THE ECOLOGICAL AND CONSERVATION GENETICS OF GARRY OAK (*Quercus garryana* Dougl. ex Hook)

by

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ABSTRACT

Garry oak (*Quercus garryana* Dougl. Ex Hook) is a deciduous tree endemic to Western North America. It is confined within Canada to only a few isolated locations in southwestern British Columbia (B.C.). Although accounting for less than 0.3% of British Columbia's entire land coverage, Garry oak-associated ecosystems support tremendous biodiversity and are home to a large number of rare species in B.C. Populations have, however, been declining since European settlement. It is estimated that only 1-5% of pre-European Garry oak ecosystems remain uncompromised in B.C. today. However, species distribution models predict the area climatically appropriate for Garry oak to triple in B.C. by the 2080's. Using a common garden experimental design, data regarding growth and biomass partitioning, bud phenology and cold hardiness were collected for two years from a total of 1700 individuals from 15 populations representing the species' entire range. Data were used to assess genetic diversity and geographic differentiation (Q_{ST}) for these quantitative traits.

Results indicate relatively weak population differentiation for most traits. However, significant genetic clines exist for height, germinant emergence date and cold hardiness. Height and germinant emergence were strongly correlated with environmental variables associated with summer aridity (mean summer precipitation, summer heat:moisture index, mean warmest month temperature), while cold hardiness was strongly correlated with temperature differential (the difference between mean warmest month temperature and mean coldest month temperature) and mean warmest month temperature.

Estimates of population differentiation for traits (Q_{ST}) were relatively low for growth related traits, bud burst and bud set (0.07-0.13) and moderate (0.30) for cold hardiness and germinant emergence. Results suggest Garry oak is a species closely adapted to conditions of intense drought and are used to recommend seed transfer guidelines and conservation strategies for current and future climates in B.C. and elsewhere.

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CHAPTER 1: Introduction and Literature Review

1.1 Introduction

Garry oak (*Quercus garryana* Dougl. Ex Hook*)*, of the family Fagaceae, is distributed from California to south-western British Columbia, where it is confined to only a few isolated locations on Vancouver Island and two disjunct mainland populations. Supported by a near-Mediterranean climate unique to this area, Garry oak ecosystems currently comprise less than 0.3% of British Columbia's entire land coverage. Supporting tremendous biodiversity, this area has simultaneously attracted rapid and extensive human settlement. As a direct result of anthropogenic global warming, temperatures are predicted to increase three to five degrees Celsius within Canada over the next one hundred years (IPCC 2007). Models of species climatic envelopes suggest that forest species will move steadily north to the extent that, disturbance, seed dispersal and seedling establishment facilitates (Hamann and Wang 2006). Based on such predictions it has been suggested that the area climatically appropriate for Garry oak will triple by 2080 (Hamann and Wang 2006).

Little research has been conducted on the evolutionary forces affecting the genetic structure of Garry oak. In this thesis I use a seedling common garden experiment to estimate levels of quantitative genetic variation for a number of juvenile fitness traits. Results from this experiment were subsequently compared to neutral molecular estimates of population differentiation (G_{ST}) in order to estimate the extent of divergent selection for this species. Results from this study will assist in predicting the potential response of

Garry oak to climate change under a range of possible scenarios and in developing seed transfer guidelines for restoration and conservation efforts.

1.2 Ecology of Garry oak

Garry Oak is a long-lived deciduous hardwood tree endemic to western North America (Pavlik et al. 1992)**.** The only oak native to British Columbia, Garry oak has the longest distribution of any of the western oaks spanning 15 degrees of latitude and 2290 metres in elevation (Pavlik et al. 1992). Although its range is extensive, it is highly fragmented, especially at the peripheries (Ward et al. 1998). This can be attributed to a number of factors including physical barriers such as mountains, human development and land use conversion, demographic attrition, and inappropriate site conditions (Ward et al. 1998, Fuchs 2001). Additionally, there is an extensive body of literature documenting, as a result of fire suppression, the mass transformation of oak habitat to closed canopy conifer (predominantly Douglas fir, *Pseudotsuga menziesii*) forest throughout its range (Towle 1979, Agee 1993, Fuchs 2001). In British Columbia Garry oak is limited to the south-eastern portion of Vancouver island, the surrounding Gulf Islands, and two disjunct mainland populations near Yale and on Sumas Mountain (Ward et al. 1998). At higher elevations in the southern reaches of its distribution a shrubby variety, Brewer's oak (*Quercus garryana* var. *breweri*) has also been identified (Pavlik 1998).

Garry oak is a monoecious species producing self-incompatible male and female flowers (Stein 1990). Seed dispersal, although not passive, is severely limited (Fuchs 2001). In addition to gravity and a variety of rodents and corvids, the principal vector of dispersal for Garry oak acorns in Canada is considered the Stellar's jay (*Cyanocitta stelleri*)(Vander Wall 1990). South of Washington state, acorn woodpeckers

(*Melanerpes formicivorus*) and western scrub jays (*Aphelocoma californica*) also contribute significantly to acorn dispersal (Grivet et al. 2005). Acorns are typically hoarded in small caches throughout the forest for later consumption (Fuchs 1998). Grivet et al. (2005) found that in the case of another western oak, valley oak (*Quercus lobata*), seed transport was limited to within a 100m radius from the maternal tree. Wind borne pollen on the other hand can travel great distances and in the case of many oak species, is considered the main avenue of among population gene flow. Additionally, it is suspected that First Nations peoples, in the past, distributed acorns great distances by hand (Fuchs 2001).

Tolerating a diverse set of soil and moisture regimes, Garry oak contributes to a number of different ecosystem types throughout its range (Ward et al. 1998, Fuchs 2001). On xeric sites, Garry oak often exists as a climax species, but in the absence of fire it is frequently out-competed on richer, mesic sites by faster growing conifer species, primarily Douglas-fir (Stein 1990, Agee 1993). Garry oak can thus be found in three distinct habitat types: closed canopy, mixed conifer, and open meadow, depending upon site conditions and disturbance regimes (Fuchs 2001).

In British Columbia, Garry oak predominantly exists as a keystone species within the endangered "Garry oak meadow" ecosystem (Fuchs 2001). Identified by the Canadian government as a hot spot of biodiversity, Garry oak ecosystems in British Columbia have been associated with 694 plant species, 104 bird species, 33 mammal species, and 7 amphibian species, of which over 100 have been officially listed as "at risk of extinction" (Ward et al. 1998, Fuchs 2001). Although home to the highest concentration of rare species in British Columbia, Garry oak and associated ecosystems

have been steadily declining since the settlement of Europeans in 1843 (Erickson 2000). As a direct result of urban and agricultural development, invasive species, and ongoing fire suppression it is estimated that only one to five percent of pre-European Garry oak ecosystems remain uncompromised within British Columbia today (Fuchs 2001).

In light of global climate change (IPCC 2007), peripheral populations such as those found in British Columbia may play an increasingly important evolutionary role. Although peripheral populations are considered to be more susceptible to climate change due to their lowered genetic variation and compromised fitness, it is expected that peripheral populations existing in northern latitudes and higher elevations of a species' range, may incur less climate change related stress, relative to their southern counterparts (Davis and Shaw 2001, Aitken et al. 2008). This is due in part, to gene flow from the centre of the range introducing alleles pre-adapted to warmer conditions. The opposite is true in the case of rear-edge peripheral populations where gene flow will likely introduce alleles pre-adapted to cooler climates, thusly reducing fitness, given future climate change scenarios (Davis and Shaw 2001, Aitken et al. 2008).

It has also been suggested that, in the absence of high levels of among-population gene flow, disjunct peripheral populations can harbour rare alleles necessary for the adaptation to novel environmental change, although they may have lower levels of genetic variation than central populations (Mayr 1982, Lesica and Allendorf 1995, Mimura and Aitken 2007). Additionally, it is suspected that, if subject to local selection pressures (as opposed to other stochastic genetic effects), peripheral populations at the leading edge may be more locally adapted and may harbour extreme phenotypes necessary for future evolutionary migration (Mayr 1982, Lesica and Allendorf 1995).

As a direct result of anthropogenic global warming, temperatures are predicted to increase three to five degrees Celsius within Canada over the next one hundred years (Hamann and Wang 2006, IPCC 2007). Based on such predictions it has been suggested that much of what is now coastal Douglas-fir forest in British Columbia will have a climate suitable for Garry oak ecosystems within the next fifty years (Hebda 1997, Hamann and Wang 2006). There is typically considerable genetic variation within and among different plant populations for outcrossing species, particularly forest trees (Huenneke 1991). Such genetic variation may prove crucial to the adaptation of *Q. garryana* in a rapidly changing climate (Aitken et al. 2008). Little research has been conducted on the climatic adaptability and evolutionary potential of Garry oak. This study attempts to fill this gap.

1.3 Quantitative trait variation and adaptation in forest trees

Quantitative trait differentiation and local adaptation of trees greatly depends on the degree of environmental heterogeneity and the equilibrium set by gene flow and selection (Savolainen et al. 2007, Morgenstern 1996). Such variation has been well documented and described for a number of temperate and boreal tree species at varying spatial scales (Morgenstern 1996, Linhart and Grant 1996). Historically, common garden experiments have been employed to understand levels of genetic variation among and within populations and also, to identify clinal genetic patterns for various quantitative traits (Rehfeldt 1989, Morgenstern 1996**,** Bower and Aitken 2008). Traits such as height, diameter, cold hardiness, transpiration rate, net photosynthesis, germination date, bud burst, bud set, root:shoot ratio are often compared to geographic variables (latitude,

longitude, elevation etc.) and modeled source climate variables (temperature, precipitation, frost fee period, etc.) in order to understand the geographic structure of genetic variation and describe the environmental variables driving evolutionary processes.

Range-wide environmental heterogeneity in combination with low levels of among-population gene flow often result in clinal patterns of quantitative variation for various traits. In the case of Sitka spruce (*Picea sitchensis*), both growth and phenology were highly correlated ($r^2 = .93$) with mean annual temperature (Mimura and Aitken 2007). Douglas-fir (*Pseudotsuga menziesii*) exhibits complex patterns of variation attributable to latitude, longitude, elevation and slope (Rehfeldt 1989, Savolainen et al. 2007). Some species, however, display relatively weak genetic clines as in the case of *Larix occidentlais*, where genetic differentiation is found only with altitudinal changes of 500 metres or more (Rehfeldt 1995, Savolainen et al. 2007). In light of ongoing climate change, understanding geographic patterns of clinal genetic variation may prove integral in predicting the potential response of species to future climate scenarios and avoiding instances of maladaptation (Aitken et al. 2008).

1.4 Estimating the effects of divergent selection in forest trees

Clinal variation in phenotypic traits can also be produced by stochastic processes such as genetic drift in combination with spatially restricted gene flow (Storz 2002). It can be difficult to separate these two evolutionary forces from one another. One means of testing whether a quantitative trait is subject to directional or homogenizing selection is to compare standardized measures of differentiation for neutral marker genes (F_{ST} or G_{ST}) to their quantitative analogue (Q_{ST}) (Spitze 1993). Although selecting neutral forces

should affect markers and quantitative traits equally, selection may act only upon the latter. Q_{ST} estimates the degree of differentiation, among populations, for quantitative traits based on the amount of additive genetic variance (Spitze 1993). G_{ST}, its neutral molecular analogue, is determined by quantifying the total neutral allelic variation among populations as a proportion of the total genetic diversity (Spitze 1993). G_{ST}, for selectively neutral markers, is considered to represent the neutral variation caused by the combination of gene flow and genetic drift, whereas Q_{ST} represents the phenotypic variation attributable to the effects of gene flow, genetic drift and selective pressures.

Accordingly, if Q_{ST} is larger than G_{ST} it is predicted that the observed differentiation in quantitative traits is under the effect of differential or divergent selection. If Q_{ST} is equal to G_{ST} the trait is considered to be neutral with respect to selection. In such a case it is difficult to determine whether quantitative differentiation is a result of drift alone or whether it is acting in concert with gene flow and weak selective pressures. Finally, if Q_{ST} is less than G_{ST} the trait is considered to be under the effect of uniform selection, or suffering from high levels of interpopulation gene flow (Spitze 1993).

Genetic differentiation can vary greatly among oak species. In previous studies of related oak species G_{ST} was estimated between 0.03 and 0.11 for isozyme markers (Table 2.5). In a range wide isozyme study, Ritland et al. (2005) estimated G_{ST} to be 0.084 for Garry oak.

The relationship between Q_{ST} and G_{ST} or F_{ST} is one that has been debated and reviewed extensively (Frankham 1999, Whitlock 1999, Merila and Crnokrak 2001, Hendry 2002, McKay and Latta 2002, Whitlock 2008). In a recent review Whitlock

(2008) warns that caution must be exercised when making inferences from such comparisons as distributions may vary, selection of traits may be overly biased in quantitative studies, and statistical tests explicitly testing differences are not always straightforward.

1.5 Thesis objectives

The primary objective of this study is to assess the quantitative genetic structure of Garry oak, the shaping evolutionary forces, and in particular, the relative effects of divergent selection on selected traits, by studying phenotypic variation among and within populations. There are a number of factors that contribute to the amount and distribution of genetic variation within a species (genetic drift, mutation, gene flow, selection, mating system) (Felsenstein 1976, Volis 2005). Although typically many factors contribute to a species' genetic architecture, understanding the relative magnitude of each contributing factor is key in assessing the evolutionary history and potential of a species.

More specifically, the objectives of this study of Garry oak are to investigate levels of genetic diversity for quantitative traits, describe patterns of differentiation for these traits, estimate the effect of divergent selection and local adaptation, and to identify and quantify any clinal patterns that may exist and the environmental gradients that these patterns reflect. Cumulatively, we endeavour to use this information to recommend seed transfer guidelines for restoration and conservation purposes and to predict the potential response of Garry oak to future climate change scenarios.

1.6 Hypothesis

In the absence of high levels of interpopulation gene flow, and within a spatially heterogeneous range, the theory of local adaptation predicts that divergent selection will play a more defining role in determining fitness optimums, resulting in high population differentiation (Williams 1966, Kaweki 2004). Both Garry oak's mating system, life history traits (limited seed dispersal, large seed size) and range are congruent with these conditions and so our initial hypothesis was that Garry oak would be subject to the effects of divergent selection and thusly exhibit high levels of population differentiation for most traits throughout its range.

1.7 Thesis introduction

Populations of Garry oak have been declining in British Columbia since European settlement in the 19th century and are currently estimated to represent only 0.3% of the province's entire land coverage. The area climatically appropriate for Garry oak however is estimated to triple by 2080 (Hamann and Wang 2006). Whereas previous studies have investigated levels of neutral genetic variation, information regarding genetic variation in quantitative traits is needed to predict the potential responses of this ecologically significant species to climate change, and to aid conservation and restoration efforts throughout its range.

In the following chapters I investigate the quantitative and ecological genetics of Garry oak. In Chapter two I employ a common garden experimental design to estimate levels of genetic diversity for a number of quantitative traits and understand and describe patterns of geographic differentiation throughout its range. In Chapter three I summarize my major findings, translate those into recommendations for seed transfer for species

restoration, discuss further research questions, and consider future conservation strategies for Garry oak in British Columbia.

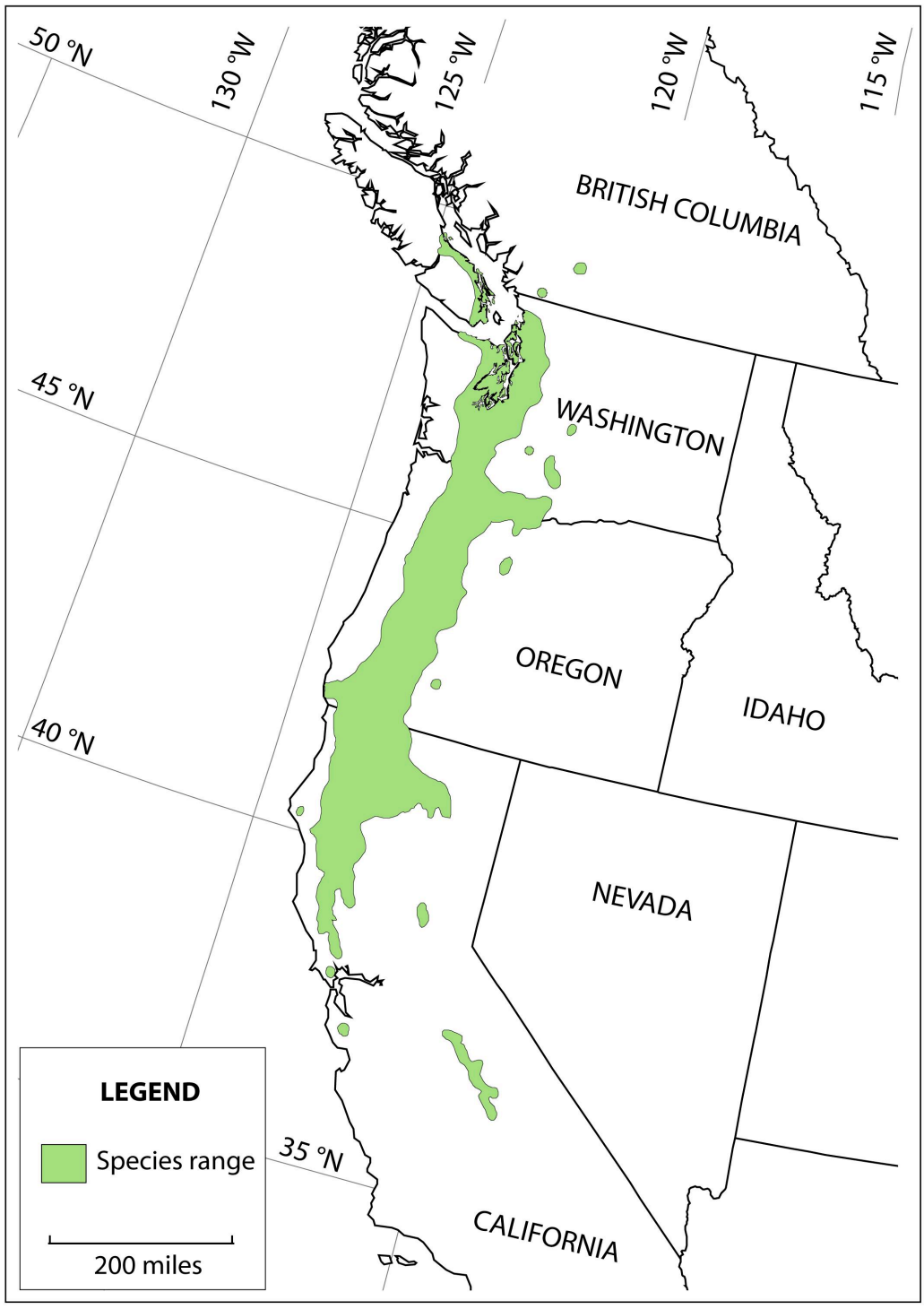

Figure 1.1 Natural distribution of Garry oak (Little 1976).

CHAPTER 2: The Ecological Genetics of Garry Oak (*Quercus garryana*)

2.1 Introduction

Genetic variability is essential for evolutionary change in tree species (Davis and Shaw 2001, Aitken et al. 2008). Unlike many other life forms, trees are unable to quickly relocate when conditions become inhospitable due to climatic or environmental change (Davis and Shaw 2001). Rather, continued survival depends jointly on their ability to adapt to changing environments, and ability to migrate (Davis and Shaw 2001, Aitken et al. 2008). This adaptive potential in trees is shaped by a balance of selection and genetic drift, the result of which is often evidenced in geographic differentiation among forest tree populations (Coyne 1992, Davis and Shaw 2001, Aitken et al. 2008).

Although molecular genetic studies are important for understanding post-glacial histories, mating systems and levels of selectively neutral genetic variation, they do not necessarily reflect the adaptive potential of a species (McKay and Latta 2002, Savolainen et al. 2007). While techniques such as association mapping and identification of quantitative trait loci are beginning to bridge this gap, common garden experiments are still necessary in understanding the geographic structure of genetic variation and the adaptive potential of a species (McKay and Latta 2002, Howe et al. 2003, St. Clair et al. 2005). For example, relatively strong adaptive differentiation among populations has been demonstrated in a number of species including Sitka spruce (*Picea sitchensis* (Bong.) Carriere), lodgepole pine (*Pinus contorta* Dougl. ex Loud), and red alder (*Alnus rubra* Bong.) (Yang et al. 1996, Hamann et al. 1998, Mimura and Aitken 2007). In all

three species, levels of population differentiation for quantitative genetic variation were not reflected in patterns of molecular variation for selectively neutral markers.

Understanding the geographic structure of quantitative genetic variation as well as the environmental factors driving selection is crucial to the management of genetic resources (St.Clair 2005). The success of such management programs is contingent upon knowledge of natural levels of genetic variation and local adaptation in species-specific quantitative traits (St.Clair 2005). Without such information, restoration projects increase the risk of maladaptation, which in turn may negatively affect adjacent native tree populations through gene flow from planted to native populations (McKay et al. 2005). Specifically, results from genecological studies assist in managing breeding and genetic conservation programs, informing seed transfer guidelines, and predicting the potential response of species to future climate change (St.Clair 2005). While most studies of this kind have been directed towards species of economic value, little information exists regarding those whose value is primarily ecological. Garry oak's (*Quercus garryana* Doug. ex Hook) declining populations in combination with its rich ecological associations warrant a pronounced need for conservation. Given the potential for the introduction of poorly adapted genotypes through such efforts, information regarding the local adaptation of this species is needed.

Garry oak is a deciduous broadleaf tree endemic to western North America and is the only oak native to British Columbia (Pavlik et al. 1992, Fuchs 2001). Displaying broad environmental tolerance, Garry oak can be found contributing to a number of different ecosystems throughout its range including closed canopy, mixed forest and open meadow, depending on site conditions and disturbance regimes (Fuchs 2001). At the

northern extent of its range in Canada, Garry oak is predominantly associated with the endangered Garry oak meadow ecosystem, where it exists as a keystone species. This ecosystem, which has been in constant decline since European settlement, is home to the largest concentration of rare species in British Columbia and represents a significant and irreplaceable portion of the provincial biodiversity (Ward et al. 1998, Erickson 2000, Fuchs 2001). As a result of ongoing fire suppression, urban and agricultural development, and the impact of invasive species, uncompromised populations in B.C. have been estimated at one to five percent of that of pre-European levels (Fuchs 2001).

Simultaneous to this documented decline, the range climatically appropriate for Garry oak in British Columbia has been predicted to triple over the next seventy years (Hamann and Wang 2006). The question of whether adaptation and dispersal can compete with modern, accelerated rates of climate change is of great concern, not only for Garry oak, but for many forest tree species (Hamrick 2004, Davis et al. 2005, Aitken et al. 2008). Without adequate knowledge of a species' genetic variation, both quantitative and molecular, it is difficult to make informed predictions regarding species' responses to climate change or to inform conservation and restoration efforts effectively (Hamrick 2004).

Thus far, studies of Garry oak have focused predominantly on its autecology and the synecology of associated ecosystems. Although the genetic variation of Garry oak has been assessed using molecular markers, levels of quantitative genetic variation and geographic differentiation in quantitative traits in Garry oak are still unknown (Ritland et al. 2005).

The specific goal of this study is to investigate levels of genetic variation and describe patterns of geographic differentiation for phenotypic traits in Garry oak throughout its range. Additionally, these quantitative estimates of population differentiation (Q_{ST}) will be compared to published estimates of G_{ST}, its neutral molecular analogue, in order to estimate the effect of differential selection and local adaptation on Garry oak. Lastly, we compare quantitative traits to source climatic and geographic variables to determine if clinal patterns exist, and if so, describe such patterns. Cumulatively, we use this information to inform seed transfer guidelines for restoration purposes and predict the potential response of Garry oak to future climate change scenarios.

2.2 Materials and methods

2.2.1 Sampling locations and techniques

Open pollinated acorns were collected from 141 individuals from 15 native populations of *Q. garryana* representing the species' entire range, during August and September of 2006 (Figure 1.1, Table 2.1). Populations were spaced a minimum of 50 kilometres apart, but in most cases distances between populations were much greater. Collection locations were determined using the Pacific Northwest Forest Inventory and Analysis database (Wadell and Hiserote 2005) and by personal communication with various U.S. Forest Service and Canadian Forestry Service employees. In order to compare results, effort was also made to collect from the same populations as those sampled for a previous isozyme study of Garry oak by Ritland et al. (2005).

Ten individuals, spaced a minimum of 50m apart from one another, were sampled in each population with the exception of populations 10 and 12, which lacked a sufficient supply of acorns. Whenever possible, 25 acorns were collected from multiple branches from each individual tree and then immediately stored in a cooler, in perforated plastic bags containing a 3:1 vermiculite perlite mixture. Latitude, longitude, and elevation were recorded for individual trees.

Although close attention was paid to collect from populations indicated as Garry oak in the Pacific Northwest Forest Inventory and Analysis database, the sub-species, Brewer's oak (*Quercus garryana* ssp. *breweri*), is known to exist in some of the collection areas (Population 1-6) and should thusly be considered when interpreting these results. As morphological differentiation of these two species (or sub-species) is difficult (based on mature size), a study of chloroplast diversity or sequence variation in the nuclear genome would be necessary to accurately differentiate subspecies at this stage of development.

2.2.2 Common garden experiment

Before sowing, all individual acorns were weighed and mean family acorn weights calculated. In the autumn of 2006, acorns were sown into individual D20 cells (Stuewe and Sons, Inc., Corvallis, Oregon) filled with a standard perennial soil mixture, and grown for their first season in a research greenhouse at the University of British Columbia. Data regarding height, diameter and emergence were collected in the first year. Additionally, above-ground and below-ground oven-dry biomass were measured for a sub-sample of 195 individuals to determine trends in root:shoot ratio. In March of

2007, 1692 one-year-old seedlings were transplanted into an outdoor field common garden experiment at Totem Field at the University of British Columbia (UBC) in Vancouver, British Columbia (49° 13'N, 123° 6'W). Before planting, a layer of Earthscape nine ounce landscape cloth (Shaw Fabric Products Inc., Wellington, Colorado) was laid and stapled over the garden site to mitigate the effect of weeds. Seedlings were then planted using a Complete Randomized Block Design (RCBD) with 12 blocks. Each family was represented once within each block for a total of 141 trees per block. Individuals were randomly assigned to both a block and position within block using a random number generator (Microsoft Excel 2001). Seedlings were planted at 60cm by 60cm spacing with a single edge row of non-experimental buffer trees surrounding the experiment. The site was weeded and watered when necessary, but no fertilizer was applied.

2.2.3 Phenotypic traits

Data regarding phenology (date of budburst, date of budset) and growth (final height, basal diameter at the root collar) were collected during the 2007/2008 growing season. In the autumn of 2008, artificial freeze testing was performed on 780 individuals using the electrolyte leakage method to determine cold injury. In total, data representing nine quantitative traits was collected including 1st and 2nd year height, 1st and 2nd year diameter, emergence date, 2nd year bud burst, 1st year bud set, root:shoot ratio and cold hardiness (Table 2.3).

The electrolyte leakage method as described by Hannerz et al. (1999) was employed to determine the cold hardiness of *Q. garryana*. Due to processing limitations (both temporal and spatial), lateral branches from a sub-sample of 780 individuals (13

populations, six families, ten blocks) were sampled in total. Also, due the same limitations, sampling was staggered. On October 26th, 2009, 390 individuals were sampled and processed. On November 3rd, 2009, the remaining 390 individuals were sampled and processed.

Three test temperatures (-22°C, -28°C, -34°C) and one unfrozen control (4°C) were employed. Test temperatures were based on preliminary freeze tests conducted one week prior. Stem samples were cut into 2mm cross sections, and two sections were placed into each test vial with 0.2ml distilled water and a few grains of silver iodide for ice nucleation. Control vials were kept at 4°C. All other samples were placed in a programmable Tenney Environmental Chamber (model T20C-3). The temperature was decreased at a rate of 4°C per hour until the first test temperature was reached. This temperature was held for one hour, following which all sample vials for that test temperature were removed and placed at 4°C to thaw. This process was repeated until all test temperatures had been reached, and held.

Following thawing, 3ml of distilled water were added to each vial and allowed to sit for 20 hours. Samples were shaken for one hour using a gravity shaker, after which conductivity was measured using a portable conductivity meter (BWR model 2052). Samples were then placed in a hot water bath at 95°C for one hour in order to heat kill tissues, and then allowed to cool at 4°C for 24 hours. Samples were shaken for one hour and heat-killed (maximum) conductivity was measured.

Injury index for each sample was calculated using the equation:

$$I_t = \frac{100(R_t - R_o)}{(1 - R_o)}$$

Where I_t is the index of injury resulting from exposure to temperature t, $R_t = L_t/L_k$, $R_O = L_O/L_d$, R_t is the relative conductance of the sample exposed to temperature t, R_O is the relative conductance of the unfrozen control sample, L_t is the conductance of the leachate from the sample exposed to temperature t, L_k is the conductance of the leachate from the sample exposed to test temperature t and then heat killed, L_O is the conductance of the leachate from the unfrozen sample, and L_d is the conductance of the leachate from the heat killed, unfrozen control sample (Flint 1967).

2.2.4 **Data Analyses**

All statistical analyses were conducted using SAS Version 9.1.3 (SAS Institute Inc. 2002-2005)**.** Analyses of variance were calculated for all traits except cold hardiness using the model:

$$y_{ijl} = u + b_i + p_j + f(p)_{jk} + b_i * p_j$$

Where b is the effect block i, p_j is the effect of population j and $f(p)_{jk}$ is the effect of family k within population j and d is the effect of date l.

To account for the difference in sampling date for cold hardiness, analysis of variance was calculated using the model:

$$y_{ijl} = u + b_i + p_j + f(p)_{jk} + b_i * p_j + d_l$$

Least squared population and family means were estimated using PROC GLM in SAS version 9.1.3 (SAS Institute 2002-2005). Family least-squares-means were estimated in addition to population least-squares-means to account for the high within population

variability observed for most traits. Using this procedure, simple linear regression coefficients of population and family means were estimated with all provenace climatic and geographic variables (including mean family seed weight) serving as independents.

Correlations among population means for quantitative traits and provenance geographic and climatic variables were estimated using PROC CORR. A sequential Bonferroni adjustment was used to ensure that an $\alpha = 0.05$ was upheld over all comparisons (Rice 1989). All climatic data was obtained using the ClimateBC model described by Wang et al. (2006).

When correlations exist among quantitative traits or in cases of complex geographic structuring, principal component analyses are useful for understanding obscured and complex relationships. PROC PRINCOMP was used to perform a principal component analysis on the population means of all quantitative traits. Utilizing an orthogonal linear transformation, principal component analysis extracts orthogonal variables that are linear combinations of the original variables such that the total variance of the data is best explained. Principal components one and two, summarizing variance in quantitative traits, were regressed as dependent variables against geographic and climatic variables using the procedure PROC REG.

Geographic and climatic variables are often intercorrelated. In such cases it can prove difficult to identify complicated spatial genetic patterns. Using the procedure PROC REG, the forward stepwise selection method was used with provenance climatic and geographic data to identify significant multivariate relationships with family means of quantitative traits.

Genetic differentiation of neutral molecular markers (G_{ST} of F_{ST}) was compared to genetic differentiation for quantitative traits (Q_{ST}) in order to compare the relative effect of divergent selection on population structuring (Merila and Crnokrak 2001). Q_{ST} for each trait was estimated following the equation:

$$Q_{ST} = \frac{\sigma^2_a}{(\sigma^2_a + 2\sigma^2_w)}$$

Where σ^2_a is the among-population genetic variation and σ^2_w is the within-population additive genetic variance (Spitze 1993). In this study the within-population variance was estimated as four times the variance component for family-within-population ($4\sigma^2_{f(p)}$). The within population genetic variation was approximated as four times the family variance due to Garry oaks high outcrossing rate (nearly 100%) and low inbreeding coefficient (0.025) (Ritland et al. 2005), thus open-pollinated progeny from one seed parent are expected to largely comprise half siblings. The SAS procedure PROC VARCOMP was used to estimate variance components for each trait using the restricted maximum likelihood method (REML).

In order to develop predictive equations for the purpose of constructing seed transfer guidelines, values of significant quantitative traits were regressed on the climate variable with the highest r^2 value. The slope of this regression provides a rate of change in the quantitative trait relative to the climate variable. Rates of differentiation along climatic gradients are interpreted relative to the least significant difference among populations at the 20% level (LSD 0.2). This reduces Type II errors – accepting no differences among populations when differences actually exist. Values of LSD for the quantitative traits were obtained from a Duncan's Multiple Range test in PROC GLM using the model for testing variation among populations described above. The rate of

differentiation of the key quantitative traits was determined as the change in the standardized climate variable. The difference in the climate variable associated with the significant genetic difference between populations was calculated as the rate of differentiation multiplied by the standard deviation of the climate variable (Rehfeldt 1991, 1994). Simple regressions of the climate variable on latitude, longitude, and elevation were used to determine the geographic distance associated with the rate of differentiation in the climate variable in order to make seed transfer recommendations.

2.3 Results

Significant differences were detected among the 13 populations analyzed for all quantitative traits except root:shoot ratio. Population, however, accounted for only a small portion of the variation in all growth related traits (height and diameter) and for some phenological traits (budset, budburst) (explaining an average of 16% of the total phenotypic variation). For cold hardiness and emergence date, population accounted for a more substantial proportion of the variance. The lack of significance for root:shoot ratio may be attributable to the smaller sample size employed (N =195).

Genetic differentiation (Q_{ST}) was weak for all quantitative traits ($0.08 \leq Q_{ST} \leq 0.13$) except cold hardiness ($Q_{ST} = 0.31$) and emergence date ($Q_{ST} = 0.30$) (Table 2.4). Comparisons of Q_{ST} to a previously published estimate of G_{ST} (Table 2.5) suggests that the weakest population differentiation in quantitative traits is similar in magnitude to the population differentiation for putatively neutral molecular markers, and that traits with the strongest differentiation show substantially more differentiation than isozymes.

Pearson correlations among population means for quantitative traits displayed strong ($r>0.75$) positive relationships between height (year one and two) and diameter (year one and two) (Table 2.6) Significant ($r>0.6$) negative relationships were evidenced between height (year one and two) and emergence date (Table 2.6).

Geographic variables describing provenance means showed strong positive correlations ($r>0.75$) of latitude with longitude and also strong ($r>0.75$) negative correlations of latitude and longitude with elevation (Table 2.7). Latitude and longitude were significantly ($r>0.6$) negatively correlated with MCMT and SH:M and positively correlated with MSP (Table 2.7). Elevation was positively correlated ($r>0.6$) with MAT, MWMT, TD, and SH:M and was negatively correlated with MSP (Table 2.7). Furthermore, significant and strong ($r>0.6$) positive correlations were detected among most temperature variables and among most precipitation variables (Table 2.7).

There were a number of significant correlations observed between population means for quantitative traits and provenance climatic or geographic variables. Height and diameter (years one and two) showed significant ($r>0.6$) negative correlations with latitude and longitude (Table 2.8). Height was also significantly ($r>0.6$) positively associated with elevation and SH:M (year one only) and negatively associated with MSP (year one only) (Table 2.8). Emergence date was significantly ($r>0.6$) positively correlated with latitude, longitude and MSP and significantly negatively correlated with elevation, MWMT and TD (Table 2.8). Cold hardiness showed significant ($r>0.6$) correlations with both TD and MWMT (Table 2.8). Finally, mean family seed weight was regressed on all climate and geographic variables. Significant linear relationships were observed for emergence date and budburst with seed weight however relationships were

weak (r^2 = 0.16 and r^2 = 0.1 respectively), suggesting a small maternal effect for some traits. Overall, populations from the higher elevations tended to grow the tallest, emerge the earliest and were generally more cold tolerant. No significant geographic or climatic patterns were observed for budburst, budset or root:shoot ratio.

Principal component analysis of growth and phenology traits for the 13 populations showed significant geographic and climatic relationships (Table 2.10). Principal component one (PC1) represented 35.5% of the total variation and generally represented traits related to growth (HT1, HT2, DM1, DM2) (Table 2.9). Principal component two (PC2) accounted for 16% of the total variation and represented traits related to phenology and in particular, budburst and emergence dates (Table 2.9). Principal component three (PC3) accounted for 14.4% of the total variation and was weighted almost exclusively towards bud set date (Table 2.9). Cold hardiness was not included in the principal component analysis due to the smaller sample size. Regression analyses were performed on the first three principal components (PC1, PC2 and PC3) whose eigenvalues were greater than one. Significant clinal relationships were revealed between PC1 and latitude, longitude, elevation, and SH:M (Table 2.10). PC2 also showed significant linear relationships with latitude, longitude, elevation, MWMT, MSP, and SH:M (Table 2.10). Regressions of PC3 did not reveal any significant linear relationships.

2.4 Discussion

2.4.1 Genetic variation

Genetically, oak is a highly variable genus, with species exhibiting a wide range for measures of molecular genetic diversity (Petit et al. 2002). Similarly, quantitative genetic studies of oak species have revealed varying results (Stowe et al. 1993, Ducousso et al. 1996, McBride et al. 1997, Uribe-Salas et al. 2008). In this study we observed significant differences among populations for all traits except root:shoot ratio. However, only differences among populations for cold hardiness and emergence date accounted for a substantial portion of the genetic variance, suggesting that these traits may play a larger role in determining fitness optimums for Garry oak. Additionally, a significant relationship was observed between mean family seed weight and mean family emergence date ($r^2 = 0.16$) and mean family bud burst date ($r^2 = 0.1$) suggesting that there may be a maternal effect on phenological traits related to initiation.

2.4.2 Effects of environment on quantitative traits

Substantial geographic clinal patterns were observed for both height and emergence date (Table 2.8), whereby individuals from the southern and eastern portions of the range tend to grow taller and emerge earlier. Latitude and longitude, however, are strongly negatively correlated with elevation (Table 2.7), suggesting that any geographic partitioning may have more to do with elevation and its associated environmental variables. Both height and elevation are strongly correlated with MSP and SH:M, suggesting that aridity influences growth in that trees experiencing drier summer conditions tend to grow taller. This may suggest an evolutionary advantage of growing taller, and to a lesser extent wider on sites more predisposed to fire.

The same is true for emergence date. Although strongly geographically correlated, individuals from acorns collected from sites experiencing more arid summer conditions (low MSP, high MWMT) (Table 2.8.) and higher continentality (TD) (Table 2.8) tended to emerge earlier. All of these climatic variables were highly correlated with elevation. Again, faster germination on sites more proned to fire may provide a stronger competitive advantage in terms of post-fire regeneration.

Finally, cold hardiness was also highly correlated with environmental variables associated with summer aridity. Specifically, individuals from sites experiencing higher MWMT and higher continentality (TD) tended to be more cold hardy. (Table 2.8).

These results support the existing evidence that Garry is relatively closely adapted to environmental conditions related to aridity and consequently, fire (Agee 1993. Tveten and Fonda 1999, Gedalof 2006), and suggest that Garry oak's competitive advantage may be more relevant within such dramatic environmental conditions. This has also been evidenced and documented by the transformation of much oak habitat to closed canopy conifer forest as a result of anthropogenic fire suppression (Agee 1993, Tveten and Fonda 1999, Gedalof 2006). It is curious, however, that geographic variation was not observed for the trait root:shoot ratio, as it is often considered a key aspect of drought tolerance. The modest sample size (N = 195) used to assess this trait may have been inadequate to detect variation for this trait.

An alternative explanation for the relationship of growth and emergence with aridity may be the effect of countergradient variation, whereby genetic selection counteracts the limiting effects of the environment on growth and emergence. The higher growth and emergence potential of more arid populations of Garry oak may be masked in

the field by stronger environmental constraints on these traits (Conover 1995). When environmental conditions are equalized, as they were in this experiment, underlying differences in growth and emergence capacity can be observed. Although possible, it is difficult to determine the effect of countergradient variation conclusively, given the confines of this experiment.

The principal component analysis is congruent with the results of the simple correlations in that PC1, which represents height and diameter and PC2, which represents emergence and budburst were both correlated with geographic and environmental variables associated with summer aridity (Table 2.10). PC3 exhibited no significant clinal patterns.

Finally, the use of multivariate stepwise regression techniques did not help to clarify the situation. The amount of variation in seedling traits explained by the inclusion of additional independent variables in the model was negligible (Table 2.11).

2.4.3 Genetic Differentiation

Genetic differentiation (Q_{ST}) for all traits except bud burst and year two diameter (excluding root:shoot ratio) was greater than the previously published estimate of population differentiation for neutral molecular markers (G_{ST}) (Table 2.5.). In all but two traits (cold hardiness and emergence date), levels of Q_{ST} were only slightly greater than G_{ST} (Table 2.4. and 2.5.)**.** Cold hardiness and emergence date, however, displayed levels of Q_{ST} substantially greater than the published estimate of G_{ST} for isozymes suggesting that these traits are under stronger effects of differential natural selection and show evidence of local adaptation. As is the case for many species of forest trees, these results suggest that traits related to cold acclimation and phenology (emergence date) have

strong differential selective pressures acting on them. (Howe et al. 2003, Savolainen et al. 2007). Oaks' propensity to hybridize may be affecting overall levels of genetic differentiation in populations south of the Siskiyou mountain range (populations 1-6) and otherwise. Seed weight may be contributing to the high Q_{ST} value for emergence date and should be considered when making conservation recommendations regarding initiation phenology.

Oak is a highly variable genus, known to exhibit substantial values for both genetic and geographic differentiation (Petit et al. 1993, Stowe et al. 1993, Ducousso et al. 1996, McBride et al. 1997, Petit et al. 2002, Savolainen et al. 2007, Uribe-Salas et al. 2008.). Levels of molecular genetic differentiation (G_{ST}) for Garry oak are comparable to values reported for other oak species (Table 2.5). The low levels of genetic differentiation observed for budset and budburst is suprising. Forest trees are known to frequently exhibit strong genetic and geographic differentiation for traits related to phenology (Howe et al. 2003, Savolainen et al. 2007). This was not observed in this study, suggesting that selective pressures are not acting differently among populations upon traits related to the initiation and cessation of growth, that gene flow is homogenizing populations, or that genetic variation is insufficient within the species for selection to act upon. In a similar common garden study of another western oak, blue oak (*Quercus douglasii*) McBride et al. (1997) found no evidence of geographic partitioning of genetic variation for any quantitative traits. No other quantitative genetic studies of western oaks have as of yet been conducted.

Molecular studies of three western oaks (Garry oak, blue oak and valley oak) have found comparatively little genetic variation (Riggs et al. 1991), and in the case of Garry

oak, levels of genetic diversity were half that of those found in other white oak species (Ritland et al. 2005). Relatively low levels of neutral genetic diversity suggest that Garry oak may have undergone a bottleneck sometime in the past and may explain the relatively weak local adaptation observed. Although speculative, this explanation is congruent with current hypotheses regarding the post-glacial history of other Pacific Northwest species (Soltis et al. 1997). A phylogeographic study of chloroplast sequence diversity would help to shed light on the post-glacial history of Garry oak.

Following a bottleneck it is expected that quantitative trait variation should recover more quickly than neutral molecular variation (Willis and Orr 1993). It has also been established that genetic variation for phenotypic traits and neutral molecular marker variation are not necessarily correlated (Reed and Frankham 2001). High levels of within population variation for most traits in this study suggest that, despite somewhat low levels of molecular genetic diversity, gene flow may be more extensive than previously suspected. Although gene flow due to seed dispersal is thought to be quite limited, pollen has been shown to travel much greater distances, especially for meadow tree species (Sork et al. 2002, Vekemans and Hardy 2004). In the case of Garry oak, pollen dispersal is likely contributing substantially to among-population gene flow. Additionally, oak's poor development of reproductive barriers between species and apparent propensity to hyrbidize may, in the case of Garry oak, also be contributing to among-population gene flow south of the Siskiyou mountain range in California where numerous oak species exist (Whittenmore and Schaal 1991, Petit et al. 2003), although no significant trends were observed across the southern populations alone.

Finally, phenotypic plasticity must be considered when interpreting results from common garden studies suggesting local adaptation. The role of phenotypic plasticity as an adaptive response to heterogeneous environments has been shown to vary widely within and among species, and is not fully understood (Bradshaw 1965, Valladares et al. 2007). Sultan (1987) argued that genetic diversity within a population or species may be the result of individual's ability to avoid selection by plastic adjustment to its environment. One way to resolve this question is to establish multiple common garden experiments in different environments in order to test the genotype by environment interaction. Although not a part of this thesis, a second common garden has been established at the Ministry of Forests and Range's Cowichan Lake Research Station on Vancouver island. Care should be given to understanding the roles of genetic and plastic adaptive responses in the case of species eligible for conservation efforts (Sultan 1987, Valladares et al. 2007).

Table 2.1 Garry oak populations sampled, geographic coordinates, and climatic information.

Site No.	Name	N	Lat. (°N)	Long. (°W)	Elev. (m)	MAT (°C)	MAP (mm)	MWMT (°C)	MCMT (°C)	FFP (days)	MSP (mm)	SH:M
1	Sequoia*	10	35.87	118.63	1224	12.3	551	21.9	5.3	181	42	521.6
2	Sierra*	10	36.80	119.08	1093	14.8	743	25.1	6.7	246	51	489.6
3	Tahoe*	10	39.10	120.85	844	13.7	1302	23.4	6.2	216	89	262.7
4	Shasta South*	10	40.35	122.94	788	14.7	916	24.5	7	314	66	369.5
5	Shasta North*	10	40.84	122.02	475	15.5	1439	24.6	6.4	270	138	177.5
6	Klamath*	10	41.84	122.83	535	9.9	618	19.5	2.4	161	72	271.9
7	Medford	10	42.46	122.61	630	10.8	708	20.4	2.8	149	131	155
8	Salem	10	45.01	123.16	191	10.8	1258	18.6	3.9	183	161	115.3
9	Mt. Hood	10	45.28	121.34	650	9.2	472	19.4	-.1	142	74	263.2
10	Kalama+	2	46.05	122.85	27	10.8	1259	18.2	3.9	205	216	82.5
11	Scatter Creek	10	46.82	123.01	63	10.4	1311	17.8	3.8	185	167	102.4
12	Gifford Pinchot+	4	46.57	121.68	467	9.2	1775	17.9	1.4	158	138	120.1
13	Duncan	13	48.79	123.69	20	9.7	1075	17.1	2.8	192	236	72.3
14	Victoria	13	48.46	123.40	25	10	871	16.5	3.9	228	42	521.6
15	Courtney	10	49.72	125.01	35	9.3	1398	17.1	2.7	192	51	489.6

*Occurs in presence of other oak species.

+ Removed from final analysis due to sample size.

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*Occurs in presence of other oak species.

+ Removed from final analysis due to sample size.

Table 2.2 Descriptions of climate variables.

Climate Variable	Description of Variable
MAT	Mean annual temperature (°C)
MWMT	Mean warmest month temperature (°C)
MCMT	Mean coldest month temperature (°C)
TD	Temperature difference between MWMT and MCMT, or continentality (°C)
MAP	Mean annual precipitation (mm)
MSP	Mean annual summer (May to September) precipitation (mm)
SH:M	Summer heat:moisture index ((MWMT/(MSP/1000))
DD<0	Degree days below 0° C, chilling degree days
DD>5	Degree days above 5°C, growing degree days
$DD5_{100}$	Julian date on which DD>5 reaches 100
DD<18	Degree days below 18°C, heating degree days
DD>18	Degree days above 18°C, cooling degree days
NFFD	Number of frost free days
FFP	Frost free period
bFFP	Julian date on which FFP begins
eFFP	Julian date on which FFP ends
PAS	Precipitation as snow (mm)
EMT	Extreme minimum temperature over 30 years
WT	Mean population seed weight

Table 2.3 Descriptions of quantitative traits.

Trait	Abbreviation	Description
First year height	HT1	From root collar to the base of the terminal bud (Year one)
Second year height	HT2	From root collar to the base of the terminal bud (Year two)
First year stem diameter	DM1	At one cm above root collar after one year
Second year stem diameter	DM2	At one cm above root collar after two years
Emergence date	EMER	Date of first visible plant material to emerge during germination
First year bud burst	BB1	Date of first visible leaf material to emerge from terminal bud at beginning of 2^{nd} growing season
First year bud set	BS1	Date of first visible terminal bud scales at end of first growing season
Cold hardiness	CH	Electolytic leakage as a measure of damage following artificial freeze test of stem segments
Root:Shoot ratio	R:S	Ratio of dry shoot rate over dry root weight

Table 2.4 Significance level of family effect and population effect in ANOVA, narrow sense heritabilities (h^2) and genetic differentiation (Q_{ST}) for eight quantitative traits and three principal components.

Variable	Family		Population		h^2	Q_{ST}
	F-value	**p-value**	**F-value**	**p-value**		
HT1	2.84	<0.0001	7.53	<0.0001	0.51	0.12
HT2	2.93	<0.0001	7.02	<0.0001	0.55	0.10
DM1	2.56	<0.0001	8.16	<0.0001	0.45	0.13
DM2	1.96	<0.0001	3.85	0.0001	0.36	0.08
EMER	3.15	<0.0001	25.42	<0.0001	0.61	0.30
BB	2.06	<0.0001	4.34	<0.0001	0.32	0.08
BS	2.4	<0.0001	8.20	<0.0001	0.43	0.12
CH	0.96	0.56	2.50	0.007	-----	0.31
PC1	3.27	<0.0001	7.12	<0.0001	0.74	0.10
PC2	2.13	<0.0001	15.45	<0.0001	0.41	0.26
PC3	2.22	<0.0001	6.76	<0.0001	0.45	0.10

Table 2.5 Reported values of genetic differentiation based on isozyme molecular markers for Garry oak and other *Quercus* species.

Populations	Species	F_{ST} or G_{ST}	Reference
42	*Q. garryana*	0.084	Ritland et al. 2005
10	*Q. rubra*	0.09	Sork et al. 1993
2	*Q. alba complex*	0.03	Kremer and Petit 1993
57	*Q. ilex*	0.10	Michaud et al. 1995
7	*Q. petraea*	0.03	Zanetto et al. 1994
7	*Q. robur*	0.02	Zanetto et al. 1994
40	*Q. suber*	0.11	Toumi & Lumaret 1998

Table 2.6 Correlations among population means for nine quantitative traits. Correlations when $|r|>0.5$ significant at $\alpha = 0.05$ after Bonferroni adjustment for number of correlations tested (n=36).

	HT1	HT2	DM1	DM2	EMER	BB	BS	CH
HT2	**0.84∗**							
DM1	**0.80∗**	**0.90∗**						
DM2	0.09	0.26	0.32					
EMER	**-0.61**	**-0.73**	-0.53	-0.10				
BB	-0.36	-0.26	-0.08	0.12	0.36			
BS	0.48	0.40	0.31	-0.002	-0.28	-0.35		
CH	0.29	0.30	0.23	0.20	-0.45	0.10	-0.36	
R:S	-0.33	-0.16	-0.34	0.18	-0.10	0.04	0.09	0.03

Bolded typeface indicates relationships stronger than 0.60.

Table 2.7 Correlations among populations for three geographic and eight climatic variables.

	LAT	LONG	ELEV	MAT	MWMT	MCMT	MAP	MSP	FFP	TD
LONG	**0.85∗**									
ELEV	**-0.94∗**	**-0.88∗**								
MAT	**-0.75**	-0.52	**0.6**							
MWMT	**-0.87∗**	**-0.65**	**0.8**	**0.93∗**						
MCMT	**-0.63**	-0.39	0.44	**0.9∗**	**0.74**					
MAP	0.34	0.47	-0.53	0.17	-0.07	0.31				
MSP	**0.81∗**	**0.77**	**-0.87∗**	-0.46	**-0.65**	-0.29	**0.71**			
FFP	-0.28	-0.05	0.15	**0.78**	0.59	**0.82∗**	0.33	-0.16		
TD	**-0.67**	-0.57	**0.75**	0.46	**0.75**	0.12	-0.41	**-0.69**	0.07	
SHM	**-0.87∗**	**-0.85∗**	**0.93∗**	0.55	**0.70**	0.46	-0.59	**-0.88∗**	0.25	**0.60**
WT	0.01	-0.01	0.08	-0.30	-0.10	-0.38	-0.53	-0.23	-0.36	0.16

∗ significant at $\alpha = 0.05$ after Bonferroni adjustment for number of correlations tested (n=55)

Bolded typeface indicates relationships stronger than 0.60.

Table 2.8 Correlations among population means for quantitative, climatic and geographic variables.

	HT1	HT2	DM1	DM2	EMER	CH	BB	BS	R:S	PC1	PC2	PC3
LAT	**-0.80**	**-0.61**	-0.51	0.35	**0.74**	-0.56	0.37	-0.36	0.27	**-0.63**	**0.72**	-0.26
LONG	**-0.89∗**	**-0.72**	**-0.64**	0.28	**0.61**	-0.48	0.29	-0.41	0.23	**-0.72**	0.57	-0.33
ELEV	**0.81**	**0.64**	0.51	-0.4	**-0.63**	0.57	-0.36	0.31	-0.36	**-0.62**	**-0.63**	0.23
MAT	0.45	0.24	0.18	-0.43	-0.52	0.50	-0.11	0.01	-0.37	0.27	-0.46	-0.05
MWMT	0.57	0.41	0.31	-0.41	**-0.63**	**0.68**	-0.19	-0.02	-0.38	0.41	-0.57	-0.1
MCMT	0.36	0.07	0.04	-0.43	-0.34	0.18	-0.15	0.25	-0.30	0.13	-0.32	0.22
MAP	-0.39	-0.49	-0.24	0.13	0.39	-0.06	0.51	-0.33	-0.01	-0.39	0.48	-0.31
MSP	**-0.60**	-0.54	-0.36	0.28	**0.67**	-0.47	0.46	-0.27	0.20	-0.52	**0.7**	-0.18
FFP	-0.04	-0.25	-0.31	-0.48	-0.08	0.08	-0.01	-0.13	-0.32	-0.23	-0.06	-0.12
TD	0.48	0.54	0.41	-0.19	**-0.60**	**0.84∗**	-0.14	-0.28	-0.27	0.47	-0.54	-0.38
SHM	**0.79**	0.56	0.41	-0.44	-0.56	0.37	-0.44	0.36	-0.29	0.55	-0.58	0.3
WT	0.22	0.36	0.23	0.36	-0.30	0.17	**-0.66**	0.01	0.05	0.34	-0.48	-0.02

∗ significant at $\alpha = 0.05$ after Bonferroni adjustment for number of correlations tested (n=99)

Bolded typeface indicates relationships stronger than 0.60.

Table 2.9 Eigenvectors of the first (PC1), second (PC2) and third (PC3) components from Principal component analysis.

	Eigenvectors		
Traits	PC1	PC2	PC3
HT1	**0.49**	0.05	0.09
HT2	**0.51**	-0.07	0.02
DM1	**0.47**	0.04	-0.11
DM2	**0.40**	-0.01	-0.06
BB	0.21	**0.72**	-0.14
BS	0.08	<0.01	**0.96**
EMER	-0.23	**0.68**	0.13
Eigenvalue	2.5	1.1	1.0
	35.5%	16%	14.4%

Table 2.10 Summary of significant results from the regression of the first two principal components on geographic and climatic variables.

Dependent Variable	**Predictor Variable**	**R^2**	**p**
PC1	Latitude	0.40	0.02
	Longitude	0.52	0.005
	Elevation	0.37	0.02
	SH:M	0.30	0.04
PC2	Latitude	0.52	0.005
	Longitude	0.32	0.04
	Elevation	0.40	0.02
	MWMT	0.33	0.04
	MSP	0.50	0.007
	SH:M	0.34	0.03

Table 2.11 Amount of variation explained by stepwise regressions of select traits on environmental variables (R^2) and environmental variables included in models and of select traits on geographic variables (R^2) and geographic variable included in models.

Trait•	R^2	Environmental variables in model	R^2	Geographic variables in model
HT1	0.4	SHM, BFFP, DD<18, MSP, MAP	0.4	LONG, $LONG^2$, LAT^2, LAT, ELEV
HT2	0.41	SHM, EFFP, DD5100, DD>18, MWMT, MSP, NFFD, EMT, DD<0, BFFP	0.23	LAT^2, LONG
DM1	0.46	TD, BFFP, DD<18, MAT, NFFD, DD5100, EMT, DD<0, EFFP, MAP, DD>18, MSP	0.27	LONG, $ELEV^2$, $LONG^2$, ELEV
DM2	0.25	BFFP, SHM, DD5100, MSP, DD>18, MWMT, MAP, EFFP, EMT, PAS, NFFD	0.06	$ELEV^2$, LONG
EMER	0.71	MSP, MWMT, EFFP, MAT DD>18, DD<0, DD<18, FFP, DD>5, MAP	0.5	LAT^2, LAT, $LONG^2$, ELEV, $ELEV^2$
BB	0.3	MAP, DD<0, PAS, DD>5, DD<18, BFFP, NFFD, SHM, EMT, DD>18	0.05	$ELEV^2$, $LONG^2$
BS	0.42	DD<0, MAP, BFFP, TD, DD<18, DD>5, EMT	0.32	$ELEV^2$, ELEV, LAT^2, LAT, LONG
CH	0.3	TD, MAP, BFFP, MSP	0.28	LAT^2, LAT, ELEV, $ELEV^2$, $LONG^2$
R:S	0.14	DD>18, PAS, BFFP, MWMT, DD5100, MAP, DD<18, SHM	0.03	$ELEV^2$, $LONG^2$
PC1	0.41	SH:M, FFP, DD<18, DD>18, TD, DD>5, MSP, PAS, MAP, DD5100, EMT	0.31	$LONG^2$, $ELEV^2$, LAT^2, LAT, LONG, ELEV
PC2	0.61	MSP, DD<0, EFFP, TD, DD>18, NFFD, EMT, FFP, DD>5, DD5100	0.35	LAT^2, $ELEV^2$, $LONG^2$, LONG, ELEV
PC3	0.37	DD<0, MAP, DD>5, DD<18, MWMT, DD5100, MSP	0.26	$ELEV^2$, ELEV, LAT^2, LAT, LONG

• See Table 2.2 for trait codes.

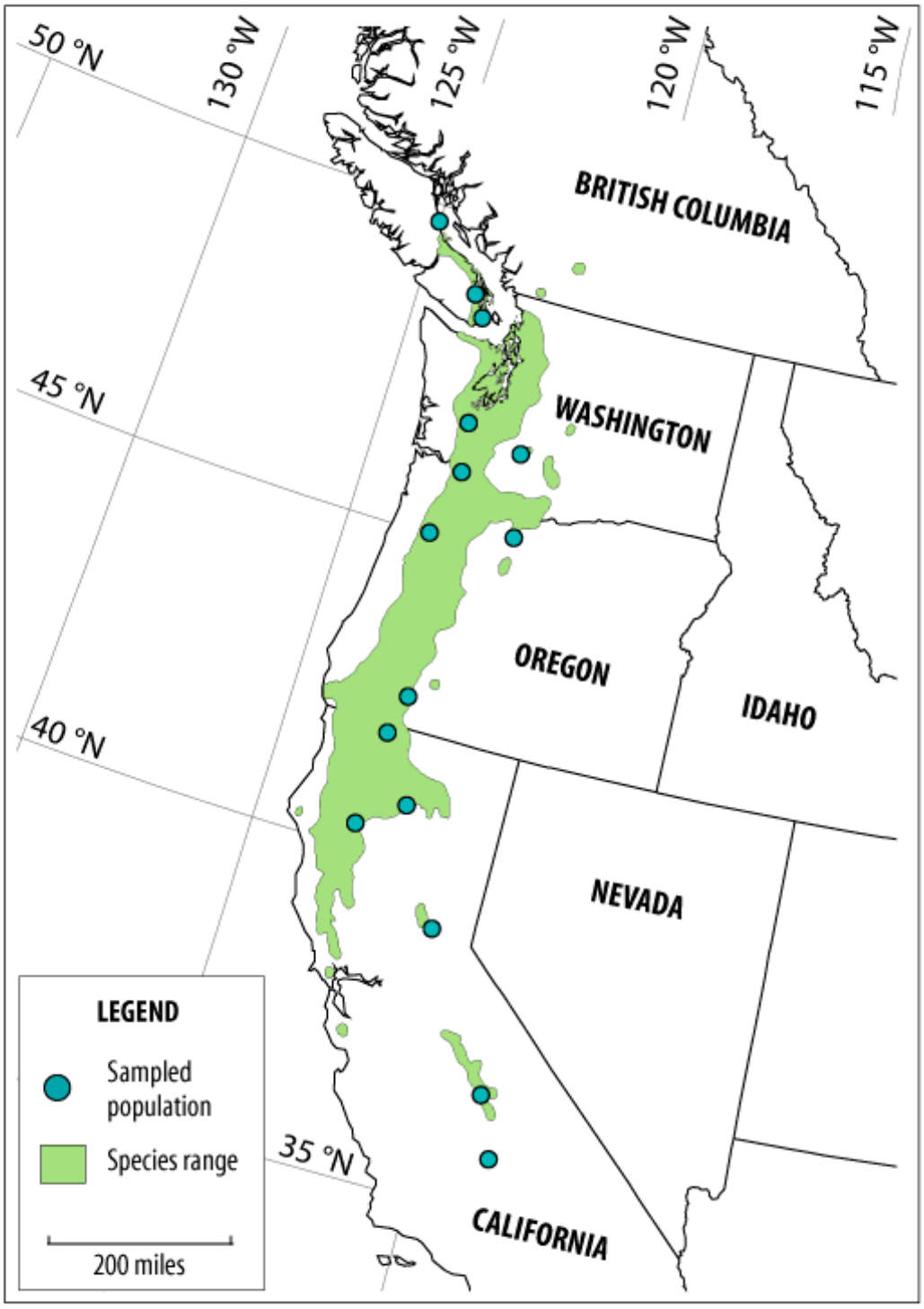

Figure 2.1 Natural distribution of Garry oak and acorn collection locations (Little 1976).

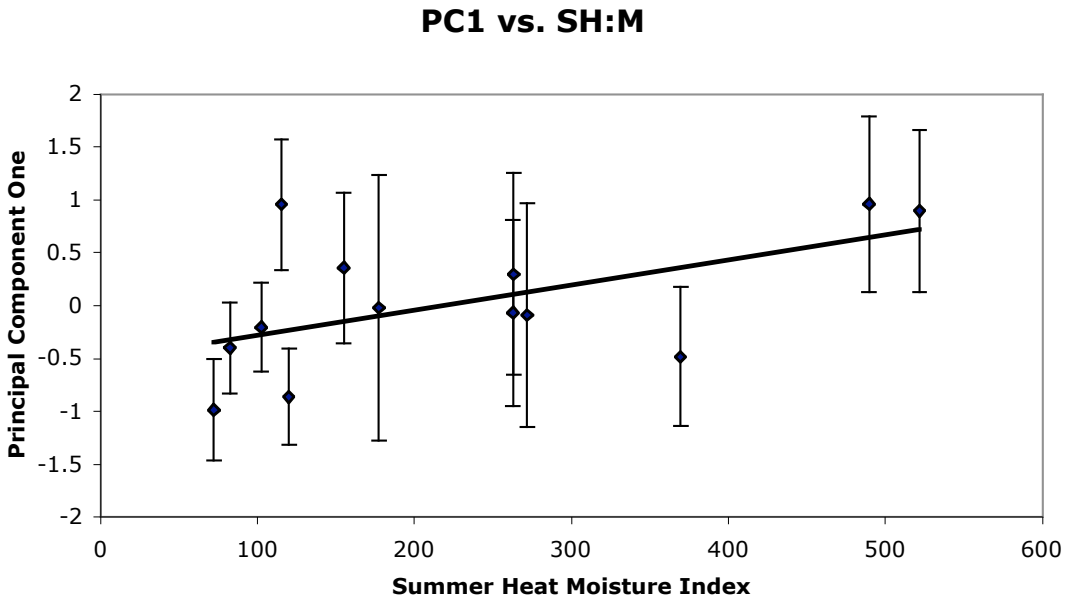

Figure 2.2 Regression of the first principal component (representing growth) on summer heat moisture index ((MWMT/(MSP/1000)) ($r^2 = 0.3$, $p = 0.04$). Data points indicate population means. Bars indicate standard error of those means.

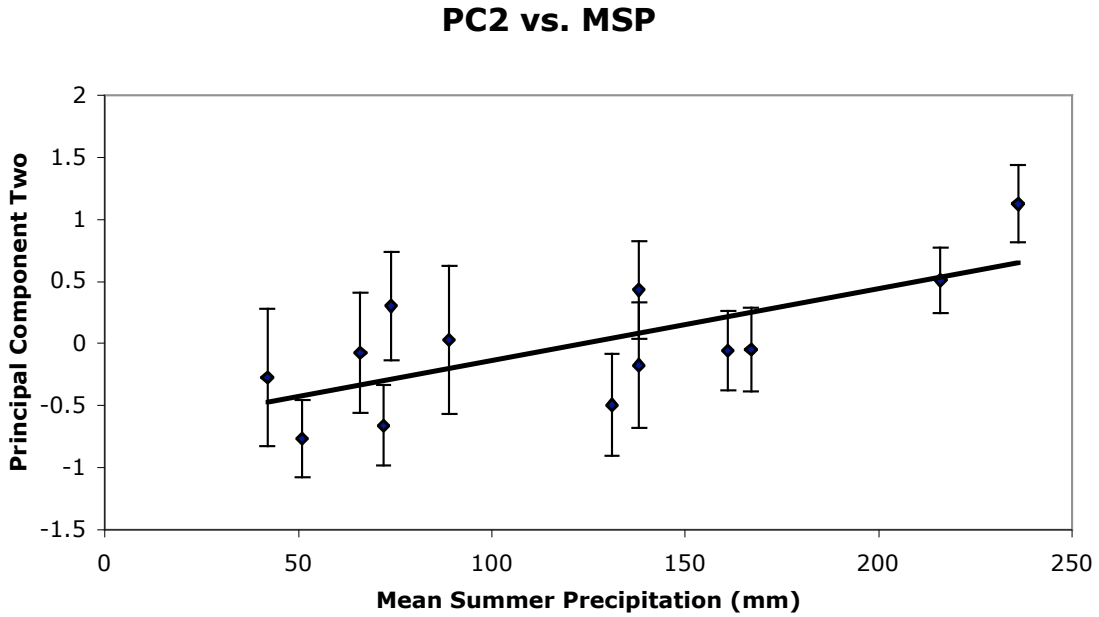

Figure 2.3 Regression of the second principal component (representing initiation phenology) on mean summer precipitation (mm) ($r^2 = 0.5$, $p = 0.007$). Higher scores on the y-axis represent later budburst and emergence. Data points indicate population means. Bars indicate standard error of those means.

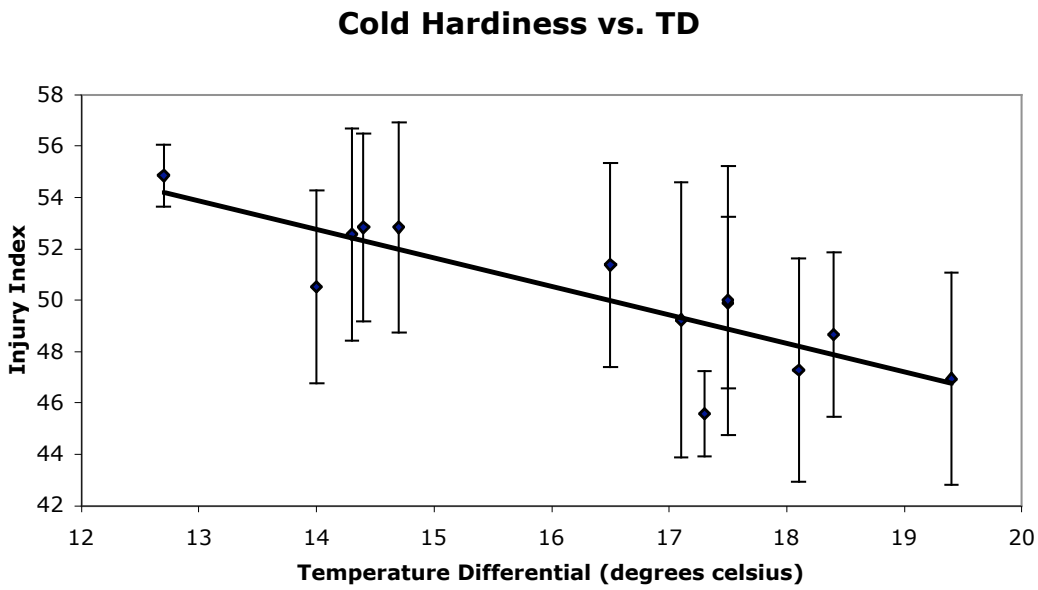

Figure 2.4 Regression of cold hardiness injury index on temperature differential (the difference between MTCM and MTWM) ($r^2 = 0.7$, $p = 0.0003$). Higher scores on the y- axis represent decreased cold tolerance. Data points indicate population means. Bars indicate standard error of those means.

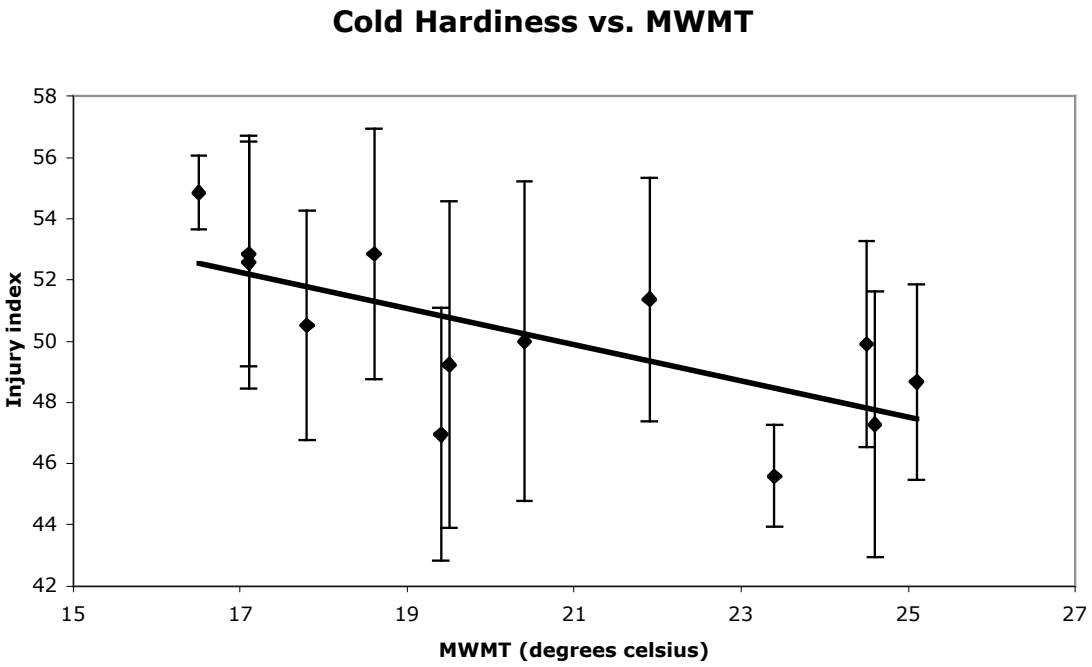

Figure 2.5 Regression of cold hardiness injury index on MWMT ($r^2 = 0.47$, $p = 0.009$). Higher scores on the y-axis represent decreased cold tolerance. Data points indicate population means. Bars indicate standard error of those means.

Chapter 3: Conclusions and Future Directions

3.1 Conclusions and conservation implications

This study reveals that 1) traits related to growth, emergence and cold hardiness of Garry oak are positively associated with provenance environmental conditions related to summer aridity, and that trees from arid environments tended to perform better; 2) cold hardiness and emergence date displayed stronger genetic differentiation than other phenotypic traits suggesting stronger selective pressures are acting on these traits; and 3) genetic differentiation for most traits was low, suggesting high gene flow in keeping with marker-based results.

Garry oak's positive association of genetic divergence among populations with summer aridity suggests that this species may be closely adapted to conditions supporting periodic forest fires and that this may have been a strong selective force in this species' evolution. Accelerated growth and emergence, presumably promoting seedling establishment and competitive ability, increase with summer aridity, suggesting that Garry oak's competitive advantage may exist predominantly in more extreme environmental conditions, such as those supporting more frequent or intense fire regimes. These results support the widely accepted evidence that oaks have evolved with fire and that many oak species possess the ability to survive and often thrive in regimes of periodic burning (Agee 1993. Tveten and Fonda 1999, Gedalof 2006). The effect of maternal seed effects or countergradient variation however, cannot be excluded and should be strongly considered when interpreting these results.

Contemporary fire suppression, however, has altered this evolutionary relationship and in many cases has resulted in the mass transformation of oak woodland to closed canopy conifer forests (Agee 1993). This conservation concern has often been

addressed through the use of prescribed burning to limit fuel build up and promote oak regeneration (Gedalof 2006). It is our recommendation that Garry oak restoration and conservation programs in British Columbia consider prescribed burning, where possible, as a tool to promote the future development and maintenance of Garry oak habitat.

Regeneration and recruitment of oaks, including Garry oak, is a common problem throughout the world, and is considered a major conservation concern (Fuchs 2000). Results from this study, linking faster emergence and increased growth with populations from high aridity environments, should also be considered when addressing the issue of oak recruitment.

Observed low levels of among-population genetic differentiation for most traits, despite our initial hypotheses, suggest that Garry oak may be experiencing high levels of among population gene flow. This suggests that Garry oak may have a strong adaptive potential due to its high within population genetic variation for most traits. Consideration should, however, be paid to the low levels of neutral molecular genetic diversity reported in other studies before making future conservation recommendations. While neutral variation for genetic markers does not reflect population differentiation for adaptive traits, levels of marker variation are considered representative of overall levels of gene diversity (Ritland and Ritland 2000).

3.2 Seed transfer guidelines and climate change

Garry oak's distribution is both extensive and heterogeneous. The results from this study suggest that particular populations are expected to be suitable for only a potion of the environmental conditions experienced throughout the species range. Under the assumption that local is best we have used the floating seed transfer model, developed by Rehfeldt (1991,1994), in concert with the results from this study to determine seed transfer guidelines for Garry oak restoration programs.

Emergence date and cold hardiness displayed the highest population differentiation (or local adaptation) levels of all phenotypic traits studied and were thus selected as the primary traits considered for calculating seed transfer guidelines. The greatest conservation risk with developing such guidelines is to conclude that populations are the same when they are, in fact, differently adapted, thus traits with the highest Qst values should be used for analysis. Differences in emergence date were predominantly related to MSP and MWMT. Garry oak seed can be moved between areas differing by up to 19 mm of precipitation in the summer or 1°C in MWMT, while maintaining an emergence date suitable for local climates and minimizing the risk of maladaptation. Differences in cold hardiness were related to temperature differential (td); however, maximum seed transfer distance based on these traits was too large to be of practical use for conservation and restoration purposes (8.2° latitude, 3.8° longitude, 667m elevation).

From a conservation and restoration standpoint, it is impractical to base seed movement on mean summer precipitation or MWMT. Rather, seed movement is traditionally based on geographic and elevational differences. In this case, the difference in mean summer precipitation and MWMT corresponds to a range of approximately 2° in latitude or 1° in longitude. Also accordingly, approximately 150m in elevation is required to distinguish genetically different populations and should be considered the limit when transferring seed from differing elevations in order to reduce the risk of maladaptation. The seed transfer guidelines may be conservative due to the possibility that maternal effects associated with provenance climates have inflated apparent genetic differentiation among populations for emergence date.

The role that climate change will play in the future of Garry oak is uncertain. Predicted increases in temperature and decreases in precipitation will likely force phenotypes to shift northward and higher in elevation to track climatic conditions to

which they are optimally adapted (Davis and Shaw 2001). Peripheral populations are considered to be more susceptible to climate change due to their lowered genetic variation and compromised fitness. However, it is expected that peripheral populations existing in northern latitudes and higher elevations of a species' range, such as those of Garry oak in British Columbia, may incur less climate change-related stress, relative to their southern counterparts (Davis and Shaw 2001, Aitken et al. 2008). This is due in part, to gene flow from the centre of the range introducing alleles pre-adapted to warmer conditions to these cooler provenances. The opposite is true in the case of rear-edge peripheral populations where gene flow will likely introduce alleles pre-adapted to cooler climates, reducing fitness to predicted future climates (Davis and Shaw 2001, Aitken et al. 2008).

In western North America, MWMT is predicted to increase 3° C by the year 2080 (Wang et al. 2006). Based on the relationship of MWMT to latitude this translates to a shift northward of approximately 4.5° latitude or 500 km over the next 70 years. This translates to approximately 7000 metres per year. Pollen records and cpDNA evidence suggest maximum historical annual migration rates of many forest trees at 100m (Mclachlan and Clark 2004, Mclachlan et al. 2007). Natural migration of such proportion is unlikely for Garry oak, especially given its current rate of habit loss and existing dispersal barriers in British Columbia (Fuchs 2001).

High levels of within-population variation for all quantitative traits in this study suggest that, irregardless of observed low levels of neutral molecular variation, Garry oak may have a strong adaptive potential, capable of dealing with such drastic environmental change. However, current rates of habitat loss in British Columbia coupled with a lack of migrational opportunity suggest that perhaps a more realistic future scenario for Garry oak is an overall range contraction resulting from extirpation in the south, in combination

with limited natural migration at the leading edge. This presents a need to investigate the potential benefit of initiating new populations through facilitated migration, north of Garry oak's current range (McLachlan et al. 2007).

3.3 Future directions

This study aimed to understand and contextualize the quantitative genetics of Garry oak. Although achieved, many questions are left in its wake. The positive association between growth, emergence and summer aridity observed in this study suggest that tolerance to drought and fire may play an important role in Garry oaks continued survival. A more extensive quantitative assessment of traits related to drought tolerance would be helpful to better understand this evolutionary relationship. Additionally, continued data collection at the Cowichan Lake Research Station common garden will help to estimate the magnitude of genotype-by-environment interaction and accurately assess the role of adaptive plasticity in this study.

Still, questions are left unanswered that quantitative genetics alone cannot address. A study incorporating genetic diversity in chloroplast DNA markers, known to be maternally inherited in oaks (Dumolin et al. 1995), would be useful in understanding the demographic and post-glacial history of Garry oak. Such a study would also afford a second index of genetic diversity for this species. Lastly, the establishment of a permanent common garden will allow for the exploitation of novel genetic techniques such as association mapping in order to better understand the genetic architecture of adaptation in Garry oak.

Literature Cited

Agee, J.K. 1993. Fire Ecology of Pacific Northwest Forests. Island Press, Washington DC and Covelo, CA. pp. 352-358.

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

Bower, A. D., and Aitken, S. N. 2008. Ecological genetics and seed transfer guidelines for *Pinus Albicaulis (Pinaceae).* American Journal of Botany. **95**(1): 66-76.

Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Gent. **13**: 115-155.

Brown, K.J., and Hebda, R.J. 2002. Origin, dynamics, and development of coastal temperate conifer rainforests of southern Vancouver Island, Canada. Can J. For Res. **32**: 353-372.

Conover, D.O., and Shultz, E.T. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends Ecol. Evol. **10**: 248-252.

Coyne, J. A. 1992. Genetics and speciation. Nature. **255**: 511-515.

Davis, M. B., and Shaw, R. G. 2001. Range shifts and adaptive responses to Quaternary climate change. Science. **292**: 673-679.

Davis, M. B., Shaw, R. G., and Etterson, J. R. 2005. Evolutionary responses to changing climate. Ecology. **86**: 1704-1714.

Ducousso, A., Guyon, J. P., Kremer, A. 1996. Latitudinal and altitudinal variation of bud burst in western populations of sessile oak (*Quercus petraea* (Matt) Liebl). Ann. Sci. For. **53**: 775-782.

Dumolin, S., Demesure, B., and Petit, R.J. 1995. Inheritance of chloroplast and mitochondrial genomes in pedunclate oak investigated with an efficient PCR method. Theoret. Appl. Genet. **91**: 1253-1256.

Erickson, W.R. 2000. Garry oak communities in Canada: classification, characterization and conservation. International Oaks **10**: 40-54.

Felsenstein, J. 1976. The theoretical population genetics of variable selection and migration. Annual Review of Genetics. **10**: 253-280.

Flint, H. L., Boyce, B. R., and Beattie D. J. 1967. Index of injury – a useful expression of freezing inury in plant tissues as determined by the electrolytic method. Can. Jo. Plant Sci. **47**: 229-230.

Frankham, R. 1999. Quantitative genetics in conservation biology. Genet. Res. **74**: 237-244.

Fuchs, M.A. 2001. Towards a recovery strategy for Garry oak and associated ecosystems. Ecological assessment and literature review. Pacific and Yukon Region, Canadian Wildlife Service, Environment Canada. Technical Report GBEI/EI-00-030.

Fuchs, M.A., Krannitz, P.G, and Harestad, A.S. 2000. Factors affecting emergence and first-year survival of seedlings of Garry oaks (*Quercus garryana*) in British Columbia, Canada. For. Ecol. Manage. **137**: 209-219.

Gedalof, Z., Pellatt, M., and Smith, M. J. 2006. From prairie to forest: Three centuries of environmental change at Rocky Point, Vancouver Island, British Columbia. Northwest Science. **80** (1): 34-46.

Grivet, D., Smouse, P.E., and Sork, V.L. 2005. A novel approach to an old problem: tracking dispersed seeds. Molecular Ecology. **14**: 3585-3595.

Hamann, A., El-Kassaby, Y. A., Koshy, M.P., Namkoon, G. 1998. Multivariate analysis of allozymic and quantitative trait variation in Alnus rubra: geographic patterns and evolutionary implications. Can. J. For. Res. **28**(10): 1557-1565.

Hamann, A., and Wang, T. 2006. Potential effects of climate change on ecosystem and tree species distribution in British Columbia. Ecology. **87**(11): 2773-2786.

Hamrick, J. L. 2004. Response of forest trees to global environmental changes. Forest Ecol. Manag. **197**: 323-335.

Hannerz, M., Aitken, S. N., King, J. N., and Budge, S. 1999. Effects of genetic selection for growth on frost hardiness in western hemlock. Can. J. For. Res. **29**(4): 509-516.

Hebda, R. 1997. Impact of climate change on biogeoclimatic zones of British Columbia and Yukon. Pp. 13-1 to 13-15 in E. Taylor and B. Taylor, eds. Responding to Global Climate Change in British Columbia and Yukon. Volume 1 of the Canada Country Study: Climate Impacts and Adaptation. Environment Canada, Ottawa, ON.

Hebda, R., and Mathewes, R.W. 1984. Holocene history of cedar and native Indian cultures of the North American Pacific Coast. Science. **225**: 711-713.

Hendry, A. P. 2002. Qst ≠ Fst? Trends Ecol. Evol. **17**: 502.

Howe, G. T., Aitken, S. N., Neale, D. B., Jerstad, K. D., Wheeler, N. C., and Chen, T. H. H. 2003. From genotype to phenotype: unravelling the complexities of cold adaptation in forest trees. Can. J. Bot. **81**: 1247-1266.

Huenneke, L.F. 1991. Genetics and Conservation of Rare Plants. Oxford University Press, Oxford UK.

Kaweki, T.J., and Ebert, D. 2004. Conceptual issues in local adaptation. Ecology Letters. 7: 1225-1241.

Keir, K. R., and Aitken, S. N. 2009. Beautiful but lacking diversity: Population genetics of pacific dogwood (*Cornus nuttallii*). Submitted for publication.

Kremer, A., and Petit, R. J. 1993. Gene diversity in natural populations of oak species. Annales des Sciences Forestieres **50**: 186-202.

Lesica, P., and Allendorf, F.W. 1995. When are peripheral populations valuable for conservation? Conserv. Biol. **9**(4): 753-760.

Linhart, Y. B., and Grant, M. C. 1996. Evolutionary significance of local genetic differentiation in plants. Annu. Rev. Ecol. Syst. **27**: 237-277.

Little, E.L. 1976. Atlas of United States trees, volume 3, minor western hardwoods. U.S. Department of Agriculture Miscellaneous Publication 1314. 13 p., 290 maps.

Mayr, E. 1982. Adaptation and selection. Biol. Zentralblatt fur Math. **101**: 161-174.

McBride, J. R., Norberg, E., Bertenshaw, J., Kloss, S., and Mossadegh, A. 1997. Genetic variation in shoot growth phenology, and mineral accumulation of northern and central Nevada populations of blue oak. USDA Forest Service Gen. Tech. Rep. PSW-GTR-160.

McKay, J. K., Christian, C. E., Harrison, S., and Rice, K. J. 2005. "How local is local?" – A review of practical and conceptual issues in the genetics of restoration. Restor. Ecol. **13**:432-440.

McKay, J. K., and Latta, R. G. 2002. Adaptive population divergence: markers, QTL and traits. Trends Ecol. Evol. **17**: 285-291.

McLachlan, J.S., and Clark, J.S. 2004. Reconstructing historical ranges with fossil data at continental scales. Forest Ecology and Management. **197**: 139-147.

McLachlan, J.S., Hellman, J.J., and Schwartz, M.W. 2007. A framework for debate of assisted migration in an era of climate change. Conservation Biology. **21**: 297-302.

Merila, J., and Crnokrak, P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J. Evolution. Biol. **14**: 892-903.

Michaud, H., Toumi, L., and Lumaret, R. 1995. Effect of geographical discontinuity on genetic variation in *Quercus ilex* L. (holm oak). Evidence from enzyme polymorphism. Heredity **74**: 590-606.

Mimura, M., and Aitken, S. N. 2007. Adaptive gradients and isolation-by-distance with post-glacial migration in *Picea sitchensis*. Heredity. **99**: 224-232

Mohammed, N. I., and Ritland, K. 2004. Genetic variation, population structure, and mating system in bigleaf maple (*Acer macrophyllum* Pursh). Can J. Bot. **82**(12): 1817-1825.

Morgenstern, K E. 1996. Geographic variation in forest trees. UBC Press, Vancouver, B.C.

Pavlik, B.M., Muick, P.C., Johnson, S.G., and Popper, M. 1992. Oaks of California. Cachuma Press and the California Oak Foundation, Oakland, California. pp. 19-72.

Petit, R. J., Kremer, A., and Wagner, D. B. 1993. Geographic structure of chloroplast dna polymorphisms in European oaks. Theoretical and Applied Genetics. **87**(1-2): 122-128.

Petit, R. J., Csaikl, U. M., Bordacs, S., Burg, K., Coart, E., Cottrell, J., Van Dam, B., Deans, J. D., Dumolin-Lapegue, S., Fineschi, S., Finkeldey, R., Gillies, A., Glaz, I., Goicoechea, P. G., Jensen, J. S., Konig, A. O., Lowe, A. J., Madsen, S. F., Matyas, G., Munro, R. C., Olalde, M., Pemonge, M. H., Popescu, F., Slade, D., Tabbener, H., Taurchini, D., de Vries, S. G. M., Ziegenhagen, B., Kremer, A. 2002. Chloroplast DNA variation in European white oaks: Phylogeography and patterns of diversity based on data from over 2600 populations. For. Ecol. Manag**. 156**: 5-26.

Petit, R. J., Bodenes, C., Ducousso, A., Roussel, G., and Kremer, A. 2003. Hybridization as a mechanism of invasion in oaks. New Phytologist **161**: 151-164.

Petit, R.J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D., and Vendramin, G.G. 2005. Comparative organization of chloroplast, mitochondrial, and nuclear diversity in plant populations. Molecular Ecololgy. **14**: 689-701.

Reed, D. H., and Frankham, R. 2001. How closely related are molecular and quantitative measures of genetic variation? A meta analysis. Evolution. **55**: 1095-1103.

Rehfeldt, G. E. 1989. Ecological adaptations in Douglas fir (*Pseudotsuga menziesii* var. *glauca*): a synthesis. For. Ecol. Manag. **28**: 203-215.

Rehfeldt, G. E. 1995. Genetic variation, climate models and the ecological genetics of *Larix occidentalis*. Forest Ecol. Manag. **78**: 21-37.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution. **43**: 223-225.

Riggs, L. A., Millar, C. I., and Delany, D. L. 1991. Genetic variation sampled in three Californian oaks. USDA Forest Service Gen. Tech. Rep. PSW-126.

Ritland, C., and Ritland, K. 2000. DNA fragment markers in plants. In Molecular methods in ecology. Blackwell Scientific, Oxford, UK. 208-234.

Ritland, K., Meagher, D.G.W.E., and Y.A. El-Kassaby. 2005. Isozyme variation and the conservation genetics of Garry oak. Can. J. Bot. **83**: 1478-1487.

SAS Institute Inc. 2005. SAS Online DocR, Verstion 9.1.3, Cary, N.C.
Soltis, D.E., Gitzendanner, M.A., Strenge, D.D., and Soltis, P.S. 1997. Chloroplast DNA intraspecific phlogeography of plants from the Pacific Northwest of North America. Plant Systematics and Evolution. **206**: 353-373.

Savolainen, O., Pyhajarvi, T., and Knurr, T. 2007. Gene Flow and local adaptation in trees. Annu. Rev. Ecol. Evol. Syst. **38**: 595-619.

Sork, V. L., Huang, S., and Wiener, E. 1993. Macrogeographic and fine-scale genetic structure in a North American oak species, *Quercus rubra* L. Annales des Sciences Forestieres **50**: 261-270.

Sork, V. L., Davis, F. W., Smouse, P. E., Apsit, V. J., Dyer, R. J., Fernandez, J. F. and Kuhn, B. 2002. Pollen movement in declining populations of California Valley oak, Quercus lobata: where have all the fathers gone? Molecular ecology **11**: 1657-1668.

Spitze, K. 1993. Population structure in *Daphina obtus*: quantitative genetic and allozymic variation. Genetics. **135**: 367-374.

St. Clair, J. B., Mandel, N. L., and Vance-Borland, K. W. 2005. Genecology of Douglas fir in western Oregon and Washington. Ann. Bot. **96**: 1199-1214.

Stein, W.I. 1990. *Quercus garryana* Dougl. ex Hook. in R.M. Burns and B.H. Honkala, tech cords. Silvics of North America. Volume 2. Agricultural Handbook 654. US Department of Agriculture Forest Service, Timber Management Research, Washington DC. pp. 650-660.

Storz, J.F. 2002. Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analyses of clinal variation. Molecular Ecology. **11**: 2537-2551.

Stowe, K. A., Sork, V. L., and Farrell, A. W. 1994. Effect of water availability on the phenotypic expression of herbivore resistance in northern red oak seedlings (*Quercus rubra L.*). Oecologia. **100**: 309-315.

Sultan, S.E. 1987. Evolutionary implications of phenotypic plasticity in plants. Evol. Biol. **21**: 127-178.

Toumi, L., and Lumaret, R. 1998. Allozyme variation in cork oak (Quercus suber L.): the role of phylogeography and genetic introgression by other Mediterranean oak species and human activities. Theoretical and Applied Genetics. **97**: 647-656.

Towle, J.C. 1979. Settlement and subsistence in the Willamette Valley: some additional considerations. Northw. Anthro. Res. Notes **13**: 12-21.

Tveten, R. K., and Fonda, R. W. 1999. Fire effects on prairies and oak woodlands on Fort Lewis, Washington. Northwest Science. **73**(3): 145-158.

Uribe-Salas, D., Saenz-Romero, C., Gonzalez-Rodriguez, A., Tellez-Valdez, O., and Oyama, K. 2008. Foliar morphological variation in the white oak *Quercus rugosa Nee* (*Fagaceae*) along a latitudinal gradient in Mexico: Potential implications for management and conservation. For. Ecol. Manag. **256**: 2121-2126.

Valladares, F., Gianoli, E., and Gomez, J.M. 2007. Ecological limits to plant phenotypic plasticity. New Phytologist. **176**: 749-763.

Vander Wall, S.B. 1990. Food Hoarding in Animals. Univ. of Chicago Press, Chicago IL and London UK. pp. 297-305.

Vekemans, X., and Hardy, O. J. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Molecular Ecology **13**: 921-935.

Volis, S., Yakubov, B., Shulgina, I., Ward, D., and Medlinger, S. 2005. Distinguishing adaptive from non-adaptive genetic differentiation: comparison of Qst and Fst at two spatial scales. Heredity. **95**: 466-475.

Waddell, K.L., and Hiserote, B. 2005. The PNW-FIA Integrated database user guide and documentation: Version 2.0. Internal Publication: Forest inventory and analysis program, Pacific Northwest Research Station. Portland, Oregon.

Wang, T., Hamann, W., Spittlehouse, D. L. and Aitken, S. N. 2006. Development of scale-free climate data for western Canada for use in resource management. Int. J. Climatol. **26**: 383-397.

Ward, P., Radcliffe, G., Kirkby, J., Illingworth, J., and Cadrin, C. 1998. Sensitive ecosystems inventory: East Vancouver Island and Gulf Islands, 1993-1997. Vol. 1. Methodology, ecological descriptions and results. Pacific and Yukon Region, Canadian Wildlife Service, B.C. Technical Report Series No. 320.

Williams, G.C. 1966. Adaptation and natural selection. University Press, Princeton, NJ. pp. 56-120.

Willis, J. H., and Orr, H. A. 1993. Increased heritable variation following population bottlenecks: the role of dominance. Evolution. **47**: 949-957.

Whitlock, M. C. 1999. Neutral addititive genetic variance in a metapopulation. Genet. Res. **74**: 215-221.

Whitlock, M. C. 2008. Evolutionary inference from Qst. Molecular Ecology. **17**: 1885-1896.

Whittenmore, A. T., and Schaal, B. A. 1991. Interspecific gene flow in sympatric oaks. National Academy of Sciences **86**: 2540-2544.

Yang, R. C., Yeh, F. C., and Yanchuk, A. D. 1996. A comparison of isozyme and quantitative genetic variation in *Pinus contorta* ssp latifolia by F-ST. Genetics. **142**(3): 1045-1052.

Zanetto, A., Roussel, G., and Kremer, A. 1994. Geographic variation of inter-specific differentiation between *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. Forest Genetics **1**: 111-123.

Appendix I – Cumulative amount of variation explained by stepwise regressions of select traits on environmental variables (R^2) and environmental variables included in models and of select traits on geographic variables (R^2) and geographic variable included in models.

Trait*	Environmental variable entered	R^2	Geographic variable entered	R^2
HT1	SH:M	0.27	LONG	0.35
	Bffp	0.30	$LONG^2$	0.38
	DD<18	0.38	LAT^2	0.38
	MSP	0.4	LAT	0.4
	MAP	0.4	ELEV	0.4
HT2	SH:M	0.14	$LONG^2$	0.22
	eFFP	0.22	LONG	0.23
	DD5100	0.27		
	DD>18	0.33		
	MWMT	0.36		
	MSP	0.38		
	NFFD	0.4		
	EMT	0.4		
	DD<0	0.41		
	bFFP	0.42		
DM1	TD	0.09	LONG	0.2
	bFFP	0.16	$ELEV^2$	0.21
	DD<18	0.3	$LONG^2$	0.21
	MAT	0.32	ELEV	0.27
	NFFD	0.35		
	DD5100	0.37		
	EMT	0.39		
	DD<0	0.41		
	eFFP	0.43		
	MAP	0.44		
	DD>18	0.45		
	MSP	0.46		
DM2	bFFP	0.06	$ELEV^2$	0.04
	SH:M	0.08	$LONG^2$	0.06
	DD5100	0.1		
	MSP	0.11		
	DD>18	0.12		
	MWMT	0.17		
	MAP	0.19		
	eFFP	0.2		

	EMT	0.21		
	PAS	0.22		
	NFFD	0.25		
EMER	MSP	0.32	LAT^2	0.4
	MWMT	0.36	LAT	0.46
	eFFP	0.41	$LONG^2$	0.47
	MAT	0.49	ELEV	0.49
	DD>18	0.58	$ELEV^2$	0.5
	DD<18	0.63		
	FFP	0.65		
	DD>5	0.67		
	MAP	0.7		
BB	MAP	0.08	$ELEV^2$	0.04
	DD<0	0.16	$LONG^2$	0.05
	PAS	0.18		
	DD>5	0.19		
	DD<18	0.24		
	bFFP	0.25		
	NFFD	0.27		
	SH:M	0.29		
	EMT	0.30		
	DD>18	0.30		
BS	DD<0	0.09	$ELEV^2$	0.07
	MAP	0.24	ELEV	0.15
	bFFP	0.34	LAT^2	0.18
	TD	0.35	LAT	0.3
	DD<18	0.38	LONG	0.32
	DD>5	0.41		
	EMT	0.42		
CH	TD	0.24	LAT^2	0.11
	MAP	0.28	LAT	0.17
	bFFP	0.29	ELEV	0.19
	MSP	0.3	$ELEV^2$	0.23
			$LONG^2$	0.28
R:S	DD>18	0.02	$ELEV^2$	0.02
	PAS	0.03	$LONG^2$	0.03
	bFFP	0.03		
	MWMT	0.04		
	DD5100	0.05		
	MAP	0.07		
	DD<18	0.09		
	SH:M	0.13		
PC1	SH:M	0.13	$LONG^2$	0.22
	FFP	0.19	$ELEV^2$	0.22

	DD<18	0.29	LAT^2	0.23
	DD>18	0.31	LAT	0.24
	TD	0.36	LONG	0.30
	DD>5	0.37	ELEV	0.31
	MSP	0.37		
	PAS	0.38		
	MAP	0.39		
	DD5100	0.4		
	EMT	0.41		
PC2	MSP	0.29	LAT^2	0.32
	DD<0	0.33	$ELEV^2$	0.34
	eFFP	0.39	$LONG^2$	0.34
	TD	0.41	LONG	0.35
	DD>18	0.54	ELEV	0.35
	NFFD	0.57		
	EMT	0.58		
	FFP	0.58		
	DD>5	0.58		
	DD5100	0.61		
PC3	DD<0	0.08	$ELEV^2$	0.05
	MAP	0.2	ELEV	0.14
	DD>5	0.3	LAT^2	0.16
	DD<18	0.35	LAT	0.24
	MWMT	0.36	LONG	0.27
	DD5100	0.36		
	MSP	0.37		

*See table 2.2 for trait codes.