1 1	0.913±0.015	0.887±0.038	0.891±0.018	0.918±0.030
Est1 2	0.004±0.000	0.019±0.000	0.005±0.000	0.020±0.000
Est1 3	0.083±0.015	0.094±0.038	0.104±0.018	0.061±0.030
Est2 1	0.896±0.029	0.962±0.021*	0.915±0.017	0.898±0.033
Est2 2	0.004±0.000	0.019±0.000	0.005±0.000	0.020±0.000
Est2 3	0.101±0.029	0.019±0.021*	0.080±0.017	0.082±0.033
Est3 1	0.915±0.023	0.865±0.045	0.940±0.019	0.917±0.038
Est3 2	0.085±0.023	0.135±0.045	0.060±0.019	0.083±0.038
Pgm 1	0.930±0.016	0.981±0.015*	0.991±0.005	0.979±0.014
Pgm 2	0.070±0.016	0.019±0.015*	0.009±0.005	0.021±0.014
Idh1 1	0.840±0.028	0.925±0.039*	0.820±0.029	0.896±0.037*
Idh1 2	0.004±0.002	0.019±0.000	0.009±0.006	0.021±0.017
Idh1 3	0.157±0.028	0.057±0.039*	0.171±0.028	0.083±0.035*
Mdh 11	0.976±0.012	0.943±0.024	0.982±0.009	0.959±0.015
Mdh 12	0.004±0.000	0.019±0.000	0.005±0.000	0.020±0.000
Mdh 13	0.020±0.012	0.038±0.024	0.014±0.009	0.020±0.014
Skd1 1	0.989±0.005	0.963±0.002	0.935±0.020	0.959±0.017*
Skd1 2	0.004±0.000	0.019±0.000	0.005±0.003	0.020±0.000
Skd1 3	0.008±0.005	0.019±0.000	0.060±0.020	0.020±0.017*
6Pg2 1	0.658±0.029	0.796±0.042*	0.678±0.034	0.840±0.051*
6Pg2 2	0.011±0.006	0.019±0.000	0.027±0.021	0.020±0.000
6Pg2 3	0.004±0.000	0.019±0.000	0.005±0.000	0.020±0.000
6Pg2 4	0.327±0.030	0.167±0.042*	0.291±0.035	0.120±0.051*

*Indicates significant differences of pollen from egg frequencies.

ancing selection). This average of 0.025 is marginally significant, as indicated by its standard error of 0.012. The results from the progeny array mating system analysis are given in Tables 2 and 3. There was a considerable lack of informative loci for mating system estimation, indicating the generally low level of diversity in Garry oak. Only one locus (6pgd2) showed good intermediate gene frequencies, and just two others (Me, Est2) had the most frequent allele less than 90%. Nevertheless, standard errors are reasonable (ca. 0.03–0.04), as many loci were used for estimation.

Table 2 shows that pollen gene frequencies often deviated significantly from egg (ovule) gene frequencies, as 14 of 32 pollen versus egg valid comparisons were significant ("valid" comparisons omit comparisons of extreme gene frequencies; cases where the bootstrap cannot give SE values when gene frequencies are unreliable; we adopted the criteria of at least one gene frequency lying within the range of 0.05–0.95). This heterogeneity is likely due to nearest-neighbour mating.

Multilocus outcrossing rates were 1.006 in Cedar Hill and 0.918 in Glen Meadows samples (Table 3). The average, 0.962, has a confidence level that overlaps with unity (SE, 0.030). Thus, mature acorns from these two populations arise predominately from outcrossing, with the possibility of a small percentage of selfing. Single-locus outcrossing rates were slightly lower (0.941, Table 3), and the difference between multilocus versus single-locus outcrossing rates (0.025) was nonsignificant. Thus, biparental inbreeding was not found in families from these two populations. The correlation of paternity (or the probability that two randomly chosen sibs are full-sibs) was a statistically significant 6%.

Discussion

Oak trees are a conspicuous feature of many drier landscapes in coastal regions of western North America. However, populations of several species are clearly endangered. While there are many documented cases of declining oak species in Europe (Führer 1998), in California, oak species have only been recently documented to suffer declines; for example, the sudden decline of three oak species from a previously unobserved and likely introduced fungus-like organism (Garbelotto et al. 2001).

A different situation exists on Vancouver Island, where Garry oak does not seem to suffer from obvious pest outbreaks or stresses such as air pollution; rather, this species, and its associated unique suite of meadow species, occurs in a prime habitat for human development. In such a situa-

Table 3. Estimates of mating system parameters in the two Garry oak (*Quercus* garryana) populations, and sample sizes used.

Parameter	Cedar Hill	Glen Meadows	Average	Total
Parental F	–0.109±0.288	–0.050±0.200	–0.080±0.175	
Multilocus (t_m)*	1.006±0.040	0.918±0.044	0.962±0.030	
Single-locus (t_s)*	0.964±0.026	0.918±0.043	0.941±0.025	
Difference ($t_m - t_s$)*	0.043±0.026	0.000±0.027	0.022±0.019	
Correlation of paternity (r_p)*	0.065±0.040	0.061±0.029	0.063±0.025	
No. of loci	11	11		11
No. of families	26	24		50
No. of offspring	260	240		500

*Values are means ± SE.

tion, fragmentation, human use, and other introduced plant species pose threats to populations (Ward et al. 1998). In addition, recent analysis indicates a history of indigenous fire management to maintain oak meadows for cultural purposes (Fuchs 2001); the disappearance of such management also places threats upon population survival. Our results about genetic diversity, population structure, and mating system provide insights into effective conservation strategies for, and the cultural significance of, populations of Garry oak.

Levels of genetic variation and inbreeding

Levels of gene diversity vary greatly among oak species (Kremer and Petit 1993). Over the sampled range of Garry oak, expected heterozygosity averaged 0.17 (within populations), and the number of alleles per locus averaged 1.84. These values are about one-half those found in other white oak species. This might suggest that Garry oak is a closely adapted species, with limited potential for adaptive response to human disturbance, to climate change, or to pests such as the fungal-like organism that causes sudden oak death in California. However, we note that variation at these specific isozyme loci are most likely not any part of the adaptive "arsenal" of this species, but rather are indicative of overall levels of gene diversity in the population, as are most molecular markers (Ritland and Ritland 2000).

Like previous studies of other oak species, we found outcrossing rates to be near 100%, based upon progeny array analysis of two Garry oak populations. Also, inbreeding coefficients of the 42 populations averaged near zero (0.025). Therefore inbreeding should not be a significant concern for management of Garry oak populations. This result contrasts with Hertel and Zaspel (1996), who found an association between stand vitality and genetic structure (at isozyme loci) in declining populations of *Q. robur* and *Q. petraea* in Germany. Such traits as branching habit, defoliation, foliage discoloration, and stem necrosis were combined into a single measure of vitality, which was found to be positively associated with heterozygosity. Their results can be explained by significant levels of inbreeding; their F values ranged from 0 to 0.16, averaging 0.081 among the nine populations surveyed. This is over three times the 0.025 F value observed for Garry oak populations in this study.

Other than estimating inbreeding, the major interest in determining oak mating systems has been the pattern of paternity, particularly as affected by population substructure. We found significant levels of "correlated mating", where siblings of a common mother also share a common father. Whereas these levels were low (5% chance that two sibs share the same father), they were statistically significant. Pollen gene frequencies also often deviated significantly from egg (ovule) gene frequencies. Together with the significant correlation of paternity, we conclude that the pollen pool of Garry oak is heterogenous, probably because of synchronous flowering and (or) nearest-neighbor mating combined with low population density (see Dyer and Sork 2001).

Population structure

Two major groupings of populations were evident in the dendrogram of Fig. 4. The strongest and most closely related group of populations was from Vancouver Island. The lack of hierarchical structure of the BC Islands populations (Fig. 4) shows that gene flow rates are high among most southern Islands sources, but not to Oak Harbor (Whidbey Island), Sequim, or Bellingham. Thus transfer of acorns, cuttings, saplings, and the like within the BC Islands region would not alter the current genetic structure of this species.

The presence of two major groups in Fig. 4 suggests that in the last glaciation there may have been two different refugia (Hebda and Mathewes 1984). Pollen records indicate that unglaciated areas existed along the west coast of Vancouver Island, from which several species have expanded. However, there is no evidence that native Garry oak occurred there (an occurrence of Garry oak near Port Alberni, on Vancouver Island's west coast, is known to be due to planting). Further genetic databased upon maternally inherited chloroplast markers are needed to substantiate the existence of separate refugia.

Patterns of allele type can also be used to delimit population groupings. The major disjunction of groupings at the Straight of Juan de Fuca (Fig. 4), indicative of a separate BC Islands refugia, is also confirmed when rare alleles are pooled (results not shown), and when the number of alleles is considered. Overall, nine alleles are unique to the USA clade, while eight alleles are unique to the BC clade (which has origins in the BC Islands refugia).

Studies of white oaks in Europe have not been as successful in identifying major clades of populations with nuclear gene markers. For example, in Ireland, *Quercus petraea* shows high nuclear genetic variation, comparable to mainland Europe, and low population differentiation within Ireland, despite this species having colonized Ireland after the last glaciation, and with more recent deforestation resulting in small, scattered forest fragments (Muir et al. 2004). Also,

in a study of three closely related white oaks native to Switzerland, *Quercus petraea*, *Quercus pubescens*, and *Quercus robur*, Finkeldey and Matyas (2003) found that in contrast with patterns of chloroplast DNA variation, glacial and postglacial population history was not reflected at nuclear gene loci. They concluded that extensive gene flow through pollen likely blurred previously existing genetic differentiation at nuclear gene loci because of glacial refugia effects.

Origins and uniqueness of the two isolated BC mainland populations

Two BC populations of Garry oak occur in the lower Fraser Valley. These populations are small and may have been founded and propagated by native peoples in the past; any evidence for anthropogenic origins are of great cultural significance. Alternatively, these populations may have arisen from long-distance dispersal of acorns by an animal, most likely a bird (Glendenning 1944; Fuchs et al. 2000), or be relics of a broader population that existed after the postglacial cooling (Hebda 1994). This alternative seems to be better supported by our results, as discussed below.

Analysis of genetic distances showed that these populations are quite distinct genetically (Fig. 4). The Sumas population was not grouped significantly with any population, and the genetically nearest populations were from northern BC Islands. The Yale population, which comprises the eastern extent of the range, grouped with the BC Islands clade. Within this clade, two northern BC Islands populations (Denman and Cape Lazo) placed closest to Yale, but this grouping was not significant. If these populations are of recent anthropogenic origin, we should expect a closer genetic affinity with another population, or at least with the "centroid" of the BC Islands population clade (if the source population was not present in our sample).

As well, these two populations contained less genetic diversity. The Sumas population showed markedly less variation than the mean, with an expected heterozygosity of 0.137, although not the least of all 42 populations surveyed. By contrast, the Yale population had values lower than average, although not consistently the lowest for all three diversity parameters. This is paradoxical, as Yale is more isolated from the main range than Sumas: Sumas to Yale is 76 km; Sumas to Bellingham is 58 km; and Yale to Bellingham is 130 km. Given this lack of correlation with geography, it seems more likely that longer-term genetic drift, instead of recent founder events, shaped the genetic diversity of these two populations.

Markers and conservation genetics

Isozymes are advantageous in many respects: they are economical and as most researchers use a common set of isozymes, their levels of genetic variability are readily compared among studies. Here, we assayed a full suite of 23 enzyme loci exhibiting 58 alleles, without regard to their polymorphism. This enabled a comparison to a very large literature for isozyme variation. In a companion paper (Ritland et al. in preparation[2]), the advantages of microsatellites will be exploited. While of limited value for comparisons among species and even among populations (Hedrick 1999), microsatellites allow detailed dissection of genealogical relationships within populations, and among populations of recent origin (Blouin et al. 1996).

While both types of markers allow identification of baseline levels of genetic diversity, and levels of inbreeding and population distinctiveness, they tell us little about the extent of local adaptation and the demographic threats to populations. We need to seek markers that are directly involved with adaptive traits, such as growth phenology, insect and fungal defense, and resource utilization. With the increasing knowledge of gene functions in model systems such as Arabidopsis, the advent of large EST (expressed sequence tag) collections from non-model species including oaks, and the development of new high-throughput genotyping methods, direct molecular genetic studies of adaptation and conservation should be increasingly possible.

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