Multivariate analysis of allozymic and quantitative trait variation in *Alnus rubra*: geographic patterns and evolutionary implications

Andreas Hamann, Y.A. El-Kassaby, M.P. Koshy, and G. Namkoong

Abstract: Frequency data from six polymorphic allozyme loci and measurements of six quantitative traits were used to examine geographic differentiation among 65 British Columbia provenances of red alder (*Alnus rubra* Bong.). Principal components analysis showed that variation in quantitative traits can be reduced to two underlying dimensions, one representing general vigor including the termination of the growing period and the other being the start of the growing period. Canonical correlation analysis among quantitative traits, allozyme frequencies, and geographic variables revealed complex associations of quantitative traits with the latitude, distance to the coast, and elevation of the seed source. There were no significant correlations among allozyme frequencies and quantitative traits, but the frequency of the most common allele at most loci decreased with latitude. Further, cluster analysis based on Nei's genetic distance revealed a strong differentiation among island and mainland provenances at one allozyme locus. This differentiation can be interpreted as a result of migration from two different refugia since the last glaciation. The island populations presumably originate solely from isolated coastal refugia west of the Cordilleran ice sheet, while mainland populations were also recruited from areas south of the ice.

Résumé : Les auteurs ont étudié la différenciation géographique parmi 65 provenances d'aulne rouge (*Alnus rubra* Bong.) de Colombie-Britannique à partir de données de fréquences alléliques pour six loci polymorphes d'alloenzymes et des mesures pour six caractères quantitatifs. Les résultats de l'analyse en composantes principales ont démontré que la variabilité des caractères quantitatifs pouvait se résumer en deux dimensions sous-jacentes, l'une représentant la vigueur générale et incluant la fin de la période de croissance et la seconde, définie par le début de la période de croissance. L'analyse de corrélations canoniques réalisée entre les caractères quantitatifs, les fréquences d'alloenzymes et les variables géographiques a fait ressortir l'existence de relations complexes entre les caractères quantitatifs et la latitude, la distance de la côte et l'altitude des sources de semences. Il n'y avait pas de corrélations significatives entre les fréquences d'alloenzymes et les caractères quantitatifs, mais la fréquence de l'allèle le plus commun diminuait avec la latitude pour la plupart des loci. De plus, l'analyse de regroupement à partir des distances génétiques de Nei a révélé une différenciation marquée entre les provenances insulaires et celles du continent pour un locus d'alloenzymes. Cette différenciation pourrait s'expliquer par le résultat d'une migration à partir de deux refuges distincts depuis la dernière période glaciaire. Les populations insulaires auraient comme seule origine présumée des refuges isolés sur la côte à l'ouest du glacier de la Cordillère, alors que les populations continentales découleraient de refuges situés au sud du front glaciaire.

[Traduit par la Rédaction]

Introduction

Red alder (*Alnus rubra* Bong.) is among the most abundant hardwood species in the Pacific Northwest. Its natural range extends from California (32°N) to Alaska (68°N) within 300 km of the Pacific Ocean, with the exception of small outlying populations in Idaho. In recent decades, red alder has attracted considerable attention as a potential reforestation species because of its fast growth and its ability to symbiotically fix nitrogen (Hibbs et al. 1994). The population genetics of this species, however, are relatively poorly studied compared with conifers of commercial value. Species with a comparable range in the Pacific Northwest that are quite variable in morphological characteristics and physiological traits show generally medium to high levels of variation in allozymes, e.g., Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Yeh and El-Kassaby 1980), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Yeh and O'Malley 1980), *Pinus contorta* Dougl. ex Loud. (Wheeler and Gurries 1982), and Sitka alder (*Alnus sinuata* (Regel) Rydb.) (Bousquet et al. 1990), while species that are morphologically more uniform such as western redcedar (*Thuja plicata* Donn ex D. Don) have been found to be predominantly

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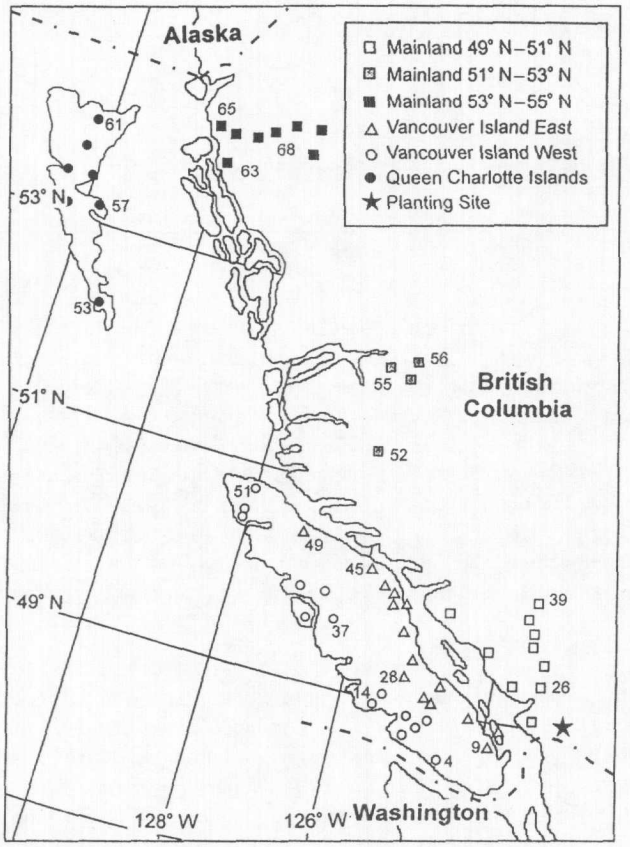
Fig. 1. Location of provenances and planting site for the British Columbia red alder provenance trial. Symbols represent regions and numbers refer to provenances assayed for allozyme frequencies.



monomorphic at allozyme loci (Yeh 1978). While red alder is morphologically remarkably uniform throughout its range, there is considerable genetic variation in adaptive traits. Range-wide provenance trials have demonstrated clinal variation over the wide latitudinal distribution of the species (DeBell and Wilson 1978; Cannell et al. 1987; Agar and Stettler 1994). This study investigates approximately one third of the species' natural range in greater detail than the previous trials. In British Columbia (48–56°N), red alder occurs over a wide range of climatic conditions, and the purpose of this study is to investigate presumed genetic differentiation of red alder provenances in adaptive and growth traits along environmental gradients. Further geographic patterns in allozyme frequencies are investigated and shall be interpreted with respect to evolutionary history since the last glaciation. Multivariate and averaging techniques are used to detect ecotypic variation in morphological and allele frequency data, while correlation techniques are employed to examine clinal variation along geographic variables. This study is restricted to the investigation of geographic patterns in morphological traits and allozyme frequencies. Partitioning of variance components for morphological data is provided elsewhere (Xie and Ying 1996), and a general analysis of allozyme frequency data with respect to diversity and population structure is provided in El-Kassaby et al. (1998).

Materials and methods

Study area and plant material

Data were obtained from a provenance trial established by the British Columbia Ministry of Forests in 1992 south of Vancouver, B.C. The locations of the 65 British Columbia provenances studied and the planting site of the provenance trial are shown in Fig. 1. The total area covered was arbitrarily subdivided into six regions: Vancouver Island Westcoast, Vancouver Island Eastcoast, Queen Charlotte Islands, Mainland 49–51°N, Mainland 51–53°N, and Mainland 53–55°N latitude. The areas are represented by symbols throughout the text to better visualize geographic differentiation. Geographic variables used were latitude, distance to the coast, and altitude. Distance to the coast was chosen instead of longitude, since longitude is confounded with latitude due to the southeast to northwest orientation of the coastline of British Columbia. The distance to the coast for mainland provenances between 49 and 51°N was measured from the west coast of Vancouver Island.

Data collection

Trees of all provenances included in the field trial were evaluated in 1996 at the end of the fourth growing season. Height, diameter at breast height, and total aboveground dry mass as well as the number of flower clusters per tree were recorded. Leaf abscission of the top five leaves and bud break of the top five buds were recorded in weekly intervals at the end of the first growing season and the beginning of the second growing season. The average day of bud break and the average date of leaf abscission were calculated as weighted averages for individual trees from these repeated measurements. Analysis in this study is based on mean values of measurements from 100 trees for each of 65 provenances except for aboveground dry mass, where only 20 trees were destructively sampled.

Allozyme polymorphisms, using horizontal starch gel electrophoresis, were evaluated for an average number of 79 trees (SE = 5.0) for each of 19 provenances. These provenances were selected to represent the entire range of red alder in British Columbia. Proteins were extracted from newly developed vegetative bud primordia according to Cheliak and Pitel (1984). Electrophoresis was conducted on 11% horizontal starch gels using lithium borate buffer (Ridgeway et al. 1970) or morpholine citrate buffer (Clayton and Tretiak 1972). The staining methods used followed those of Conkle et al. (1982). A locus was included in this study if the most common allele had a frequency of less than 95%, namely aconitase (ACO, EC 4.2.1.3), alcohol dehydrogenase (ADH, EC 1.1.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.3.), and uridine-5′-diphosphoglucose dehydrogenase (UGP, EC 1.1.1.22), which were single-locus enzyme systems. Further, the more cathodally migrating locus of two aspartate aminotransferase (AAT, EC 2.6.1.1) loci and the intermediately migrating locus of three malate dehydrogenase (MDH, EC 1.1.1.37) loci were included.

Statistical analysis

As a means to compare patterns of similarity among provenances based on the morphological and allozyme data sets, and to identify groups of genetically similar provenances, cluster analysis was applied to both data sets separately. Only the 19 provenances for which allozymic and quantitative data were available were included. The unbiased genetic distance (D_N) among provenances according to Nei (1978) was generated from allele frequency data. As an analogous measure, a matrix of Mahalanobis distances (D_M) among provenances was calculated from normalized data for quantitative traits (Dillon and Goldstein 1984). Based on these distance measures, dendrograms were constructed to better visualize allozymic and morphological differentiation among provenances. The unweighted group average method (UPGMA) was used to

Fig. 2. Dendrogram of 19 British Columbia red alder provenances using the UPGMA clustering technique for Nei's unbiased genetic distance. Symbols refer to regions specified in Fig. 1.

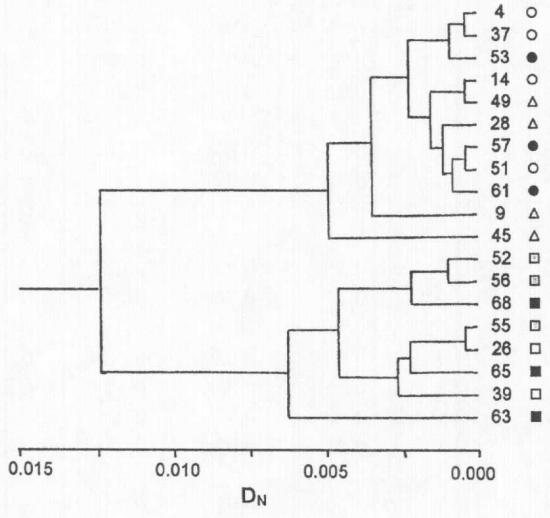
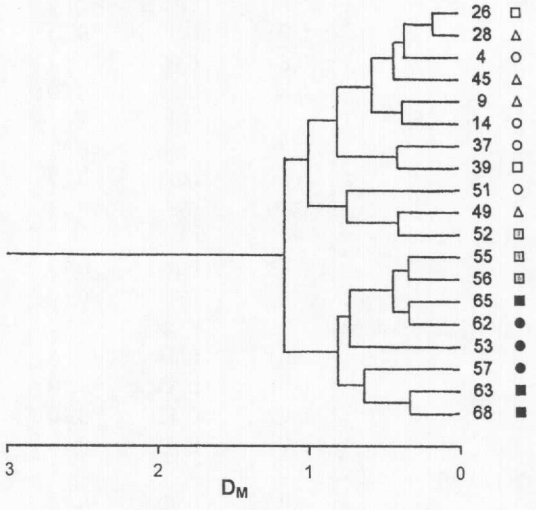


Fig. 3. Dendrogram of 19 British Columbia red alder provenances using the UPGMA clustering technique for Mahalanobis distances derived from quantitative traits. Symbols refer to regions specified in Fig. 1.



construct dendrogams (Sneath and Sokal 1973). Another descriptive tool, principal components analysis, was applied to quantitative data including all 65 provenances. Orthogonal variates, which are linear combinations of the original variables that best account for the total variance in the data set, were extracted from correlation matrices. Only principal components with eigenvalues greater greater than 1 were retained. The first two variates were plotted against each other to highlight different dimensions in the original data set. Calculations were made using the CLUSTER procedure and the PRINCOMP procedure of the SAS statistical software package (SAS Institute Inc. 1988). The IML procedure was used to generate distance matrices.

In addition to the descriptive multivariate techniques above, canonical correlation analysis and canonical redundancy analysis were used to investigate relationships among geographic variables, morphological variables, and allozyme frequencies. Canonical correlation analysis generates pairs of linear combinations from two sets of original variables so that the correlation is maximal among the two variates (Dillon and Goldstein 1984). Because the number of variables (alleles) was large relative to the number of samples (provenances), only the most frequent allele of each locus was used in the analysis. Since the frequencies of the other alleles at the same locus are not independent, little information was lost and the statistical power was enhanced for canonical analysis by lowering the degrees of freedom in the numerator (Gittins 1985). Canonical redundancy analysis was used to determine what proportion of the variation in one original data set, e.g., morphological variables, can be accounted for by a canonical variate of another data set, e.g., geographic variables (Cooley and Lohnes 1971). Calculations were made with the CANCORR procedure of the SAS statistical software package (SAS Institute Inc. 1988).

Results

Population differentiation

The dendrograms produced by the UPGMA clustering technique based on Nei's genetic distance for allozyme allele frequencies and Mahalanobis distance for quantitative measurements are shown in Figs. 2 and 3, respectively. Using allele frequency data, the provenances cluster into two groups of island and mainland provenances. The average distance between island and mainland provenances is 0.0149, considerably larger than differences within these regions, averaging 0.0029. Provenances from the Queen Charlotte Islands and from Vancouver Island cluster closely together, although they are separated by a large geographic distance. Only one locus, *AAT*, is responsible for the differentiation of island and mainland provenances. Table 1 shows that *AAT* is fixed or nearly fixed in island provenances, while it is segregating in mainland provenances. When this locus is removed from the cluster analysis, no separation among island and mainland provenances is retained. In contrast, distances based on quantitative data are larger between northern and southern provenances than within these groups. Mainland and island provenances from comparable latitudes are not distinct.

The principal components analysis identified two variates with eigenvalues greater than 1.0 that accounted for 72.3% of the variation in six quantitative traits. The first variate is interpreted as representing all variables except bud break in roughly equal proportions, while the second variate represents primarily the date of bud break (Table 2). Figure 4 shows a plot of the 65 provenances over the two composite variates. Regional differences are expressed by both variates. Latitudinal differences are apparent mostly from the right to the left representing the first principal component, with dark symbols representing provenances from further north and light symbols provenances from the south. Clinal differences with distance from the coast are apparent by ordering the provenances of the three regions Vancouver Island Westcoast, Vancouver Island Eastcoast, and Mainland 49–51°N along the axis of the second principal component.

Simple correlations among variables

Table 3 shows simple correlations among variables, and Table 4 shows summary statistics for quantitative traits and regression coefficients from simple linear regression analysis of each quantitative trait with latitude, distance to the coast, and altitude. For simple correlations among quantitative

Table 1. Allele frequencies for 19 red alder populations from British Columbia.

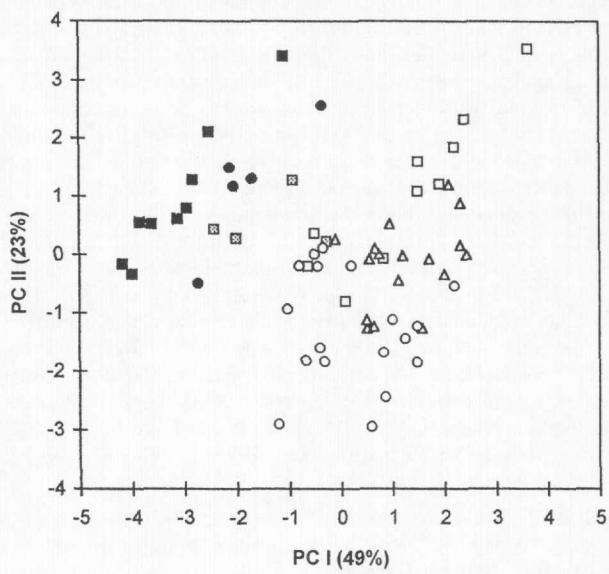
Population	*ACO*	*ADH*			*AAT*	*GDH*		*MDH*		*UGP*
No.	Allele 1	Allele 1	Allele 2	Allele 3	Allele 1	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1
Mainland 53–55°N										
63	0.73	0.37	0.25	0.21	0.59	0.54	0.33	0.78	0.22	0.76
65	0.84	0.60	0.17	0.10	0.71	0.72	0.22	0.91	0.08	0.85
68	0.80	0.51	0.20	0.20	0.29	0.70	0.26	0.84	0.16	0.90
Mainland 51–53°N										
52	0.75	0.66	0.11	0.14	0.40	0.77	0.17	0.83	0.10	0.86
55	0.81	0.53	0.27	0.13	0.46	0.68	0.28	0.87	0.11	0.78
56	0.80	0.67	0.13	0.12	0.37	0.79	0.12	0.86	0.10	0.88
Mainland 49–51°N										
26	0.83	0.69	0.13	0.13	0.59	0.67	0.25	0.91	0.07	0.88
39	0.79	0.58	0.19	0.13	0.69	0.79	0.17	0.76	0.24	0.91
Vancouver Island Eastcoast										
9	0.64	0.81	0.06	0.06	1.00	0.94	0.03	0.92	0.08	0.92
28	0.71	0.65	0.19	0.11	0.99	0.72	0.25	0.85	0.12	0.85
45	0.72	0.42	0.10	0.25	0.99	0.56	0.30	0.74	0.20	0.87
49	0.71	0.72	0.15	0.05	0.99	0.71	0.22	0.92	0.05	0.85
Vancouver Island Westcoast										
4	0.90	0.70	0.15	0.07	1.00	0.77	0.20	0.92	0.05	0.91
14	0.68	0.73	0.15	0.03	1.00	0.80	0.20	0.90	0.10	0.83
37	0.93	0.67	0.13	0.10	1.00	0.76	0.15	0.88	0.12	0.91
51	0.73	0.55	0.15	0.17	0.99	0.72	0.17	0.81	0.15	0.93
Queen Charlotte Islands										
53	0.84	0.69	0.18	0.09	0.90	0.77	0.16	0.92	0.07	0.86
57	0.76	0.61	0.21	0.13	1.00	0.80	0.14	0.89	0.11	0.89
61	0.73	0.58	0.14	0.15	0.99	0.68	0.26	0.83	0.17	0.88

Note: Monomorphic loci and the least frequent allel of each polymorphic locus are ommitted.

Table 2. Component loadings of the first two principal components for six quantitative traits.

	Eigenvectors	
Trait	PC I	PC II
Height	0.47	0.02
Diameter at breast height	0.44	0.11
Dry mass	0.51	0.13
Date of bud break	–0.01	–0.81
Date of leaf abscission	0.41	–0.50
Number of flowers	0.38	0.22

Fig. 4. Plot of the first two principal components for 65 British Columbia red alder provenances based on six quantitative traits. Symbols refer to regions specified in Fig. 1.



traits, it is notable that all growth traits are correlated with the date of leaf abscission but not with the date of bud break. After sequential Bonferroni adjustment for simultaneous inference (Rice 1989), allozyme frequencies are not significantly correlated with quantitative traits or geographic variables (data not shown). The planting site is located at a southern, low-elevation, inland position. Therefore, geographic variables of provenances represent the effects of a transfer from a certain location to this planting site on quantitative traits. Diameter and total aboveground dry mass decrease on average by 9 and 6%, respectively, for every degree of latitude a provenance is transferred south to the planting site. Bud break occurs approximately 1 day later and leaf abscission 2 days later per degree of latitude. Transferring provenances from the coast to further inland results in decreased diameter and biomass production and a lower number of flowers. The effect of elevation is not significant for most traits. Only diameter changes significantly with elevation, increasing by 6 mm for every 100 m a provenance is transferred from a higher elevation to the planting site.

Table 3. Simple correlations among quantitative traits and geographic variables based on population means.

Trait	HT	DIA	TDM	BUD	LAB	FLW	LAT	DIST	ALT
HT	—	0.58*	0.70*	–0.12	0.50*	0.31*	–0.27	0.11	–0.15
DIA	0.58*	—	0.55*	–0.05	0.56*	0.37*	–0.46*	0.35*	0.29*
TDM	0.70*	0.55*	—	–0.10	0.59*	0.59*	–0.44*	0.43*	–0.02
BUD	–0.12	–0.05	–0.10	—	0.44*	–0.19	–0.63*	–0.18	0.07
LAB	0.50*	0.56*	0.59*	0.44*	—	0.39*	–0.80*	0.24	0.06
FLW	0.31*	0.37*	0.59*	–0.19	0.39*	—	–0.28	0.53*	0.09

Note: HT, height; DIA, diameter at breast height; TDM, total aboveground dry mass; BUD, date of bud break; LAB, date of leaf abscission; FLW, number of flowers per tree; LAT, latitude; DIST, distance to the coast; ALT, altitude.

*Significant r value at $p < 0.05$ after sequential Bonferroni adjustment (Rice 1989) for quantitative traits ($k = 15$) and for correlations among geographic and quantitative traits ($k = 18$).

Table 4. Summary statistics and regression coeffients for quantitative traits based on population means.

					Regression coefficient		
Trait (unit)	Mean	(SD)	Min.	Max.	D/LAT	D/DIST	D/ALT
Height (m)	4.89	(0.26)	4.11	5.65	ns	ns	ns
Diameter at breast height (cm)	2.95	(1.11)	2.26	3.84	–0.26	–0.35	0.06
Dry mass (kg)	1.24	(0.22)	0.93	1.64	–0.06	–0.08	ns
Date of bud break (day)	84	(2.57)	78	89	–0.95	ns	ns
Date of leaf abscission (day)	329	(5.09)	317	335	–2.12	ns	ns
Number of flowers	3.2	(1.54)	0.8	8.4	ns	–1.22	ns

Note: Min., smallest population mean; Max., largest population mean; D/LAT, change in trait if provenance transferred 1° latitude south; D/DIST, change in trait if provenace transferred 100 km inland; D/ALT, change in trait if provenance transferred 100 m to lower elevation; ns, coefficient not significantly different from 0 at $p < 0.05$ after sequential Bonferroni adjustment (Rice 1989).

Canonical correlation and redundancy analysis

The canonical structure is summarized in Table 5, providing the correlation of the original variables with their canonical variates. Tests for significance of correlations among canonical variates are shown in Table 6. The first three rows of Tables 5 and 6 investigate the relationship of growth and adaptive traits with the geographic origin of the provenance. The three canonical correlations are 0.90, 0.62, and 0.46, all significantly different from zero. The first pair of canonical variables demonstrates mainly the effect of latitude, since latitude has the highest correlation with its canonical variable (Table 5). It can be seen that trees from lower latitudes are growing larger in height and diameter, break bud later, and abscise leaves later than provenances transferred to the planting site from further north. The second canonical pair represents the effect of distance to the coast. Provenances from further inland utilize a longer growing season at the planting site and grow larger in diameter and height compared with those provenances transferred from coastal areas. The third pair represents the effect of altitude and to some degree the distance from the coast. Provenances from higher elevation further inland grow shorter in height and larger in diameter, with an overall reduced biomass production. At the planting site, they exhibit a reduced growing season with a late date of bud break and an early date of leaf abscission. Canonical redundancy analysis shows that the three canonical variates, representing the effects of latitude, distance to the coast, and altitude, account for 22, 10, and 5% of the total variation in the quantitative data, respectively.

Geographic trends of allozyme frequencies are investigated in the fourth to sixth rows of Tables 5 and 6. The first linear combinations of allozyme and geographic variables are significantly correlated. The frequency of the most common allele increases at all loci with decreasing latitude and distance from the coast. This increase is significant for *ADH*, *AAT*, *GDH*, and *MDH* but not for *UGP* and *ACO*. This implies that the overall expected heterozygosity increases with latitude and distance to the coast, which can be seen in Fig. 5, where the expected heterozygosity is plotted over latitude. Coastal provenances and inland provenances are represented by the usual symbols. The correlation of the second pair of canonical variates is "almost significant", indicating that the frequency of the most common allele at locus *UGP* increases with distance from the coast. The three canonical variates account for 29, 3, and 1% of the total variation in allele frequencies.

The seventh to ninth rows of Tables 5 and 6 represent associations of allozyme data with quantitative traits. The coefficients themselves are rather high, with approximately 0.8 for the first canonical pair and 0.6 for the second pair of each comparison. Although none of the canonical coefficients is statistically significant, there appears to be a trend that for large trees that utilize a growing season with late bud break and late leaf abscission, the most common allele of each locus occurs at higher frequencies than for small trees except for locus *ACO*.

Discussion

Differentiation in quantitative traits

Clinal variation was found along all three investigated environmental gradients. When interpreting the results in detail, it is important to keep in mind that the planting site is located at a southern, low-elevation, inland position. Thus, local sources are observed in comparison with provenances

Table 5. Correlations between original variables and their canonical variates.

Pair	Variable set 1						Variable set 2					
	HT	DIA	TDM	BUD	LAB	FLW				LAT	DIST	ALT
1	0.34*	0.47*	0.52*	0.71*	0.92*	0.32*				–0.98*	0.21	–0.19
2	0.03	0.46*	0.50*	–0.50*	0.09	0.75*				–0.06	0.98*	–0.14
3	–0.46*	0.31*	–0.50*	0.28*	–0.15	–0.32*				–0.19	0.41	0.91*
	ACO	*ADH*	*AAT*	*GDH*	*MDH*	*UGP*				LAT	DIST	ALT
4	0.11	0.66*	0.85*	0.46*	0.57*	0.29				–0.75*	–0.52*	0.28
5	0.02	0.17	–0.04	0.14	–0.39	0.49*				–0.56*	0.83*	0.62*
6	0.73*	–0.43	–0.02	–0.20	–0.28	0.20				0.34	–0.13	0.72*
	HT	DIA	TDM	BUD	LAB	FLW	*ACO*	*ADH*	*AAT*	*GDH*	*MDH*	*UGP*
7	0.29	0.29	0.84*	0.79*	0.55*	0.18	–0.39	0.63*	0.44*	0.41*	0.18	0.55*
8	0.00	–0.13	–0.32	0.00	0.24	–0.41	0.20	0.00	0.36	–0.51*	0.35	–0.38
9	–0.06	0.24	–0.12	–0.34	–0.65*	–0.09	0.04	–0.56*	–0.17	–0.51*	–0.47*	0.35

Note: HT, height; DIA, diameter at breast height; TDM, total above ground dry mass; BUD, date of bud break; LAB, date of leaf abscission; FLW, number of flowers per tree; LAT, latitude; DIST, distance to coast; ALT, altitude; *ACO*, *ADH*, *AAT*, *GDH*, *MDH*, and *UGP*, frequencies of most common allele at allozyme loci.
*Significant r value at $p < 0.05$.

Table 6. Correlation analysis of the relationship between pairs of canonical variables.

Pair	Coeff.	SE	F	dfn	dfd	$p > F$
1	0.90	0.02	11.6	18	156	0.001
2	0.62	0.08	4.8	10	112	0.001
3	0.46	0.10	3.7	4	57	0.009
4	0.81	0.08	2.3	18	29	0.024
5	0.74	0.11	2.2	10	22	0.057
6	0.66	0.13	2.4	4	12	0.104
7	0.89	0.05	1.4	36	34	0.189
8	0.83	0.07	1.1	25	31	0.405
9	0.67	0.13	0.8	16	28	0.671

Note: Coeff., canonical correlation coefficient; F, F value approximation; dfn, dfd, degrees of freedom (nominator/denominator); $p > F$, probability of greater F values.

transferred from north to south, from the coast to the inland, and from high to low elevation. Along the latitudinal gradient, the length of the growing season in the north is shorter and average temperatures are lower. Provenances can be distinguished by their response in vegetative phenology and growth traits. The northern provenances break bud earlier and drop leaves earlier than local provenances, which is commonly observed in other tree species when transferred south (Morgenstern 1996). Since local sources perform better than those transferred from the north, it can be inferred that adaptation with respect to the synchronization with the seasonal cycle has taken place. Transferred provenances do not fully utilize the end of the available growing season at the planting site, resulting in generally smaller trees. The early start of the growing period in transferred trees has apparently no advantage and is perhaps premature for the local climatic conditions. The magnitude of latitudinal effects on adaptive traits (Table 4) corresponds well to results for red alder from Cannell et al. (1987), who observed that frost hardening occurred 2 days earlier for each degree latitude north.

The second important trend is observed along a gradient from the coast to approximately 300 km inland, which represents a cline from a maritime climate with high precipitation and little amplitude in daily and seasonal temperature extremes to a continental climate with low precipitation and high temperature amplitudes. Local sources begin their growing period earlier and perform better with respect to growth traits than those transferred from coastal sites further inland. Apparently, coastal sources have a higher heat sum requirement to break bud, which results in a delayed start of the growing period at the planting site, which is not a typical result. Bud break is expected to be later in provenances from mountain and inland regions, since late-spring frost events are more likely at high elevations and under continental climate at the same latitude (Morgenstern 1996). The reduced performance of coastal sources at an inland site could also be due to lower water use efficiency and stomata control of coastal sources, leading to water stress during the summer months. However, an investigation of ecophysiological parameters could not clearly demonstrate such a differentiation (Dang et al. 1994).

A third trend, which would have gone unnoticed without utilizing multivariate techniques, is the effect of elevation of the seed source. Trees from high elevations grow smaller in height but larger in diameter than the average and have a reduced aboveground biomass. They break bud later and abscise leaves earlier, although the last observation was not significant. These growth patterns are expected for high-elevation provenances, but have rarely been demonstrated (Morgenstern 1996).

Allozymic differentiation and evolutionary history

This analysis revealed a clinal trend toward greater heterozygosity at higher latitudes, which was observed in all six loci, although the correlation with the canonical variate was not significant for *ACO* and *UGP* (Tables 5 and 6, pair 4). A similar continuous trend was observed in Norway spruce (*Picea abies* (L.) Karst.), the northern European populations showing a greater heterozygosity than the central

Fig. 5. Expected heterozygosities for 19 British Columbia red alder provenances plotted as a function of latitude. Symbols refer to regions specified in Fig. 1.

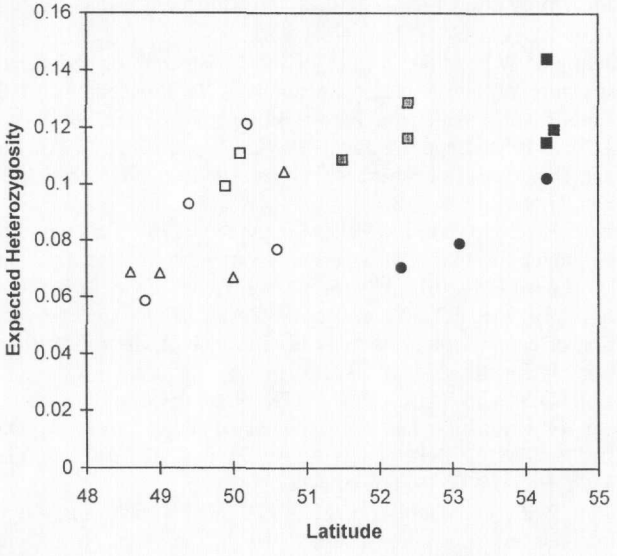
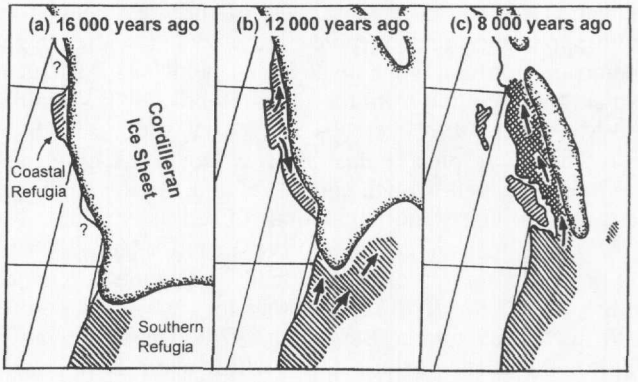


Fig. 6. Hypothetical refugia and migration of red alder since the height of the last glaciation (adapted from Pilou 1991).



and southern European populations (Lagercrantz and Ryman 1990). In the latter study, it was concluded that the southern European populations originated from refugia that experienced restrictions in population size in the Dinaric Alps, while the northern provenances originated from large refugia further east, which later mixed to form a continuous cline. Such an explanation seems inappropriate in case of red alder, since a population "bottleneck" was more likely in the coastal refugia than in the area south of the ice. Also, Yeh and O'Malley (1980) found significant correlations of the most common allele at several loci with latitude, longitude, and altitude in Douglas-fir. Similar to this study, the majority of loci had negative correlation coefficients with latitude, of which two were significant (p values not adjusted for simultaneous inference). Yeh and O'Malley (1980) cautiously suggested that these frequencies were due to selection. Using multivariate methods comparable with this study, Yeh et al. (1985) also observed a rich structure of genetic variation associated with geographic variables in lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.), which was not detected in earlier investigations of this species by Wheeler and Gurries (1982) and Dancik and Yeh (1983). The previous studies suggest that geographical factors are weakly associated with allozyme frequencies, and these associations may not be easily detected unless a rather large number of populations over a wide geographic range are investigated. Since the expected heterozygosity is consistently higher in northern provenances in this study, a possible explanation would be different historic effective population sizes as a result of limited pollen or seed dispersal over the observed environmental gradient. However, a decrease of variation due to genetic drift as a consequence of smaller effective population sizes would be expected in the north rather than the south, where climatic conditions could result in limited pollen or seed dispersal. An alternative explanation may be that effective population sizes in northern populations were larger in the past, considering that red alder is an aggressive colonizer of recently disturbed or impoverished sites. Recently disturbed areas sustaining large populations of red alder may have been more frequent in northern environments, while red alder populations further south may have been more transient prior to large-scale human disturbance in the Pacific Northwest.

Canonical correlations among quantitative traits and allozyme frequencies were not significant, but some significant simple correlations among allozyme loci and quantitative traits (prior to sequential Bonferroni adjustment) were found in this study as well as in others (Yeh and O'Malley 1980; El-Kassaby 1982, 1983). However, it appears that there is no functional relationship among the two data sets. It is rather plausible that the environmental gradient (as measured by geographic variables) have selective effects on quantitative traits as well as influences on effective population sizes, either due to effects on seed and pollen dispersal or by favoring or restricting the overall competitiveness of a species in a specific habitat.

Cluster analysis revealed nonclinal variation in allele frequencies at locus *AAT*. In provenances from Vancouver Island and the Queen Charlotte Islands, the most common allele is fixed or at very high frequencies whereas it ranges between 30 and 70% in mainland provenances. The genetic similarity of the geographically distant populations on Vancouver Island and the Queen Charlotte Islands may be explainable by the reinvasion history after the most recent glaciation. British Columbia was entirely covered by the Cordilleran ice sheet, and it is generally assumed that during the coldest period of the Pleistocene the current species survived in refugia south of the ice front (Critchfield 1984). There is, however, evidence, in sediments found on the east coast of the Queen Charlotte Islands (Warner et al. 1982; Josenhans et al. 1997), of coastal refugia west of the ice that sustained a woody flora and that persisted throughout the entire glaciation. The climate in these refugia was thought to be relatively mild, since arctic currents were blocked by the Beringian land bridge. Red alder could have reinvaded the coastal areas of British Columbia from such a refugium, as shown in Fig. 6 (adapted from Pilou 1991). When the ice started to retreat (Fig. 6*b*), Vancouver Island and the Queen Charlotte Islands were connected to the coastal mainland due to isostatic rise of the coastal area and a low sea level, forming a continuous coastal strip that was still separated from areas south of the ice by glaciers covering the Olympic Mountains. Under this hypothesis, red alder reinvaded the

coastal mainland subsequently also from southern refugia, when the islands were already separated (Fig. 6*c*). A further indication for coastal refugia west of the ice shield is the rapid appearance of lodgepole pine woodlands after deglaciation on northern Vancouver Island, which is difficult to explain by migration from the south (Hebda 1983; Wainman and Mathewes 1987). Perhaps the coastal variety of lodgepole pine, shore pine (*Pinus contorta* Dougl. ex Loud. var. *contorta*), evolved in such coastal refugia, similar to the genetically distinct island populations of red alder.

Allozyme loci are commonly considered selectively neutral or nearly neutral, but occasionally, allozymes are apparently selected for or linked to other loci, as in the case of *APH* in Norway spruce (Bergmann 1978). The same may be true for the alleles at locus *AAT*, which could have experienced selection pressure leading to fixation of one allele for this locus on the island populations. Since there is no indication of environmental conditions particular to the islands today, if such selection occurred, it must have been during glaciation in a refugia west of the ice shield. Allozyme frequencies comparable with those at locus *AAT* have not been found in any other species investigated in this area. Considering that allozyme loci that are influenced by selective forces are rather exceptional, it probably requires a different approach to test our hypothesis. Pollen and fossil records, for example, could be specifically inspected for the timing of reappearance of woody species along the coast.

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