POST-GLACIAL MIGRATION, LIMITATIONS TO POLEWARD RANGE EXPANSION, AND GROWTH RESPONSES TO FUTURE CLIMATES OF PLANTS IN THE GARRY OAK ECOSYSTEM

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by

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Abstract

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A key goal in ecology is to understand the factors limiting species’ distributions. Important range-limiting factors are often difficult to generalize, however, because organisms have many different life-history traits, evolutionary histories, and diverse interactions with other species. Climate is often implicated as the most important range-limiting factor in modern species distributions. Yet many species are not or not yet exhibiting range changes associated with anthropogenic climate change. A potentially important non-climatic range-limiting factor is dispersal limitation. Recently, some researchers have concluded that dispersal limitation is likely as strong a range limiting factor as climate. One way to tackle the limits to generalization is to investigate range limiting factors and patterns of range shift for well-chosen taxa in a comparative fashion to glean general principles.

My research uses a comparative approach to investigate patterns of post-glacial colonization, factors involved in geographic range limitation, and species responses to
Travis D. Marsico

future climates using genetic techniques, a field experiment, and a chamber experiment, respectively. All studies were conducted on species associated with the Garry oak ecosystem in the Pacific Northwest of North America and focused on four plant taxa: *Quercus garryana* var. *garryana*, the flagship species of the Garry oak ecosystem, and three *Lomatium* species, *L. dissectum* var. *dissectum*, *L. nudicaule*, and *L. utriculatum*.

An overall conclusion from this dissertation is that related, co-occurring species provide an appropriate comparison for determining species- and trait-based generalization. *Lomatium* chloroplast genetic data suggest that abundance is important in determining the ability of long-distance seed dispersal. The field experiment shows that dispersal limitation is currently important in determining range boundaries for species no matter their regional abundance. The field experiment also shows that closely related species may differ in their competitive abilities and responses to competitors/facilitators. My genetic survey on *Q. garryana* provides evidence that generalizations about range changes in oaks as a taxonomic group seem to be relatively universal, no matter the historical landscape conditions. The chamber experiment provides evidence that some responses to global change will be unpredictable, making certain generalizations difficult. Given these findings, humans may consider accelerating species migration through purposeful translocation outside species’ ranges to overcome dispersal barriers.
This dissertation is dedicated to my mom, whom I hope is looking down from above with pride. I also want to dedicate this dissertation to Mr. Randy Flanagin, my high-school biology teacher, who encouraged me in my scientific interests so much that I have now completed a doctoral dissertation in biology.
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One of the common definitions of ecology is ‘the study of the abundance and distribution of organisms’. Therefore, a key goal in ecology is to understand the factors that limit species’ distributions. This understanding is often difficult to generalize, however, because living organisms are so diverse and have many different life-history traits, evolutionary histories, and diverse interactions with other species. There are many factors of the environment and differing barriers to dispersal that limit species distributions and influence range changes (Sauer, 1988; Gaston, 2003), but anthropogenic climate change has set a new imperative to making ecological generalization for conservation reasons (e.g., Thomas et al., 2004). One way to tackle the limits to generalization is to investigate range limiting factors and patterns of range shift for well-chosen taxa in a comparative fashion to glean general principles (Parmesan et al., 2005). The set of abiotic and biotic factors that may control species ranges are finite but non-trivial including physical barriers to dispersal, climatic conditions, soil properties, disturbance regimes, predation, parasitism, competition, availability of food resources, mutualism, or a combination of factors (Gaston, 2003). When viewing peripheral populations in a metapopulation context, these range limiting factors can be simplified to
those that decrease habitat availability whereby overall colonization rates are less than
local extinction rates, increase local extinction rates, or decrease local colonization rates
(Holt & Keitt, 2000; Holt et al., 2005).

The fossil record tells us that climate often is important in setting the range limits
of species. Plant species tracked climatic changes after the Last Glacial Maximum as
glacial ice retreated from temperate and boreal regions (Davis & Shaw, 2001). Fossil
pollen and plant macrofossils are often used to reconstruct paleoclimatic conditions, as
plant species can be good indicators of regional climate conditions (Whitlock, 1992;
Jackson et al., 2000). For these reasons, climate is often implicated as a range-limiting
factor in modern species distributions (Woodward, 1987). If climate were the most
important factor limiting species ranges, then peripheral populations should experience
increased local extinction rates relative to core populations since conditions toward the
range periphery are expected to be less suitable than in the core (Maurer & Taper, 2002).

There is evidence species are shifting their geographic ranges in accord with
contemporary anthropogenic climate change as peripheral conditions become more
suitable for certain taxa (Parmesan & Yohe, 2003; Walther, 2004). Yet many species are
not or not yet exhibiting range changes. For example, 35-40 percent of studied butterflies
in Europe have not extended their northern boundary in accordance with predictions
based on observed climatic changes (Parmesan et al., 1999). Therefore, factors other
than climate or that interact with climate must have a key role to play in some cases.

One of these non-climatic, range-limiting factors is dispersal limitation, and until
recently it has received relatively little attention in the paleoecological and modern
climate change literature. Clark (1998) showed that long-distance dispersal events could
have allowed for the observed rapid rates of geographic range shift in response to Quaternary climate change. He found a lack of difference in migration rate from taxa with large differences in life-history (including dispersal), which he attributed to migration rates not reaching their full range-shift potential. Clark’s study, therefore, indicated that dispersal limitation was not likely important in rates of range shift during Quaternary migrations. In direct contrast, however, Svenning and Skov (2005, 2007) and Svenning et al. (2008) have shown that post-glacial ranges in Europe are predicted not only by climate, but by dispersal limitation. These authors have concluded that dispersal limitation is likely as strong a range limiting factor as climate. In addition, when dispersal estimates are incorporated into range change models based upon climatic changes, changes in geographic range sizes have been shown to more closely approximate the null migration model (in which species cannot shift at all) rather than the full migration model (in which dispersal is unlimited) (Midgley et al., 2006). Therefore, the role of climate versus dispersal limitation as primary factors controlling species ranges is not yet resolved.

All four of the studies presented within this dissertation relate to the ecology and biogeography of species at their poleward range limits and responses to previous and future climatic changes. My goal was to use information about past migration along with contemporary range limitation to better understand how these species will likely respond to future changes. While all of the data chapters included in this dissertation are derived from my interests in species’ responses to global change, each chapter specifically addresses a facet of this topic by answering questions using different methodological approaches. By gaining an understanding of how species differ in their responses to past,
current, and future conditions at their poleward range limits, we gain a more thorough understanding of similarities and species-specific differences to global changes.

In my research, I addressed patterns of post-glacial colonization and factors involved in geographic range limitation for species associated with the Garry oak ecosystem in the Pacific Northwest of North America. My research focused on four plant taxa: the flagship species of the Garry oak ecosystem, *Quercus garryana* Douglas ex Hook. var. *garryana*, and three *Lomatium* species, *L. dissectum* (Nutt.) Mathias & Constance var. *dissectum*, *L. nudicaule* (Pursh) J.M. Coul. & Rose, and *L. utriculatum* (Nutt. ex Torr. & A. Gray) J.M. Coul. & Rose. *Quercus garryana* is a large, open-grown, long-lived tree species, and it is the only oak species found in the Pacific Northwest, north of central Oregon. The three *Lomatium* species are long-lived perennial herbs, widely distributed in the Pacific west. Each of these species is shade-intolerant and is found growing alongside other meadow and savanna plants, including members of the Liliaceae (primarily native) and Poaceae (primarily non-native) as dominant herbaceous species. At the northern range boundary of *Q. garryana* (Northern Washington, USA and Vancouver Island British Columbia, Canada) where it is the dominant tree species in savanna habitats, these areas are known as Garry oak ecosystems. As with other Mediterranean-type savanna ecosystems (Hayhoe et al., 2004), the Garry oak ecosystem is expected to be strongly influenced by current anthropogenic climate change. The seasonal precipitation pattern is likely to become more pronounced, and temperatures are expected to rise. The expectation at the shared northern range limit for these species is for expansion of their geographic ranges under increasingly favorable environmental conditions (Hamann & Wang, 2006).
Range expansion was the outcome for species in the Garry oak ecosystem following glaciation. However, the past cannot be used as a direct surrogate for the present, as conditions are different. First, the niches that may become available due to the changing climatic conditions are, at present, already filled with other species that do not allow for coexistence. Additionally, the habitat matrix, both natural and anthropogenically modified, is not the same as in the wake of a retreating glacier. Therefore, because of the rapid pace of current anthropogenic climate change and habitat loss and fragmentation, dispersal limitation may be key to determining future species’ distributions.

Elements of the research within this dissertation aim to help clarify the role of dispersal at range boundaries. I focus on testing factors that may affect population establishment beyond the northern extent of the geographic range of the three species of *Lomatium* (Chapter 2). In doing so, I was able to indirectly test for dispersal limitation in historically limiting colonization of suitable habitat patches outside the range. In my field experiment, I planted *Lomatium* seeds within and north of their current geographic distributions, in experimental treatments that reduced competitors and excluded herbivores. Results of this experiment were used to determine the range-limiting factors of these species and species-specific differences in response to the given treatments. This work also speaks to conservation strategies that may be needed to facilitate or enable poleward range expansion in these species.

An important corollary to understanding the mechanisms determining the limits to geographic ranges is understanding the patterns of geographic range shift and species migration once range limitations are overcome. As opposed to many north temperate
species in Europe and eastern North America, the species of the Garry oak ecosystem have the bulk of their current geographic ranges within non-glaciated areas.

Paleoecological data show that even during the height of the Last Glacial Maximum, these species retained populations within the unglaciated northern portion of the species’ ranges (see e.g., Hansen & Easterbrook, 1974). In addition, the species expanded their distribution a distance of at least 200 km, colonizing the once glaciated Vancouver Island. Therefore, we can use the genetic signatures of relatively short-distance migration as a surrogate process for range expansion that may occur in the future.

I am interested in seed dispersal of the three *Lomatium* study species at their northern periphery on Vancouver Island, British Columbia, and adjacent islands (Chapter 3). These species colonized the peripheral island locations after ice retreated during deglaciation. Using chloroplast microsatellites, I determined patterns of seed dispersal within the post-glacial peripheral islands. I hypothesized that species abundance would correlate with haplotype richness and diversity, population differentiation and distribution of haplotypes, and the correlations between genetic and geographic distances. One of the generalities known about rare-common differences is that rare taxa have reduced nuclear genetic diversity relative to more abundant taxa (Karron, 1987). Genetic patterns related to rarity are often attributed to differences in pollen flow, (Petit et al., 2005), but far less is known about how genetic diversity and differentiation patterns exclusively from seed dispersal relate to species abundance. It is intuitive that large populations should export a greater number of seeds than small populations. Still, interspecific comparisons are necessary to determine the extent of the effect that abundance has on genetic diversity and differentiation resulting from differential seed dispersal.
For *Q. garryana*, I asked questions related to the population genetic structure after post-glacial migration (Chapter 4). Specifically, I was interested in determining if populations at the northern periphery were genetically distinct from populations in the center of the geographic range, if genetic diversity was reduced in the periphery relative to the core populations, and if nuclear and chloroplast markers indicate different patterns of pollen flow and seed dispersal. For species that migrated great distances following glaciation, typically the highest genetic diversity is found within the refugial range with decreasing diversity along the migratory path (Hewitt, 2000). In contrast to post-glacial colonization in Europe, much of the *Q. garryana* range was not glaciated at the Last Glacial Maximum (LGM), providing an interesting opportunity to investigate the signature of post-glacial colonization for a taxon that did not shift its geographic range very far poleward. Because known glacial refugia were close to the post-glacial colonization sites, I expected this difference to be less than is typically found for oak species, resulting from relatively high seed dispersal to areas that now represent the furthest reaches of the species’ range. I also expected the genetic differentiation across sampling sites to be higher in the chloroplast markers than the nuclear markers due to the strictly maternal inheritance of the chloroplast markers and the difference in pollen flow and seed dispersal (Petit et al., 1993a; Ennos, 1994; Grivet et al., 2008).

In a chamber experiment aimed at investigating how the three *Lomatium* species may respond to future conditions without shifting their ranges, I tested the effects of elevated CO₂ and average temperatures on the growth of the three *Lomatium* species (Chapter 5). A focus of this chapter was to compare the seedling growth responses of the species to determine how general their responses are. Previous research has examined
the response of contrasting species to elevated CO₂ and climatic change, such as C₃ vs. C₄ plants and trees vs. shrubs vs. herbs vs. grasses (Carter & Peterson, 1983; Oechel & Strain, 1985; Poorter, 1993; Ainsworth & Long, 2005; Cleland et al., 2006; Housman et al., 2006). Different responses among these groups are attributed to differences in physiological processing of CO₂ (in the case of C₃ vs. C₄ plants) or to different baseline growth rates for species with different growth habit. There also is evidence, however, that populations within species respond differently to elevated CO₂ and increased temperature, in some cases showing qualitative differences but in others showing responses in the same direction with only differences in magnitude of the response (Garbutt & Bazzaz, 1984; Wulff & Alexander, 1985; Bazzaz et al., 1995; Thomas et al., 1999; Ziska et al., 2005; Stinson et al., 2006). Given such population-level findings, it seems reasonable to hypothesize that closely related species may qualitatively differ in their growth responses to changing CO₂ and climate, and that species could show differential responses greater than among populations. My study uses the three Lomatium to determine if, and to what degree, similar species vary in their response to global change. On the one hand, related, similar taxa may be expected to perform similarly if selective forces have acted to conserve similar traits (niche conservatism). On the other hand, competition for the same resources may have allowed for evolution of different responses in similar, related taxa (overdispersion) (see Cavender-Bares et al., 2004; 2006). The similarity in outcome of responses to a changing climate will depend upon what taxonomic scale climate forces act.

The research here addresses important ecological questions at range boundaries taking a comparative approach among closely-related species with similar life histories in
order to determine levels of generality that can be gleaned for making predictions in response to global change. My chosen *Lomatium* study species differ in their regional abundance at their northern range periphery, allowing for inference about the role of abundance in determining responses to global change. The inclusion of *Q. garryana* as a study species allows for comparison of it with other, better studied oak species in eastern North America and Europe.
2.1. Introduction

Changes in climate alter the geographic distributions of many species, presumably because climate limits range expansion (Davis & Shaw, 2001; Parmesan & Yohe, 2003). Given that range limits often are set by climate (Woodward, 1987), it is feasible that species may expand poleward under regional warming if colonization of newly-suitable sites can be achieved. In the case where habitats become (or already are) suitable but a species cannot naturally migrate to those areas, purposeful introductions outside the current range boundaries could advance poleward migration under climate change (McLachlan et al., 2007; Hoegh-Guldberg et al., 2008).

Because habitat near and outside the range boundary of many species is patchy and fragmented (Gaston, 2003), it is possible that seed dispersal is a predominant factor limiting poleward range expansion (Matlack, 1994; Cain et al., 1998; Grashof-Bokdam & Geertsema, 1998; Svenning & Skov, 2005, 2007). Dispersal limitation has been demonstrated at even very local scales of a few meters (Primack & Miao, 1992; Eriksson,
1998). However, plant populations successfully colonized habitats over long distances following glaciation, indicating that dispersal is often not completely limiting, at least not over thousands of years when human barriers to migration are absent (Davis & Shaw, 2001).

A wide range of climate-related mechanisms of range limitation have been reported in plants including death from minimum cold temperatures or gradually accumulating sub-optimal weather events and slow pollen tube growth from cool summer temperatures resulting in unfertilized ovaries (Burke et al., 1976; Davison, 1977; Pigott & Huntley, 1981). Studies on the recent dynamics of species ranges have observed changes in range boundaries that correlate with climatic changes, suggesting that climate change relaxes climatic limitation (Parmesan & Yohe, 2003; Walther, 2004 and citations within). In addition, it is known that species moved to follow post-glacial climate changes more often than adapting to new conditions in place (Bradshaw, 1991; Davis & Shaw, 2001).

It has long been recognized, however, that factors other than climate can limit species’ ranges (Griggs, 1914, 1940; Prince & Carter, 1985). Competition can be an important limiting factor, for example, and recruitment may be poor for species entering diverse, competitive communities (Elton, 1951; Tilman, 1997). Recipient habitats may have little open niche space and limit establishment due to crowding, limited light, and reduced nutrient availability (Naeem et al., 2000; but see Levine & D’Antonio, 1999 and Fridley et al., 2007 for discussion of the “invasion paradox”). Repeated colonization attempts, however, might overcome limitations to establishment due to competition (i.e., increased propagule pressure as in Kolar & Lodge, 2001). Also, competition alone may not be responsible for limiting species’ distributions, but an interaction between
competition and climate. Certain species may be poor competitors in climates under which they are not ideally adapted (Woodward, 1975). Likewise, climatic conditions that do not outright kill species may confer a competitive disadvantage (Woodward, 1987; Loehle, 1998).

While there is little evidence that herbivores limit the geographic ranges of plant species, herbivory is known to affect the distribution of species at smaller spatial scales (Cantor & Whitham, 1989; Bruelheide & Scheidel, 1999). On one hand, generalist herbivores could limit the establishment of populations outside the historic range, sometimes as effectively as competitors, by favoring novel food sources (Maron & Vilà, 2001; Levine et al. 2004). On the other hand, plant species moving outside their ranges may be released from herbivores in their historic range, allowing for colonization (Maron and Vilà, 2001; Keane & Crawley, 2002). There is experimental evidence that herbivores sometimes prefer native plant species and other times non-native species (e.g., Knapp et al., 2008), but herbivores may facilitate the spread of colonizing species more than they limit their establishment (Maron and Vilà, 2001).

This research examines the range limits for three *Lomatium* species at their poleward geographic range boundary. This study 1) investigates the potential for range change, 2) determines if related and co-occurring species respond to the same range-limiting factors, and 3) determines species-specific differences in early-life stage survivorship when transplanted to novel locations.

The three study species offer the potential to reveal differences among range limiting factors and ability for poleward establishment in a phylogenetically controlled way. The three species—*Lomatium utriculatum* (Nutt. ex Torr. & Gray) J.M. Coult. &
Rose, *L. nudicaule* (Pursh) J.M. Coult. & Rose, and *L. dissectum* (Nutt.) Mathias & Constance var. *dissectum*—belong to the same genus and share important life history traits (flowering time, dormant season, perennial habit, herbaceous form, insect pollination, gravity or non-specific seed dispersal). One obvious difference among them is abundance, a characteristic likely to be linked to the ability for geographic range shift (Iverson et al., 2004).

I hypothesized that the *Lomatium* species are dispersal limited at their poleward geographic range boundaries. I tested this hypothesis by adding seeds to plots within the current distribution and to seemingly-suitable habitat in areas north of the current maximum northward extent. Equal or better survivorship outside the range would indicate dispersal limitation, as the species would be expected to grow in the outside-range sites if they were able to reach these locations. I tested alternatives to this hypothesis as well. If survivorship outside the range was less than within the range, the result could be due to abiotic differences between the regions (e.g., climatic differences) or biotic differences. Given similar life histories and genetic relatedness of the species, I hypothesized that range-limiting factors and species’ responses to those factors would be qualitatively the same. Underlying differences in abundance among the species, however, suggested that the species could have different baseline germination and reemergence rates.

This experiment examines climatic factors, competition, and ungulate herbivory given their potential importance in new population establishment (above). Other factors such as insect herbivores, mutualists, parasites, pathogens, and seed predators can be important in population establishment and persistence (Thompson, 1985; Packer & Clay,
These factors, however, were not studied here based on a related study in the literature. Thompson (1998) conducted a long-term study on herbivore and pathogen attack of a population of *L. dissectum* in eastern Washington and found that 36 of 103 individuals were alive after 10 years of monitoring. Of the plants that did not survive, nearly half of the deaths were attributed to pocket gophers feeding on the roots. Other deaths could not be assigned a cause. Additionally, over the ten years of study, each individual was attacked only by a mean of 1.6 above-ground herbivores or pathogens per year out of a total of five common herbivores and pathogens (mammalian herbivores, one moth, two flies, and a rust). These results suggest that above-ground herbivory and pathogen attack is a regular event, but it cannot readily be implicated in plant death or lack of establishment. I also expect that plant species are more likely to be released from pathogens outside their native range, rather than negatively impacted them (Mitchell & Power, 2003). It is possible, however, that parasites or diseases could emerge under climate change or over the years following population establishment outside their current ranges.

2.2. Materials and Methods

2.2.1. Location and study species

This study was conducted on Vancouver Island, British Columbia, Canada, in Garry oak ecosystems and similar open sites north of the distributional limit of *Quercus garryana* Douglas ex Hook. (Garry oak). The climate is sub-Mediterranean, with cool, wet winters and dry summers. North of this region, mean temperatures are cooler and annual precipitation is higher (see Results). Species within the Garry oak ecosystem are
hypothesized to expand northward within southwestern British Columbia, as warmer
temperatures and more extreme seasonal variation in precipitation patterns are expected
for the coastal Pacific Northwest (Fuchs, 2001; Hamann & Wang, 2006). The Garry oak
ecosystem is characterized by varying degrees of open oak canopy and a diverse
understory of ground flora (MacDougall et al., 2004). Many plants within this ecosystem
range from California to a northern limit on Vancouver Island. Some typical ground
flora associates are found beyond the northernmost Garry oak populations, and these
northern herbaceous communities are less invaded by exotic grasses. Large mammalian
herbivore communities consist of dense populations of Columbian black-tailed deer
(Odocoileus hemionus columbianus) in both regions and elk (Cervus elaphus) in open
sites north of the current extent of Garry oak. In many Garry oak habitats, plants are
browsed heavily by deer. At the northern limit of Garry oak and its associated flora,
habitat patches are typically smaller and more isolated than patches further south. The
three Lomatium study species are long-lived perennial herbs that are shade intolerant and
grow in association with oak in savanna, rock outcrop, or prairie habitats.

2.2.2. Germination trials and seed measurements

To investigate germination rates in a competitor- and predator-free greenhouse
environment, seeds of all three species were collected from five sites for each species on
Vancouver Island and brought back to the University of Notre Dame in 2005. Seeds
came from 30 L. utriculatum, 20 L. nudicaule, and 21 L. dissectum plants and numbered
886, 394, and 447, respectively. Seeds were placed in cold stratification: 2 weeks for L.
utriculatum, which germinates earlier than the other two species, and 7 weeks for both L.
nudicaule and L. dissectum. Optimum cold stratification times were determined in 2004 for L. utriculatum and L. nudicaule using purchased nursery seed. Lomatium dissectum seed was unavailable for cold stratification trials, but it has a natural germination time similar to L. nudicaule so I provided it the same amount of cold stratification. Ten seeds were planted per 6” pot with Complete Potting Soil by Fafard with extended release fertilizer (0.09-0.04-0.06). Planted seeds were placed in a greenhouse set at 15°C days and 7°C nights with 12 hours enhanced light. Number of germinants was recorded through 19 weeks. Final germination proportions were recorded and compared across species using a one-way ANOVA.

Before the greenhouse experiment was conducted in 2005, >340 seeds per species were measured and massed, and seed area was calculated using the formula for the area of an ellipse, \[ A = \frac{1}{2} \text{(length)} \times \frac{1}{2} \text{(width)} \times \pi. \]

2.2.3. Field experiment

Six planting sites were chosen in spring 2006 to conduct a transplant field experiment. Three sites within the species range were grouped to form the “inside range” region, and three sites northward of the current range boundary were grouped to form the “outside range” region (Fig. 2.1). Sites within a region were separated by at least 4 km, and sites across study regions were separated by at least 186 km. Sites both inside and outside the range have a history of Euro-descendant land modification including introduction of European native pasture grasses and livestock grazing, but the extent of this use varies between regions and among sites. The three inside-range study sites lack woody canopy cover and have a high proportion of exotic herbaceous species, whereas
Figure 2.1. Map of study region on Vancouver Island, British Columbia, Canada, including the six *Lomatium* planting sites (black circles). Three study sites are within the current geographic range boundary of the three *Lomatium* species, and three sites are beyond the northern range limit of all three species. The black squares labeled with species names indicate the most northerly known location of each species. The most northerly within-range planting location coincides with the known northern location of *L. dissectum*. The sites marked with an “X” are the airport weather reporting stations that collected data compiled in Figure 2.7.
the sites outside the range have been less modified by recent human activity. Fire has been suppressed in both regions. Sites also vary in soil depth with two shallow soil/rock outcrop sites and one deeper soil/pasture-like site in each region. The outside-range sites were chosen as the most likely to be occupied by shifting species of the Garry oak ecosystem as they are not shaded by coniferous forests, are within 200 km of the current range limits of the study species, and are not separated from the current range by a significant water body. The region occupied by these sites has been predicted to be among the first to become suitable for colonization by Garry oak-associated species under climate change (Hamann & Wang, 2006).

Twenty 1m² plots in each of the six sites were stocked with seed: 18,000 seeds of *L. utriculatum*, 10,320 of *L. nudicaule*, and 5,520 of *L. dissectum* were collected from four to six sites per species on Vancouver Island. Seeds were attached to toothpicks with non-toxic, water-soluble glue for ease of transport, planting, and tracking within the field plots. One hundred and fifty *L. utriculatum*, 86 *L. nudicaule*, and 46 *L. dissectum* seeds were placed into each 1m² plot using the same randomized design (seed placement determined using a random number generator). Seeds were planted at or just below the soil surface under a 1m² grid of plastic mesh (deer fence) staked to the ground. The deer fence grid was comprised of 900 6.25-cm² cells, 282 of which were planted with seeds.

Each 1m² plot was treated with one of two levels of a competition (vegetation reduced and vegetation intact) and an herbivory (herbivores excluded and open to herbivores) treatment. Treatments were randomly assigned to the plots such that one quarter were controls, one quarter were given a vegetation reduction treatment, one quarter were given a herbivore exclusion treatment, and one quarter had vegetation
reduced and herbivores excluded. Each treatment combination was replicated five times at each site, and sites were replicated three times within each study region (i.e., within and outside the species’ range).

I reduced interspecific competition in vegetation reduction (VR) plots by hand-pulling plants and digging out roots, rhizomes, and bulbs near the soil surface before seed planting. This VR treatment was a pulse experimental treatment level, as it was only applied once before seed additions. To avoid dramatic soil temperature differences due to dark colored soil and the potential for wind or water erosion in VR plots, the VR plots were covered with a thin layer of removed plant material. Mechanical VR has the consequence of confounding reduction of competition with a soil disturbance treatment (see Discussion). The other half of the plots received no manipulation, which provided natural levels of competition and undisturbed soil. The herbivore exclusion (HE) plots were covered with deer fence supported by a ½” PVC cage (1 m x 1 m x 0.5 m) to limit access by herbivores. Cages were kept over the plots for the entire study period, constituting a press experimental treatment. Herbivores may limit population establishment if they consume *Lomatium* plants. Alternatively, they may reduce the abundance of *Lomatium* competitors.

*Lomatium* seeds were planted in July 2006. Germination was assessed in April 2007. When ungerminated seeds were readily found, they were recorded so that the fate of as many seeds as possible was known. Reemergence was assessed in April 2008. The proportion of germination and reemergence was calculated for each species in each plot by dividing the number of living individuals by the number of seeds planted. The vegetative community was assessed in the plots in both 2007 and 2008 for species
richness, proportion of vascular plant coverage, proportion of each species’ coverage, and mean height of the vegetation.

2.2.4. Field experiment statistical analyses

First, I conducted a three-factor repeated-measures analysis of variance (ANOVA) to determine the influence of region, vegetation, and herbivory on the vegetative communities (“vcANOVA”), irrespective of *Lomatium* performance. This statistic also was used to compare the vegetative community between 2007 and 2008. To determine a single competition metric for use in the vcANOVA, we performed a Principle Components Analysis (PCA) using four variables representative of competition levels within the plots measured in both 2007 and 2008: species richness, proportion of vascular plant coverage, proportion of grasses, and mean plant height. We measured these four variables with the assumption that plot-level assemblages with higher values in any or all of the measured variables would exhibit more intense competition for light, water, and nutrients than assemblages with lower values. Species richness was assumed to be an indicator of niche utilization such that plots with more species would more effectively compete for all available resources than plots with fewer (Naeem et al., 2000). Proportion of vascular plant coverage is an indication of the physical space available to be filled by vegetation. The proportion of plot coverage by grasses was used as a competition variable because grasses with their dense, fibrous root systems are effective competitors of surface water and soil nutrients (Simmons et al., 2007). The vast majority of grass in Garry oak ecosystems is non-native (MacDougall et al., 2004). Finally, mean
plant height is a measure of light competition, as taller plants more effectively shade the *Lomatium* seedlings than shorter plants (see Results).

Once it was determined with the vcANOVA that the VR did reduce the variables assumed to be important in interspecific competition (see Results), region, vegetation, and herbivory treatments applied to the plots were used as main effects in a three-factor repeated-measures ANOVA to determine the influence of the applied treatments on *Lomatium* establishment (“esANOVA”). Proportion germinated (i.e., survivorship from seed) was the response variable in 2007, and proportion reemerged (i.e., continued survivorship from total seed planted) was used in 2008.

*Lomatium* species identity was not treated as a factor in the esANOVA because it did not represent an experimental factor. Still, it was important for us to determine if differences in survivorship exist across the three study species. To examine differences among species, four one-way ANOVAs were conducted with species as the only factor in each model. Each ANOVA used survivorship data from 2008 as the response variable from each of the region and vegetation treatment combinations (i.e., inside range, vegetation intact; inside range, VR; outside range, vegetation intact; outside range, VR). Tukey post-hoc tests were conducted, and statistical analyses were performed using Systat 12.

2.2.5. Environmental measurements and analyses

Light is likely the primary limiting resource for the establishment of native plants in the Garry oak ecosystem (MacDougall & Turkington, 2007). The amount of light reaching the plots was determined at two locations, one within-range (April 2007) and
one outside-range (May 2007), with a Quantum meter (Apogee Instruments, Inc.). Light levels reaching the soil surface at these sites also were calculated and the difference between light reaching the plot and light reaching the ground was analyzed to determine the effect of shading by competitors. A two-sample t-test was conducted to determine significant differences between vegetation-intact and VR plots.

Soil moisture data were collected to 1) assess the change in soil moisture over the growing season, 2) determine if experimental treatments had an impact on soil moisture, and 3) determine if site-level *Lomatium* survivorship was related to soil moisture. Soil moisture was determined with a Hydrosense TDR (Campbell Scientific) using 12 cm probes at four points within each plot in April, May, and June 2007. The four plot-level readings were averaged to obtain a plot-level measurement. Pearson correlations of soil moisture were conducted between each pair of months sampled (April/May, May/June, April/June) to determine the consistency among plots in soil drying over the growing season. I also conducted a three-factor ANOVA on June moisture ("smANOVA"), the driest month sampled. Region, vegetation, and herbivore treatments were included as factors in the model to determine if the treatments imposed on the plots impacted soil moisture. I then regressed site-level mean 2008 survivorship for each species against site-level mean June soil moisture levels to determine if there was a significant relationship between soil moisture at the end of the first growing season with survivorship in the second growing season.

Finally, to determine if climatic conditions outside the current *Lomatium* distribution have recently changed to become more like those within the range, climate data inside and outside the range were gathered from Environment Canada National
Climate Archive (http://www.climate.weatheroffice.ec.gc.ca/) for Victoria International (YYJ) and Campbell River (YBL) Airports for 1965-2006 (Fig. 2.1). Mean annual temperature, total annual precipitation, annual snowfall, and annual extreme minimum temperature were regressed against time to examine recent climatic changes in each region. Additionally, we used available monthly data to determine how climatic variables during the experiment related to long-term averages.

2.3. Results

2.3.1. Germination trials and seed measurements

The first flush of germination happened within two weeks after planting for all three species, and within four weeks all *L. dissectum* germination was complete. Only four additional (0.7%) *L. utriculatum* germinated after week 4, but 25% of *L. nudicaule* germinated after week 4. Proportion of seeds germinated after 19 weeks averaged 0.61 (se=0.03) for *L. utriculatum*, 0.39 (se=0.06) for *L. nudicaule*, and 0.03 (se=0.02) for *L. dissectum*. ANOVA results and Tukey post-hoc comparisons show that germination rates among the species were significantly different ($F_{2,68}=58.6$, $P<0.001$).

Seed sizes and shapes are similar for all three species, but *L. utriculatum* < *L. nudicaule* < *L. dissectum* for seed mass and area (Fig. 2.2). *Lomatium nudicaule* seeds are relatively longer and narrower than the other two species (Fig. 2.2).

2.3.2. Field Experiment

In 2007, species richness, plant coverage, grass coverage, and plant height, were highly loaded on a single PC axis (PCcomp), which explained approximately 68% of the
Figure 2.2. Seed characteristics of the three *Lomatium* study species. (a) Representative examples of *L. utriculatum* (left), *L. nudicaule* (middle), and *L. dissectum* var. *dissectum* (right) seeds (photo by J.D. Dzurisin) and (b) graphical comparison of seed shape and (c) size. Values were so consistent within species that standard error bars are not visible beyond the borders of the mean marker.
variation in these variables. In 2008, vascular coverage, plant height, and proportion grasses were loaded on PC\textsubscript{comp}, which explained 58\% of the variation in the four variables. Species richness was highly loaded on a second axis that explained 28\% of the variation. Species richness was not loaded on PC\textsubscript{comp} in both years because by 2008 VR plots regained species richness levels similar to unmanipulated plots. Species richness, however, was higher at the sites within the current \textit{Lomatium} range than outside the range. From the vcANOVA I determined that in both 2007 and 2008, competition was lower outside the range than within the range and lower in VR plots than in plots with vegetation intact (Fig. 2.3). The VR applied to the plots, therefore, had the effect of reducing the strength of competition within each region. The relationship in the effect of this VR did not change between 2007 and 2008, indicating that the initial pulse experimental treatment had lasting effects on competition. Herbivory also had an effect on the competitive community that only appeared in 2008 (Table 2.1). Competition was reduced in plots where herbivores had access to the vegetation.

From the esANOVA I determine that region and vegetation treatments, either individually as main effects or in an interaction, significantly affected germination and survivorship for each of the three \textit{Lomatium} species. Herbivory, however, was not an important factor in the first two years of growth, nor was any interaction involving herbivory (Table 2.2). The species’ responses to the significant treatments differed in the following ways: \textit{L. utriculatum} showed no significant interaction between the region and vegetation treatments and no change in the relationship between years. Both region and vegetation main effects were significant (Table 2.2; Fig. 2.4). The regional treatment effect showed significantly higher germination and reemergence within the current
Figure 2.3. Compiled competition measurement ($PC_{\text{comp}}$) in each region and vegetation treatment combination. White bars are competition values from 2007, and gray bars are from 2008. Error bars are standard error.
TABLE 2.1

THREE FACTOR REPEATED-MEASURES ANOVA FOR FACTOR PCOMP AS THE RESPONSE VARIABLE

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>$F$-ratio</th>
<th>$P$-value</th>
<th>$F$-ratio</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1</td>
<td></td>
<td>&lt;0.001</td>
<td>&gt;0.999</td>
<td></td>
</tr>
<tr>
<td>Region (R)</td>
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<td>422.6</td>
<td>&lt;0.001</td>
<td>1.262</td>
<td>0.264</td>
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<tr>
<td>Vegetation (V)</td>
<td>1</td>
<td>166.845</td>
<td>&lt;0.001</td>
<td>0.084</td>
<td>0.773</td>
</tr>
<tr>
<td>Herbivory (H)</td>
<td>1</td>
<td>5.339</td>
<td>0.023</td>
<td>10.696</td>
<td>0.001</td>
</tr>
<tr>
<td>R x V</td>
<td>1</td>
<td>2.642</td>
<td>0.107</td>
<td>0.507</td>
<td>0.478</td>
</tr>
<tr>
<td>R x H</td>
<td>1</td>
<td>0.048</td>
<td>0.827</td>
<td>1.015</td>
<td>0.316</td>
</tr>
<tr>
<td>V x H</td>
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<td>0.882</td>
<td>0.325</td>
<td>0.570</td>
</tr>
<tr>
<td>R x V x H</td>
<td>1</td>
<td>3.081</td>
<td>0.082</td>
<td>3.198</td>
<td>0.076</td>
</tr>
<tr>
<td>Error</td>
<td>112</td>
<td></td>
<td></td>
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</table>

NOTE: Bold font $P$-values are significant at $P<$0.05.
TABLE 2.2

THREE-FACTOR REPEATED-MEASURES ANOVA FOR GERMINATION AND REEMERGENCE OF EACH *LOMATIUM* SPECIES

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Treatment effects</th>
<th>Comparison across sampling time</th>
<th>Treatment effects</th>
<th>Comparison across sampling time</th>
<th>Treatment effects</th>
<th>Comparison across sampling time</th>
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</thead>
<tbody>
<tr>
<td>Year</td>
<td>1, 112</td>
<td></td>
<td>0.001</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Region (R)</td>
<td>1, 112</td>
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<td>0.162</td>
<td>0.107</td>
<td>0.024</td>
<td>0.003</td>
<td>0.027</td>
</tr>
<tr>
<td>Vegetation (V)</td>
<td>1, 112</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.098</td>
<td>0.957</td>
<td>0.005</td>
<td>0.001</td>
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<tr>
<td>Herbivory (H)</td>
<td>1, 112</td>
<td>0.479</td>
<td>0.162</td>
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<td>0.081</td>
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</tr>
<tr>
<td>R x V</td>
<td>1, 112</td>
<td>0.107</td>
<td>0.387</td>
<td>0.005</td>
<td>0.695</td>
<td>0.002</td>
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</tr>
<tr>
<td>R x H</td>
<td>1, 112</td>
<td>0.784</td>
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<td>0.571</td>
<td>0.723</td>
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</tr>
<tr>
<td>V x H</td>
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<td>0.866</td>
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<td>0.500</td>
<td>0.035</td>
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<tr>
<td>R x V x H</td>
<td>1, 112</td>
<td>0.874</td>
<td>0.784</td>
<td>0.560</td>
<td>0.984</td>
<td>0.440</td>
<td>0.894</td>
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</table>

NOTE: Bold font *P*-values are significant at *P*<0.05. Missing values in 2008 make the error degrees of freedom 106 for *L. utriculatum* and for 110 for *L. nudicaule*. 
Figure 2.4. Germination (2007) and reemergence (2008) for each species for the region and vegetation treatments. Solid lines are vegetation-intact treatments, and dashed lines are vegetation-reduction treatments.
species range than outside, but the regional effect is driven by poor survivorship in outside range, VR plots only (Fig. 2.4). *Lomatium utriculatum* responded negatively to the VR treatment in 2007. Upon reemergence, *L. utriculatum* had fewer plants in all region-vegetation treatment combinations, but there was a greater proportional loss in both regions from those plants grown with the vegetation community intact. *Lomatium nudicaule* displayed an interaction between vegetation and region in both growing seasons: surviving significantly less in the inside-range, vegetation-intact treatment combination, the treatment combination with the highest competition level (Table 2.2; Fig. 2.4). *Lomatium dissectum* showed a significant interaction between region and vegetation treatment in germination, but that relationship changed for reemergence (Table 2.2; Fig. 2.4). Germination was least successful outside the range when vegetation was reduced but similar in each of the other three treatment combinations. Reemergence of *L. dissectum* was similar in all four treatment combinations, which eliminated the interaction observed after germination.

I found species-level differences in reemergence in each region and vegetation treatment combinations (Table 2.3; Fig. 2.5). The only consistent pattern among all treatment combinations was that *L. dissectum* exhibited the lowest survivorship (Fig. 2.5).

2.3.3. Environmental measurements

The amount of light intercepted by the vegetation was 662 μmol/m²/sec ± 69SE for intact vegetation plots and 393 ± 41SE for VR plots. The lower number here for VR plots indicates that more light was reaching the soil surface (t=3.35, df=38, P=0.002). On
TABLE 2.3

SPECIES COMPARISONS ACROSS ALL FOUR REGION BY VEGETATION TREATMENT COMBINATIONS

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>inside range, vegetation intact</td>
<td>2, 82</td>
<td>8.534</td>
<td>&lt;0.001</td>
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<tr>
<td>outside range, vegetation intact</td>
<td>2, 87</td>
<td>8.341</td>
<td>&lt;0.001</td>
</tr>
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<td>inside range, vegetation reduced</td>
<td>2, 84</td>
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<td>&lt;0.001</td>
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<tr>
<td>outside range vegetation reduced</td>
<td>2, 87</td>
<td>12.472</td>
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</table>

NOTE: After significant differences were determined with ANOVA, a Tukey test for pair-wise comparisons was conducted (see Fig. 2.4).
Figure 2.5. Species-level differences in reemergence (2008) for each of the region and vegetation treatment combinations. (A) inside range, vegetation intact; (B) outside range, vegetation intact; (C) inside range, vegetation reduced; (D) outside range vegetation reduced. Error bars are standard error. Different letters above bars indicate significant differences among species determined by Tukey pair-wise comparisons.
average, the vegetation in the intact plots intercepted over 1.5 times the amount of light as the vegetation in the VR plots.

Progressive soil drying was observed at all sites from April through June. Pearson correlations for monthly soil data showed that plot-level soil moisture values in April were highly positively correlated with values in both May (r=0.947) and June (r=0.830). May, likewise, was highly correlated with June values (r=0.825). The smANOVA showed that region had a significant effect on soil moisture (P<0.001), but vegetation removal (P=0.446) and herbivore exclosure (P=0.388) did not and neither did any interaction. Soils had a higher water content outside the range than inside the range, but this was driven by two of the three sites (Fig. 2.6). For *L. utriculatum* and *L. dissectum*, higher levels of soil moisture trended toward lower survivorship, but the relationships were not statistically significant (*L. utriculatum*: n=6, R^2=0.15, P=0.24; *L. nudicaule*: n=6, R^2=0.00, P=0.54, *L. dissectum*: n=6, R^2=0.14, P=0.25).

Linear regressions showed an increase in mean annual temperature at both YYJ (inside range) and YBL (outside range) from 1965 through 2006 (YYJ R^2=0.481, \(F_{1,40}=38.9, P<0.001\); YBL R^2=0.413, \(F_{1,38}=28.4, P<0.001\); Fig. 2.7). Using the regression line as a mean value, the annual mean temperature in 2006 at YBL is equivalent to the annual mean temperature at YYJ in 1976 (Fig. 2.7). Extreme minimum temperature has not significantly changed at YYJ, but it has increased significantly in recent years at YBL, though variability is high and is not well predicted by year (R^2=0.082, \(F_{1,38}=4.5, P=0.041\)). Minimum temperatures in any given year between the two locations are always colder at YBL, though across years there is substantial overlap in minimum temperature. Snowfall has seen no consistent change at YYJ, but it has
Figure 2.6. June 2007 soil moisture by *Lomatium* planting site. Sites are displayed from south to north, such that the left three sites are within-range locales and the right three sites are outside-range sites. Error bars are standard error.
Figure 2.7. Trends in annual mean temperature in Victoria and Campbell River, British Columbia, from 1965 through 2006. $R^2=0.48$ for Victoria and 0.41 for Campbell River. The mean temperature given by the regression line in present day Campbell River is the same as the mean temperature for 1976 in Victoria.
decreased at YBL (R²=0.120, F₁,₃₉=6.5, P=0.015). YBL saw an average of 71 cm more snowfall than YYJ from 1965-1995, but between 1996-2006 the difference shrunk to 54 cm, and in 2005 YYJ received more snowfall than YBL. Accumulated annual precipitation shows no pattern with time at either location over the last 41 years. Precipitation is so much higher at YBL than at YYJ (1454 vs. 880 mm on average per year), that only four times in the last 41 years have record dry years at YBL fallen within the range of precipitation at YYJ.

During the duration of the experiment (July 2006-April 2008), monthly weather variables only deviated from long-term climatic averages a few times. At both YBL and YYJ, July and August 2006, were warmer than the long-term average, and July 2007 was warmer at YYJ (data not available for YBL). YYJ recorded a record high temperature in July 2007 of 36.3°C. Mean monthly minimum temperatures were at or above the climatic average except for January 2007, and November 2007, through April 2008, at YYJ. During the course of the experiment, monthly extreme minimum temperatures remained well above the all time coldest values in the climate record in either location. November 2006, was cooler than the climatic average at YBL and for both YBL and YYJ recorded rainfall was 1.5 and 2.2 times the long-term average. Additionally that month there was 6 and 12 times the average snowfall at YBL and YYJ, respectively. Both locations received more precipitation than average during the winter of 2006-2007. I conclude that weather was not extreme during the experiment and thus represents conditions within the normal climatic range since 1965 for both regions.
2.4. Discussion

Field plots with the vegetation community intact outside the geographic range had equal survivorship to plots within the current distribution. This is consistent with my hypothesis of dispersal limitation. This study also suggests that the intact community of shade-intolerant, herbaceous plants northward of the current range of Garry oak associated species can be invaded, and seed additions can overcome dispersal limitation.

Inadequate long-distance dispersal may be determining the current poleward range limit of these *Lomatium* species in one of two ways. First, the *Lomatium* populations may have never realized their full fundamental climatic niche following their migrations onto Vancouver Island after the Last Glacial Maximum. If this is the case, habitat that has long-been suitable for population establishment has remained uncolonized. This scenario is likely because suitable habitat patches north of the current range are small and isolated. Second, a changing climate may have recently allowed for sites north of the current distribution to become suitable. My climate reconstruction provides evidence that the climate of the sites near Campbell River, British Columbia, is becoming more similar to the recent past climate of sites near Victoria, specifically with respect to mean annual temperature. The prediction based on bioclimate modeling is that these areas will only become more favorable for species associated with coastal meadow ecosystems (Hamann & Wang, 2006). In either case, whether historical or recent, I infer that dispersal limitation has prevented range expansion into suitable habitat beyond the current northern limit of these three *Lomatium* species.

All three species responded to the same treatment effects, but each in a unique way. Though deer herbivory recently has been shown to be exceedingly important to
some species in the Garry oak ecosystem (Gonzales and Arcese, in press), it was not a factor important in *Lomatium* survivorship. Instead, each species responded to region, vegetation, or their interaction. *Lomatium utriculatum* reemerged significantly lower in the combined situation of outside-range and vegetation reduced (the lowest observed level of competition), but *L. nudicaule* germinated and reemerged significantly lower in the combined situation of within-range, vegetation-intact (the highest observed level of competition). These opposing responses to competition suggest that *L. nudicaule* is a weaker competitor at early stages of establishment. During germination, *L. dissectum* survivorship was significantly reduced in the outside-range and vegetation-reduction treatment combination. This result for *L. dissectum* is more similar to *L. utriculatum* than to *L. nudicaule*. In both *L. utriculatum* and *L. dissectum*, the differences between responses to the treatments became less pronounced upon reemergence, completely removing vegetation effects for both species and region effects from *L. dissectum* in 2008.

For *L. utriculatum* and *L. dissectum* in 2007, I found positive impacts from an intact vegetative community, indicating that vegetation can have a facilitation effect on germination or that soil disturbance negatively impacts germination in these species. The mechanisms of facilitation include buffering individuals from herbivores or environmental extremes. Recently, it has been recognized that facilitation is generally widespread and important, particularly in environments toward the edge of a species’ tolerances (Choler et al., 2001; Callaway et al., 2002; Bruno et al., 2003; Brooker et al., 2008). The outside-range, VR treatment combination resulted in the lowest amount of competition (which would also include the same factors involved in facilitation), as
determined by PC\textsubscript{comp}. Because \textit{L. dissectum} only germinated significantly less under this treatment combination, facilitative effects from the surrounding vegetation seems to be a fitting explanation for the observed germination patterns. For \textit{L. utriculatum}, however, I observed low germination in the VR treatment both inside and outside the range. Since measures of competition were lower outside the range regardless of the vegetation treatment, I would have expected to see poorer germination outside the range from the vegetation-intact treatment, if facilitation from other plants aided \textit{L. utriculatum}. Because I observed similarly reduced germination in the VR treatment in both regions, however, facilitation of \textit{L. utriculatum} germination is not the clear mechanism for the observed germination differences in this species.

Dismissal of facilitation as important to \textit{L. utriculatum} germination, however, should be made with caution. A complicating factor is that \textit{L. utriculatum} germinated earlier than its competitors/facilitators emerged in 2007. This means that the buffering capabilities of the vegetative community at the time of \textit{L. utriculatum} germination would be less than depicted in PC\textsubscript{comp}, particularly for vegetation-reduction plots. Vegetative community assessments were conducted more than one month after the germination assessment to obtain a depiction of species richness and vegetative coverage in the plots at the peak of the growing season. As an alternative to facilitation, \textit{L. utriculatum} may have been negatively responding to an element of the soil disturbance caused from the VR, but this would be a unique response for a germinating species because other research shows only positive responses in germination from soil disturbance (Isselstein et al., 2002; Hierro et al., 2006). For both \textit{L. utriculatum} and \textit{L. dissectum}, any facilitation effect provided by the vegetative community during germination was reduced upon
reemergence, even though the vegetative communities between vegetation intact and vegetation reduced treatments remained distinct. This indicates a transition from facilitation during germination toward competition in later growth.

Vegetation reduction did not confer an advantage to any of the three species outside the range, possibly because competition for resources outside the range is less intense than within the range. Within the range, only *L. nudicaule* benefited from having vegetation reduced, potentially because interactions with neighboring plants are not negative during early life stages of the other two species. It has been suggested that native herbaceous species in Garry oak habitats are not inferior competitors to non-native taxa, but that they are not adapted to the current disturbance regime and have lower propagule pressure than non-natives (MacDougall et al., 2004; MacDougall & Turkington, 2005). My data show successful establishment given seed additions, indicating that these species may be able to successfully establish new populations given enough propagule pressure.

The fact that none of the species responded to the herbivore exclosure treatment suggests that ungulate herbivory is not a driving force in population establishment for these species near the edge of their range. Nevertheless, I observed that the remainder of the vegetative community was significantly reduced by the effects of herbivores in 2008 (see also Gonzales and Arcese, in press). While young *Lomatium* plants are not directly affected by herbivores, their surrounding community is. If competing species are eaten by herbivores more frequently than *Lomatium*, herbivory has the potential to effectively reduce competition, providing an advantage to the establishing *Lomatium* over the long-term.
In both the greenhouse and field experiment, my data were consistent with the hypothesis that the rare species, *L. dissectum*, has the lowest germination and reemergence. This is despite the fact that *L. dissectum* often produces a larger number of seeds per plant than either *L. nudicaule* or *L. utriculatum* (unpublished data). Thus, these results suggest that more seeds will be required from *L. dissectum* to obtain the same number of seedlings as the other two species (e.g., for assisted migration projects outside the current distribution). This may be difficult due to the low number of seed producing plants on Vancouver Island. Constraints on reproductive output are generally the rule for rare species (Gaston & Kunin, 1997; Murray et al., 2002). In contrast, lower germination and survivorship of rare taxa, as found here, is equivocal.

Episodic weather events have the ability to constrain species ranges (Yaqub, 1981; Crozier, 2004; Battisti et al., 2006). During this study, monthly mean weather conditions were similar to recent averages, except for an exceptionally wet and cool November 2006, and warm July and August 2006, and July 2007. During the warm times in 2006, the seeds had been recently planted in the plots and were still dormant, and by July 2007, the plants had senesced. Extremely wet conditions during the first winter when the seeds had not yet germinated, in contrast, could have impacted germination success, but observed germination rates were greater than those previously recorded for these species (Thompson, 1985; A. McDougall, personal communication). Periodic extreme cold events, which were not experienced by these young plants during the experiment, could be involved in range limitation. This is unlikely in this system for three reasons. First, because extreme events are infrequent, plants would have an opportunity to become established during benign weather years if dispersal was adequate.
to suitable northern locations. Second, mature plants would be hard to kill with cold, particularly during the dormant season, but they would also have adequate root reserves to flush if tender spring vegetation were killed by freezing. Third, these species experience colder conditions on other parts of their ranges (see http://plants.usda.gov and http://www.usna.usda.gov/Hardzone/).

A number of caveats are important to consider regarding the design of this experiment. One factor not explicitly accounted for in this experiment is the impact of small mammals, including seed predators and root herbivores, and insect seed predators. Studying *L. dissectum* in eastern Washington, Thompson (1985) found that after 10 months (July to May), ~10% of seeds set out in piles remained, only 0.76% germinated, and all of those plants died within two months. Two years later, when populations of seed predators were higher, more than 95% of seeds were eaten within four months. The levels of seed predation in this study are far lower, as seen from the high germination success. Further, for both *L. nudicaule* and *L. dissectum*, germination in the field was higher than that observed in the predator-free greenhouse environment. Lastly, I occasionally found intact, ungerminated seeds during our germination assessment. At the site with the highest amount of bare soil (an outside of range location), where ungerminated seeds were likely to be most easily seen (presumably by both myself and seed predators), I found 14% of ungerminated *L. dissectum* seeds. Though, seed shape and size is similar for each of the study species, *L. dissectum* does have the largest seed of the three, making it easier to see and, perhaps, a more valuable food resource (see also Thompson, 1985). Yet, at the site where seeds were easiest to find, I observed a mean germination of 37% and a recovery of 14% of the ungerminated seeds. Therefore, while
we cannot account for unfound, ungerminated seeds, it is likely that many more were present, that is, not eaten, at other sites than I am aware of. I also observed some plots at a few sites with active tunneling by voles (*Microtus*). Vole activity, tunneling and root-feeding, has the potential to significantly damage the large taproot of the *Lomatium* species, but in this study only very few plants were affected by root damage because of their small size.

I did not measure reproductive output in this experiment because the *Lomatium* species do not usually reproduce in the field until they are at least four years old. Tracking survivorship through reproduction is important to capture fitness differences among the study regions and experimental treatments. Survival to reproduction also is critical to population persistence without relying on inputs of seed from outside sources.

It is important to understand the factors involved in range limitation to determine the need for assisted migration and craft its implementation. This study provides evidence that an assisted migration strategy may be viable for species that are dispersal limited, and protected areas outside the historical distribution of target species could be used for establishment. Seed additions alone also may be relatively inexpensive. Assistance in moving species poleward may be important in establishing local seed source populations in new areas early, allowing for more short-distance dispersal events to take place if the vegetation structure changes due to climate change (Hebda, 1997). Experimental populations would have to be monitored after seed additions to determine if growth and survivorship of the planted individuals results in a reproductive, self-sustaining population.
CHAPTER 3

THE RELATIONSHIP BETWEEN ABUNDANCE AND POST-GLACIAL SEED DISPERSAL REVEALED IN THREE *Lomatium* (APIACEAE) SPECIES

3.1. Introduction

Seed dispersal is emerging as crucial to understanding post-glacial plant migration and predicting distributions of species under future climate change (Svenning & Skov, 2005; 2007; Williams et al., 2007; Thuiller et al., 2008). It is likely that historically-limited dispersal plays a major role in determining the current distributions of species that shifted out of glacial refugia, rivalling the importance of climate as a range-limiting factor (Svenning & Skov, 2005; Svenning et al., 2008; Chapter 2). Yet, long-distance dispersal events and patterns of post-glacial migration remain poorly understood (Clark, 1998; Jackson et al., 2000; Nathan, 2006). To complicate matters, not all species possess equal dispersal capabilities (e.g., Matlack, 1994). Some species disperse longer distances than others due to seed size traits, for example (Leishman & Westoby, 1994; Leishman et al., 1995). Even for species with similar dispersal mechanisms, effective seed dispersal can differ due to differential abundance of source populations. Large populations should export a greater number of seeds than small populations, which would result in a greater number of successful immigrants (Clark et al., 1999). By extension, species with large
populations should have greater interpopulation seed dispersal than species with small populations due to a larger number of seed donors (Iverson et al., 2004).

Genetic differentiation should increase as interpopulation dispersal decreases (Austerlitz et al., 1997). The population differentiation statistic, $F_{ST}$, inversely depends on the migration rate ($m$) and the effective population size ($N_e$) (Wright, 1943). Therefore, among taxa with similar migration rates, species with larger populations should have decreased population differentiation relative to less abundant species. Species-level genetic diversity also should increase with increasing abundance because the population mutation rate ($\theta$) depends directly upon effective population size ($N_e$) and number of mutations per generation ($\mu$) ($\theta=4N_e\mu$; Watterson, 1975). Generally, decreased genetic diversity and increased population differentiation have been found using neutral nuclear markers in small populations relative to large populations (Ellstrand & Elam, 1993; Karron, 1997). Far less, however, is known about how genetic diversity and differentiation patterns from seed dispersal alone are related to species abundance. Therefore, it is not clear if limited pollen flow (indistinguishable from seed dispersal in nuclear markers) or limited seed dispersal (observed alone in chloroplast markers) creates the spatial patterns of genetic diversity that have been observed.

The maternally-inherited chloroplast genetic markers of angiosperms are ideally suited for tracking colonizations and range expansions due to seed dispersal (Birky, 1976; Petit et al., 1997, 2002; Provan et al., 2001; Grivet et al., 2006). It is widely accepted that these markers exhibit a higher level of population differentiation than nuclear markers for the same organisms (see Petit et al., 2005; Moyle, 2006). This higher differentiation occurs even if pollen and seed gene flow rates are equal due to a smaller effective
population size from maternally-inherited markers (Hamilton & Miller, 2002). Rates of pollen flow, however, are often found to be greater than seed dispersal, commonly by an order of magnitude or more (Hamilton & Miller, 2002; Petit et al., 2005). Related taxa often share similar life-history traits involved in dispersal and reproduction, and taxonomic relationship has been found to be the strongest predictor of population differentiation for both biparentally- and maternally-inherited markers (Duminil et al., 2007).

In this study, I compared the maternally-inherited genetic structure of three closely-related species that share similar life histories to determine the relative influence of species abundance and seed movements by humans on the spatial partitioning of genetic diversity. Three study species—*Lomatium utriculatum* (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose, *L. nudicaule* (Pursh) J.M. Coult. & Rose, and *L. dissectum* (Nutt.) Mathias & Constance var. *dissectum*—belong to the Apiaceae and are long-lived perennial herbs that grow in open habitats, often associated with oak savanna on the west coast of North America. They co-occur at their shared northern range boundary on Vancouver Island, British Columbia, Canada. Shared life-history traits include herbaceous growth form, perennial habit, generalist insect pollination, wind or gravity seed dispersal, mating system weighted toward outcrossing, hermaphroditic breeding system, and overall geographic range size (Douglas et al., 1998; Thompson 1985). The species differ in their germination rate and subsequent survival (Chapter 2), but this difference is offset somewhat by the number of seeds produced per plant. On average, *L. dissectum* produces more seed per plant than the other two species but has a lower survivorship (Chapter 2).
The primary difference among these three species is regional abundance, a demographic characteristic important for determining range shift potential (Iverson et al., 2004). On islands at the northern edge of its range, *L. dissectum* is rare, known from only nine locations on Vancouver Island with a total of ~7000 individuals. *Lomatium nudicaule* is found in many locations but in a patchy distribution within sites, with a mean of ~1000 individuals growing at the sampled sites. *Lomatium utriculatum*, similarly, grows in many locations but often grows in great numbers with a mean of ~50,000 individuals within the sampled sites. One sampled *L. utriculatum* population in this study (site 17) contains an estimated 600,000 individuals in an area of 7.5 ha. This difference in abundance could be due to increased variability in successful seed-set for the less common taxa, increased mortality due to inherent species differences (Chapter 2), increased mortality due to species-specific responses to environmental variables (e.g., Chapter 5), or post-glacial history of dispersal.

In addition to differential abundance, these *Lomatium* species have had different relationships with indigenous people in the region of their common northern range. Indigenous peoples of the coastal Pacific Northwest had the potential to influence the post-glacial migration of *Lomatium* species, particularly *L. nudicaule*, to and within Vancouver Island. It is often thought that plants important for human use would be shared and traded among groups, thereby allowing for rapid spread (MacDougall, 2003). *Lomatium nudicaule* is known to have been a highly valued source of medicine for coastal peoples, having been used to cure ailments from colds and sore throats to skin infections and body aches (Turner & Bell, 1971, 1973). So important was *L. nudicaule* that it was traded north of its known range limit to northern Vancouver Island (Turner &
Bell, 1973). In contrast, Compton (1993) provides evidence that *L. utriculatum* was considered inedible, and *L. dissectum* does not appear in the ethnobotanical literature, likely due to its rarity. If human use effectively increased seed dispersal of *L. nudicaule* through trade, we might expect it to show population differentiation at levels lower than expected by its abundance. Likewise, because there was less (or no) trade in *L. utriculatum*, genetic patterns should represent natural dispersal.

Herein, I investigated whether past seed dispersal and colonization within the range periphery differs among the three *Lomatium* study species in order and magnitude of their relative abundances. Specifically, I tested the hypotheses that greater species abundance would result in 1) decreased population differentiation, 2) increased haplotype richness and diversity, and 3) positive correlations between genetic and geographic distance over greater spatial scales than for less abundant taxa. I tested this abundance hypothesis against an alternative that indigenous people played a large role in altering natural seed dispersal patterns of *L. nudicaule*, the single ethnobotanically important species in this comparison. To test my hypotheses, I sampled individuals of the three *Lomatium* species with different abundances from multiple populations at their northern periphery in areas that were once glaciated. I developed and used five chloroplast microsatellites to investigate patterns of seed dispersal over the 250 km post-glacial range. Under both hypotheses, *L. dissectum* is expected to have reduced haplotype richness and increased population differentiation relative to *L. utriculatum*. Under the abundance hypothesis, *L. nudicaule* would have haplotype richness and population differentiation between the other two species, but under the human movement hypothesis *L. nudicaule* would have a similar or greater number of haplotypes and a similar or
decreased population differentiation relative to *L. utriculatum*. In other words, the human movement hypothesis would be supported by higher *L. nudicaule* migration than expected by its abundance alone.

3.2. Materials and Methods

3.2.1. Geologic history of study region and species distribution

During the peak of the most recent glaciation (Vashon Stade ~19-13 kya), Vancouver Island and the adjacent Gulf and San Juan Islands were glaciated by the Juan de Fuca lobe, an extension of the Cordilleran ice sheet (Alley & Chatwin, 1979; Herzer & Bornhold, 1982; Huntley et al., 2001). At the time of maximal ice coverage, temperate communities were advancing northward toward the ice (Whitlock, 1992). Within 200 km to the south of Vancouver Island, areas remained unglaciated (Waitt and Thorson, 1983; Whitlock, 1992). These unglaciated areas could have supported refugial populations of many species currently inhabiting Vancouver Island and other neighbouring islands. Based on pollen record data, plants began colonizing Vancouver Island over 12 kya, and *Quercus garryana* Dougl. ex Hook., the dominant tree species currently in Vancouver Island *Lomatium* habitats today, likely colonized just under 11 kya (Brown and Hebda, 2002). Therefore, it seems likely that many plant species colonized the deglaciated islands more than 10 kya.

The three *Lomatium* species overlap in the north-western portion of their geographic ranges (Fig. 3.1). *Lomatium dissectum* var. *dissectum* grows from northern California northward to southeast Vancouver Island, primarily west of the Cascade Ranges, but it is found in a few counties in north-central Idaho. *Lomatium nudicaule* has
Figure 3.1. Geographic ranges of the three *Lomatium* study species. *Lomatium dissectum* var. *dissectum* distribution is contained within solid lines, *L. nudicaule* within dashed lines, and *L. utriculatum* within dotted lines. Overlap of all three species is shaded. Data gathered from Douglas et al. (2002) and USDA, NRCS (2008).
a broader east-west distribution and grows farther east than *L. dissectum* var. *dissectum*
over a greater portion of its range. It can be found in open meadow or grassland habitats
from central California east to Northern Utah and north to southeast Vancouver Island
and portions of Interior British Columbia. *Lomatium utriculatum* extends farther south
than the other two species from northern Mexico northward to southeast Vancouver
Island, chiefly west of the Sierra Nevada and Cascade Ranges (Douglas et al., 1998;
USDA, NRCS, 2008).

3.2.2. Sample collection

Leaf material of the *Lomatium* study species was collected from the glaciated
range periphery at sampling sites in the San Juan Islands, Washington, and Vancouver
Island, Saturna Island, and Hornby Island, British Columbia, in May, 2005, and April-
May, 2007 (Table 3.1, Fig. 3.2a-c). For *L. dissectum*, 76 total samples were collected
from nine sites (1-13 samples/site); for *L. nudicaule*, 199 total samples were collected
from 17 sites (4-21 samples/site); and for *L. utriculatum*, 204 total samples were
collected from 16 sites (2-24 samples/site). The nine sampled *L. dissectum* sites
represent all known populations on Vancouver Island. All sampled individuals were
growing at least 1 m apart to assure the collection of unique individuals, though samples
typically were collected from individuals separated by at least 20 m. Leaves collected in
2005 were frozen at -80°C. In 2007, leaves were cut and dried on silica gel prior to
laboratory analyses. An additional 46 *L. utriculatum* samples were collected from the
Washington mainland south of Puget Sound (Fig. 3.2c). Though not a main focus of this
study, these samples were collected to determine the relation between peripheral island

51
### TABLE 3.1

LOCATIONS OF SAMPLING SITES AND NUMBER OF SAMPLES PER SPECIES

<table>
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<tr>
<th>Site number</th>
<th>Latitude</th>
<th>Longitude</th>
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**NOTE:** Sites are numbered south to north. Mainland Washington *L. utriculatum* site numbers are preceded with an “M”. 
Figure 3.2. Location of *Lomatium dissectum* genetic sampling sites (a) and geographic distribution of post-glacial *L. nudicaule* (b) and *L. utriculatum* (c) haplotypes in coastal Washington and British Columbia. Sites are numbered from south to north (see Table 1). In (b) and (c), haplotypes for the numbered sites are shown nearest to the site number. For *L. nudicaule*, haplotype N1 is black, N2 is white, and N3 is gray. For *L. utriculatum*, haplotype U1 is black, U2 is white, U3 is gray, and U4-U8 are labelled individually as each was found only at one location. Mainland haplotypes (U9-U12) are not mapped. The *L. dissectum* haplotype distribution is uniform for a single haplotype and is not shown.
haplotypes and putative colonization sources. Because of its lack of peripheral island genetic diversity (see Results) and its absence from Garry oak habitats in mainland Washington, mainland *L. dissectum* were not collected. Mainland *L. nudicaule* samples also were not collected, though in a future study it would be interesting to determine the sources of Vancouver Island haplotypes, especially with regard to testing hypotheses of human seed dispersal from the mainland Interior of British Columbia.

3.2.3. DNA extraction and genotyping

Genomic DNA was extracted using 1x CTAB extraction buffer. Leaf material in 700µl extraction buffer was first shaken in a 1.5-ml tube with a 1/4” ceramic bead (Qbiogene, Inc.) in a FastPrep FP 120 (Qbiogene, Inc.) for three cycles of 20 seconds each to physically break apart leaves and lyse cells. Emulsified leaves were incubated at 56°C for 1 hour. 700µl of 25:1 chloroform:isoamyl alcohol solution was added to the extraction buffer. After a 15 minute centrifugation step, the aqueous layer was removed and two volumes of ice-cold isopropyl alcohol were added. Samples were then frozen at -20°C for a minimum of 20 minutes. Alternating centrifugation and washing steps with 70% ethanol resulted in a DNA pellet that was suspended in AE buffer (Qiagen).

Extracted DNA was diluted to 5-30 ng/µl and stored at 4°C.

Chloroplast microsatellites comprised of mononucleotide repeats were used in this study because they had the greatest potential to show sequence variation in recently diverged, post-glacial populations (Provan et al., 2001). The fully-sequenced genome of *Daucus carota* L. (Ruhlman et al., 2006), also in the Apiaceae family, was obtained from GenBank (http://www.ncbi.nlm.nih.gov) and analyzed for repeats of 10 bases or more.
(mono- and dinucleotide) from non-coding regions (see Jakobsson et al., 2007). Thirty potential candidate markers including a minimum 10 bp repeat were selected from across the D. carota chloroplast genome for testing in the target species. In most cases, primer sequences were selected in regions that were conserved for other sequenced species more distantly related to Lomatium than D. carota. Twenty-three markers amplified appropriate-sized (90-300 bp) fragments in all three Lomatium species. Only five of these markers, however, showed polymorphism in tests with a subset of the sampled individuals (Table 3.2).

PCR was performed for chloroplast markers with an Eppendorf Mastercycler in a total volume of 25 μl containing 0.5 U GoTaq Flexi (Promega Corporation), 1X colourless GoTaq Flexi buffer, 2.5 mM MgCl₂, 200 nM each dNTP (Fermentas, Inc.), and 5 pM forward and reverse primer. The PCR protocol consisted of a 2 min initial denaturing step at 95°C followed by 35 cycles of 30 sec at 95°C, 30 sec at 50°C, and 30 sec at 72°C, then a 10 min final elongation at 72°C. Initial detection of polymorphism to determine appropriate markers was achieved by direct sequencing of samples, as follows. After PCR was run, amplified fragments were purified using an Invitrogen PureLink PCR Purification Kit. Purified PCR products were then sequenced using the Applied Biosystems BigDye Terminator Cycle Sequencing kit according to manufacturer specifications. Sequences were analyzed on an Applied Biosystems 3730 DNA Analyzer. Microsatellites were sequenced in both directions using the original primers from PCR (Table 2). Polymorphism was detected by aligning within-species sequences in Sequencher (Gene Codes Corporation), and the reference sequences were deposited in GenBank (Table 2). Once the five polymorphic microsatellites were identified, PCR was
<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences (5′→3′)</th>
<th>Repeat motif</th>
<th>Map position</th>
<th>GenBank Accession</th>
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<td>FJ490685-</td>
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<td></td>
<td>R: GGAAAACCTCTTGCTCT</td>
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<td>rpoB</td>
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<td></td>
<td>UGC—</td>
<td>FJ490684</td>
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</table>

NOTE: Primer sequences are displayed 5′→3′. The region of the *Daucus carota* chloroplast genome where these SSRs map are indicated (see Ruhlman et al., 2006).
run on all samples (as described above), and fragment lengths were measured using forward primers labelled with WellRED dye (Sigma-Proligo) on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc.). All markers provided clearly discernable single peaks except for LomC16 in *L. utriculatum* (see Results).

Every sample was run once with each marker on plates containing six positive controls and at least one negative control. This approach allowed us to assess consistency in fragment length scores and ensure reagents free of contamination. Over the course of genotyping, the six positive controls (two from each species) were run with each marker eight times. These controls were correctly scored in each run. In addition, each time a new haplotype was encountered, a sample with that haplotype was re-run to ensure its correct assignment, which in all cases confirmed the original haplotype identification. Therefore, I consider the genotyping error rate to be negligible.

### 3.2.4. Statistical analyses

I assembled chloroplast haplotypes using the fragment lengths of the five microsatellite markers. I determined genetic diversity (*H*$_S$ and *H*$_T$) and differentiation among populations (*G*$_{ST}$ and *R*$_{ST}$) within each species using the program CPSSR (www.pierroton.inra.fr/genetics/lab/Software/PermutCpSSR/index.html) following Pons & Petit (1995, 1996). *G*$_{ST}$ and *R*$_{ST}$ were calculated and compared to determine if phylogeographic structure could be detected at the studied scale (Pons & Petit, 1996; Grivet et al., 2006). Pairwise population *F*$_{ST}$ values for use in a Mantel test, which correlates the *F*$_{ST}$ values with geographical distance among sample sites (correlation coefficient=$r_M$) were calculated in Arlequin 3.1 (Mantel, 1967; Smouse et al., 1986;
Excoffier et al., 2005). \( F_{ST} \) values were also used to compare population size estimates for the species, given equal migration rates. Haplotype networks were created using the program TCS (Clement et al., 2000) based upon the methodology of Templeton et al. (2000).

### 3.3. Results

Amplification was successful for *L. dissectum* and *L. nudicaule* at all five markers. Marker LomC16, however, could not be easily scored for *L. utriculatum* and was excluded from analyses for this species. The loss of a marker from the most abundant species has the potential to reduce the number of observed haplotypes, but because this occurs for the species with the highest number of expected haplotypes, it provides a conservative estimate of haplotype diversity. Post-glacial populations yielded a total of one *L. dissectum*, three *L. nudicaule*, and eight *L. utriculatum* haplotypes (Table 3.3, Fig. 3.2a-c). In addition, four unique *L. utriculatum* haplotypes were discovered on the Washington mainland. Across the markers, microsatellite length combinations (i.e., haplotypes) were unique within species (Table 3.3). The single *L. dissectum* haplotype was not analyzed for genetic diversity or population differentiation. The lack of an information-rich data set for *L. dissectum*, however, is by no means uninformative. As there are likely fewer than 10,000 *L. dissectum* plants on Vancouver Island, the single *L. dissectum* haplotype indicates that after colonization there has not been enough time given the few, small populations for the genesis of new haplotypes.

Haplotype diversity and population differentiation differed between *L. nudicaule* and *L. utriculatum*. *Lomatium nudicaule* exhibited lower within-population diversity
### TABLE 3.3

**DESCRIPTION OF HAPLOTYPES BASED UPON FRAGMENT SIZE OF EACH AMPLIFIED CHLOROPLAST MICROSATellite**

<table>
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<tr>
<th>Species</th>
<th>Haplotype</th>
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<th>LomC18</th>
<th>LomC19</th>
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Note: Haplotypes in the shaded portion of the table were found exclusively on the mainland of Washington.
than *L. utriculatum* (*H*$_S$=0.041 vs. 0.269, respectively). This pattern is driven by the greater number of sites with only one haplotype for *L. nudicaule* (15/17 vs. 5/16) (Tables 3.4 and 3.5). Total diversity (*H*$_T$) was similarly low between the two species (*L. nudicaule*: 0.444, SE=0.118; *L. utriculatum*: 0.297, SE=0.058) because each species had a single predominant haplotype. The partitioning of the haplotype diversity differed between species (*L. nudicaule*: *G*$_{ST}$=0.909, *R*$_{ST}$=0.899; *L. utriculatum*: *G*$_{ST}$=0.095, *R*$_{ST}$=0.101). The *G*$_{ST}$ value near one for *L. nudicaule* indicates highly differentiated populations, but the value close to zero for *L. utriculatum* indicates that interpopulation connectivity via seed is relatively high. Using Wright’s (1943) island model for haploid loci, our *F*$_{ST}$ values (not shown, but similar to *G*$_{ST}$ values) indicate a 60-fold larger population size for *L. utriculatum* than for *L. nudicaule*, given the same migration rates. This 60-fold difference is supported by population size estimates that indicate populations of *L. utriculatum* on Vancouver Island often contain 50 times more individuals than *L. nudicaule* populations (see Introduction).

For both *L. nudicaule* and *L. utriculatum*, *G*$_{ST}$ equals *R*$_{ST}$, indicating no phylogeographic structure. Mantel tests confirmed the lack of correlation between genetic and geographic distances for both species at the spatial scale tested (*L. nudicaule*: *r*$_M$=-0.072, *P*=0.718; *L. utriculatum*: *r*$_M$=0.022, *P*=0.446). For both *L. nudicaule* and *L. utriculatum*, the two most common haplotypes of each species are found across the sampled island periphery (Fig 2b, c).

For *L. nudicaule*, haplotype N2 was found at all but four locations, and it was the only haplotype found at 11 of those 13 sites where it occurs. Each of the four locations
### TABLE 3.4

DISTRIBUTION OF *L. NUDICAULE* HAPLOTYPES OVER ALL SITES SAMPLED

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### TABLE 3.5

**DISTRIBUTION OF *L. UTRICULATUM* HAPLOTYPES OVER ALL SITES SAMPLED**

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without haplotype N2 was monomorphic for haplotype N1 (three sites) or N3 (one site) (Table 3.4, Fig. 2b). Haplotype N2 is too many mutational steps away from haplotype N3 to be connected in a single network with a parsimony probability of 0.95. Therefore, two different networks at probability of parsimony 0.90 (Fig. 3.3a) and 0.95 (Fig. 3.3b) were calculated. Haplotypes N1 and N3 are a single mutational step away from each other.

*Lomatium utriculatum* mostly had two or more haplotypes at each location, and haplotypes U1 and U3 were found at eight sites and four sites even though each was only represented by 19 and 9 individuals, respectively (Table 3.5). U2, the predominant haplotype, was found at all 16 sampling locations. Haplotypes U4-U8 were found only at one location each (Table 3.5, Fig. 3.2c). All *L. utriculatum* haplotypes from the post-glacial island periphery are a maximum of one mutational step away from another haplotype (Fig. 3.3c) at 0.95 parsimony probability. By contrast, three mainland Washington haplotypes (U10, U11, U12) are connected by a single mutational step, but the two other haplotypes (U9 and the island predominant U2) are five or more steps away from each other and from the most common mainland haplotype sampled (Fig. 3.3d). Haplotype U2 was the only haplotype from the post-glacial range found on the Washington mainland, and was represented by a single individual. A haplotype network of the relationship between mainland Washington and peripheral island haplotypes shows haplotype divergence between the two regions (Fig. 3.3d).
Figure 3.3. Haplotype networks for *L. nudicaule* (a and b) and *L. utriculatum* (c and d). At a 0.90 probability of parsimony, the three *L. nudicaule* haplotypes are connected in a single network due to a maximum number of four mutational differences (a), but at a probability of 0.95, the number of mutational differences between haplotypes N2 and N3 (i.e., 3) is greater than the number allowed by the parsimony criterion (i.e., 2) (b). The *L. utriculatum* haplotype network from the post-glacial island periphery is shown with a probability of parsimony of 0.95 (c). The *L. utriculatum* haplotype network from the Washington mainland allowed for only two mutational differences at a probability of 0.95, so only U10, U11, and U12 would have been connected, keeping U2 and U9 as outgroups. Instead, we forced connections by setting our criteria to a minimum of 6 mutational steps for mainland Washington samples (d). Within each panel, labelled rectangles (highest outgroup probability) and ovals (other haplotypes) are sized proportionally to their frequencies. Each line connecting haplotypes represents one mutational step, and an unlabeled circle represents an additional mutational step not found in the sampled populations.
3.4. Discussion

Differential regional abundance is correlated with a pattern of reduced seed dispersal from less abundant taxa. *Lomatium nudicaule* and *L. utriculatum*, the two species that had multiple chloroplast haplotypes, showed striking differences in population genetic differentiation. Reliable ethnobotanical evidence indicates that *L. nudicaule*, a species less abundant than *L. utriculatum*, was purposefully translocated by indigenous people. Still, the type of human assistance that *L. nudicaule* received did not increase the genetic diversity within populations, suggesting that *L. utriculatum* has been able to disperse among populations more readily than *L. nudicaule* even without the help of humans. Therefore, the influence of pre-European human activities on *L. nudicaule* dispersal was not great enough to overcome effects of its relatively low abundance.

Comparisons across plant species with different life-history traits have shown that life-histories weakly predict population differentiation (Duminil et al., 2007). Reproduction type is important for population structure of maternally-inherited markers, and to a lesser extent seed dispersal mode, seed mass, and geographic range size influence among-population differentiation (Duminil et al., 2007). Each of these life-history traits relates to effective population size (reproduction type [sexual only vs. both asexual and sexual]), migration rate (seed dispersal mode and seed mass) or both (geographic range size), and for each of these characters, the traits that result in smaller population sizes or lower migration rates have more highly differentiated populations. In this study, however, life-history traits were controlled for by using closely related species that are adapted to similar environmental conditions with similar seed dispersal mechanisms and seed sizes and shapes. Neither life-history traits nor divergent
evolutionary histories, therefore, explain the observed differences. Less abundant species are expected to produce fewer emigrants from populations than more abundant taxa. It has been demonstrated through a simulation model that species abundance has a large impact on the ability for species to shift their ranges in response to climate warming (Iverson et al., 2004). In this model, rare species were shown to have nearly no migration over the 100-year simulated time interval. Thus, *L. utriculatum*, the more abundant species, would have a greater probability of sending its propagules more often to distant locations, and the less abundant *L. nudicaule* would be expected to colonise new sites less frequently, potentially dispersing only a single maternal lineage to a new location.

If long-distance dispersal events were common, we would expect nearby sites to be more similar to one another than more distant sites, as the probability of dispersing to nearby sites is orders of magnitude greater than dispersing very long distances (Davis et al., 2004; Skarpaas et al., 2005). Of the two species with haplotype diversity, neither showed a significant relationship between genetic and geographic distance in the post-glacial portion of the species ranges, but this synonymy occurs despite a different spatial pattern of haplotype distribution for each species. For *L. nudicaule*, some of the populations in close proximity to each other are fixed for different haplotypes, and distant populations are as equally likely as nearby populations to share the same or exhibit different haplotypes when compared in a pairwise fashion. Therefore, interpopulation dispersal events seem rare for *L. nudicaule*. In contrast, *L. utriculatum* shows low population differentiation, and populations in close proximity may share similar haplotypes, but distant populations share these same haplotypes as well. *Lomatium*
*utriculatum*, therefore, appears to disperse very long distances at high enough frequencies to wash out a signal of genetic and spatial correlation.

The haplotype networks suggest origins of unique, low-frequency haplotypes. Low-frequency haplotypes that are few mutational steps from neighbouring haplotypes are likely to be recent, in situ mutations. All of the *L. utriculatum* haplotypes found at a single site are one mutational step away from another more abundant haplotype occurring at the same location with the exception of U6. Haplotype U6, found only at site 3, is two steps away from the only other haplotype (U2) found at that location. Interestingly, the nearest sites with the most closely related haplotypes (U3 and U5) were found 40 km to the north. The origin of haplotype U6 at site 3 cannot be determined clearly. It is not possible to differentiate among descent from a low frequency, unsampled haplotype at that site, descent from a maternal lineage farther north, dispersal from unsampled or mainland populations, or simultaneous mutation at two markers in an individual. Results from mainland sampling, however, does provide additional evidence for *in situ* origins for all low-frequency haplotypes found in the island periphery. Haplotype U2, the most common haplotype in the peripheral island populations, was found once in a mainland sample, but other mainland haplotypes were distinct from island forms. It is not clear if U2 is an uncommon mainland haplotype that colonized the glaciated island periphery, or if haplotype U2 originated in the range periphery and back-colonized into mainland populations. In either case, the haplotype data indicate that the plants likely colonized the glaciated range in a single or few colonization event(s).

Two different *L. nudicaule* haplotype networks are produced depending upon the probability of parsimony. In order to connect all three haplotypes in a single network
with two unsampled haplotype steps between the outgroup N2 and N3, the parsimony probability was set to 0.9. At a more stringent parsimony probability of 0.95, N2 remains unconnected, and N1 is the outgroup of an N1-N3 connection. With so few haplotypes overall, it is more likely that N1 and N2 independently colonized the peripheral island locations from mainland sources, rather than one resulting from the other through a series of mutational steps. Under this scenario, the highly restricted N3 could have descended from nearby N1 populations on southern Vancouver Island, rather than giving rise to the more widely distributed N1. In addition, because N3 is restricted to one sampled location with a small population size (~30 individuals), it likely represents a recent divergence that has not had enough time to colonise other locations. It is possible that trade among indigenous people allowed for *L. nudicaule* colonization of the peripheral islands from multiple sources.

Several assumptions underlie my interpretations of historical seed dispersal. First, I assume that each dispersed chloroplast haplotype had an equal probability of establishing at a newly-colonized location. Because the haplotypes are derived from non-coding regions of the chloroplast genome, I feel this is a valid assumption. An additional assumption is that haplotypes are identical by descent and are not due to homoplasy. Again, I think this is a valid assumption because the short time since *Lomatium* recolonization of the peripheral islands has not allowed for a high amount of chloroplast mutation, and, in support of this, I found a relatively low number of haplotypes overall. Also, because of thorough sampling within a substantial number of populations, low haplotype richness is not due to sampling effort.
Because I used one fewer marker for *L. utriculatum*, there is a possibility that I underestimated *L. utriculatum* haplotype richness at its range periphery. However, adding haplotypes to *L. utriculatum* with the addition of another marker would only heighten the haplotype richness differences between it and its less common congeners. I sampled the number of *L. nudicaule* and *L. utriculatum* populations nearly equally to avoid a sampling effect in the results (see Pons & Petit, 1995), but sampling of *L. dissectum* was limited by the number of populations that occur on Vancouver Island. Thus, the monomorphic haplotype for *L. dissectum* is a result of its actual rarity and is not a sampling artefact.

Anthropogenic climate changes may incite people to purposefully move rare species for their conservation (McLachlan et al., 2007; Hoegh-Guldberg et al., 2008). This study suggests that more abundant species may have greater success than rare taxa in shifting their geographic ranges on their own, and that careful planning of seed source collections will be important to create genetically diverse populations.
4.1. Introduction

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

For most angiosperm taxa, the path of post-glacial colonization has been examined using maternally-inherited chloroplast markers because they provide information only on the dispersal of seed (Birky, 1976; Dumolin et al., 1995). Patterns of population differentiation for oak species observed using chloroplast markers have
Figure 4.1. *Quercus garryana* Dougl. ex Hook. species range map inset into study area in the Pacific Northwest. Numbered dots are sampling sites (n=22). Capital letters are pollen sediment sampling sites from the literature (n=12). Estimate of ice sheet extent at the LGM drawn from Waitt and Thorson (1983). Range map inset reprinted from Burns & Honkala (1990). Base map created using GPSVisualizer (www.gpsvisualizer.com).
typically been attributed to population responses during and following the period of high variability of climates of the Pleistocene (Petit et al., 1993b; Dumolin-Lapègue et al., 1997; Grivet et al., 2006). Typically, the highest genetic diversity is found within the refugial range (Hewitt, 2000). Genetic diversity is often lost along the migratory path, permitting inference of the path and the source population through genetic analysis of the descendant populations. Diversity is initially lost because post-glacial recolonization is not from a random draw of seed (Feng et al., 2008). Instead, dispersing seeds are most frequently drawn from the poleward edge of the shifting distribution. Once a post-glacial region is colonized, newly-established populations often limit establishment from other sources by filling the niche space (Taberlet et al., 1998; Hewitt, 2000). Further, it is likely that a few colonizing individuals contribute the bulk of seed to newly-established populations because colonization events are infrequent (Petit et al., 1997). Under this model, one expects low genetic diversity within populations and an overall loss of genetic diversity with increasing geographic distance from the original source (Cwynar & MacDonald, 1987; Dumolin-Lapègue et al., 1997).

Nuclear markers, in contrast, provide a biparentally-inherited view of biogeographical patterns, incorporating both pollen flow and seed dispersal. Wind-pollination in trees can be effective over great distances, and pollen donors may frequently come from outside a recipient population (Liepelt et al., 2002; Bacles et al., 2005; Craft & Ashley, 2006, 2007). High within-site nuclear diversity is a common finding for oaks, and it is usually attributed to long-distance pollen flow (Dow & Ashley, 1996; Streiff et al., 1999; Aldrich et al., 2003b). For wind-pollinated species, there is also evidence that pollen flow can be enhanced in a fragmented or open landscape, as
would be found in the wake of a retreating glacier (Bacles et al., 2005, but see Sork et al., 2002). For example, in an open, fragmented landscape Craft & Ashley (2007) could not detect population structure for *Q. macrocarpa* Mich. over 200 km geographic distances.

In addition, Muir et al. (2004) found little nuclear differentiation among peripheral populations of *Q. petraea* (Mattuschka) Liebl. in sites in Ireland that were island colonizations and in a highly fragmented landscape. This high within-population genetic diversity and low population differentiation has the effect of obscuring source populations of post-glacial locations while mitigating potentially deleterious genetic consequences of small founding populations.

In many systems, species are thought to have travelled great distances from their glacial refugia to colonize their post-glacial ranges (Brewer et al., 2002). European oaks were relegated to refugia south of the Alps, a major migratory barrier (Hewitt, 1999; Petit et al., 2002), and chloroplast genetic signatures reflect the loss of diversity with northward migration in distinct latitudinal lineages (Grivet et al., 2006). The pattern in eastern North America was thought to be similar to Europe, with temperate species restricted to southern refugia, but gaps in fossil data made determining glacial distributions difficult (Jackson et al., 2000). Evidence is emerging that oak and other temperate species may have maintained refugial populations relatively close to the ice sheet, potentially allowing some species to persist at low densities much farther north than the pollen or macrofossil records suggest (McLachlan & Clark, 2004; McLachlan et al., 2005; Magni et al., 2005; Loehle, 2007; Birchenko et al., in press). In the Pacific Northwest of North America, the pattern of species distribution was greatly influenced by the Laurentide ice sheet much further east. But by the time the Cordilleran ice lobes were

73
reaching their greatest extent on Vancouver Island and in the Puget Trough, temperate
communities were advancing towards the ice (Whitlock, 1992). Maximum ice extent
clearly did not restrict taxa to distant refugia. In addition, the Cordilleran ice sheet did
not reach its maximum extent in the lowlands until alpine glaciers were retreating (Waitt
& Thorson, 1983). The varied topography of the Pacific Northwest, including its north to
south trending mountain ranges, probably allowed for many areas where small
populations could have waited out unfavourable conditions of the LGM.

I used pollen records to complement this phylogeographic study because they
provide a temporal and spatial context for interpreting results (Cruzan & Templeton,
2000; Petit et al., 2002; López de Heredia et al., 2007). Pollen record data indicate that
oak populations were present at low densities in Oregon and Washington before and
during the Fraser glaciation (25-10 kyr BP; Fig. 1; Table 1), a period when lowland ice
lobes reached their maximum extent (15 kyr BP). These data suggest that during what
would appear to be region-wide unfavourable conditions (15-14 kyr BP), *Q. garryana* was
not extirpated from even the northern parts of its mainland geographic range (compare
Fig. 1 and Table 1). For example, high pollen levels were found at Mosquito Lake Bog
in northern Washington (48.77°N, 122.12°W, 198 m) from 15-9 kyr BP, providing
evidence that *Q. garryana* maintained populations close to the ice sheet (Hansen &
Easterbrook, 1974). The only portion of the current range of *Q. garryana* that was
glaciated during the LGM is the Puget Sound region and San Juan Islands of Washington
northward into British Columbia (Brubaker, 1991; Brown & Hebda, 2002). As the Juan
de Fuca lobe of the Cordilleran ice sheet retreated, marked increases in oak at most
**TABLE 4.1**

**PRESENCE OF QUERCUS GARRYANA DOUGL. EX HOOK. POLLEN IN LAKE SEDIMENTS BY 500 YEAR INTERVAL**

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**SOURCES:** Data were compiled from the The National Climatic Data Center (www.ncdc.noaa.gov/paleo/pollen.html). Original sources for data are (A) Sea & Whitlock, 1995; (B) Worona & Whitlock, 1995; (C) Sea & Whitlock, 1995; (D) Barnosky, 1985b; (E) Barnosky, 1985a; (F) Barnosky, 1981; (G) Cwynar, 1987; (H) Hansen & Easterbrook, 1974; (I) Mathewes, 1973; (J) Brown & Hebda, 2002; (K) Brown & Hebda, 2002; (L) Brown & Hebda, 2003.

**NOTE:** Locations are marked with letters as in Fig. 4.1. Sites are organized from south to north on the mainland (A-I) and on Vancouver Island (J-L). A plus (+) means at least one grain of oak pollen was present within the 500 year interval, an empty table cell means pollen was absent, and a cell shaded grey indicates no data collected.
peripheral sites occurred from 10-4 kyr BP due to regional warming and drying (Barnosky, 1985a; Sea & Whitlock, 1995). *Quercus garryana* probably colonized Vancouver Island at this time from nearby locations, as the oldest oak pollen record from Vancouver Island is from just under 11 kyr BP.

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

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

Given previous literature, the two genome approach used here, and the existence of pollen record data for our study region, my motivation for studying the biogeography of *Q. garryana* was to infer seed dispersal and pollen flow patterns to better understand post-glacial colonization of peripheral regions, particularly where refugial populations
were in close proximity to the post-glacial expanded range. Based on previous research and population models, I had different expectations for each marker system. I expected the genetic differentiation across sampling sites to be higher in the chloroplast markers than the nuclear markers due to the strictly maternal inheritance of the chloroplast markers and the difference in pollen flow and seed dispersal (Petit et al., 1993a; Ennos, 1994; Grivet et al., 2008). Because known glacial refugia were close to the post-glacial colonization sites, I expected this difference to be less than is typically found for oak species, resulting from relatively high seed dispersal to areas that now represent the furthest reaches of the species’ range. However, the unstable climatological history of the region made it difficult to predict how many recognisable regional clusters I would find.

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

4.2. Materials and Methods

4.2.1. Study species and study region
Quercus garryana is the sole oak species in oak-savanna ecosystems of the Pacific Northwest region of North America. The current distribution of Q. garryana ranges from isolated mountain areas in the Sierra Nevada, California, to the coastal regions and valleys of northern California and Oregon, along the Columbia River region of Oregon and Washington, the Puget Trough, Washington, and southern British Columbia (Fig. 4.1 inset). It is the only native oak species north of southern Oregon (Glendenning, 1944). At the northern range boundary on Vancouver Island, British Columbia, populations occur in fragmented patches surrounded by coniferous forest. At its extreme northern limit, Q. garryana occurs on rocky outcrops and on southerly and westerly facing slopes where Douglas-fir (Pseudotsuga menzesii (Mirb.) Franco) grows poorly. Toward the center of the species’ range, populations of Q. garryana are less isolated and fragmented sometimes forming extensive woodlands.

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

4.2.2. Sample collection

Twigs of Q. garryana were collected from 22 sampling sites throughout the northern half of the species range in May 2005 and February and April 2007 (Fig. 4.1).
Sampling locations included Siskiyou Mountain and Rogue River regions, Willamette Valley, Puget Trough, the San Juan Islands, Vancouver Island, and Hornby Island. At each sample site, twigs were collected from 9-22 trees at least 20 m apart. Twigs collected in 2005 were shipped to the laboratory on water ice and frozen at -80°C until DNA extraction. In 2007, most twigs were cut and dried on silica gel to maintain integrity of the DNA, though a small subset of the 2007 samples were shipped fresh to the laboratory.

4.2.3. DNA extraction and genotyping

Fresh, frozen, and dried twigs were ground under liquid nitrogen using a Freezer/Mill 6850 (Spex CertiPrep). Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Inc.) according to the manufacturer’s protocol with the following modification: twig powder in extraction buffer was shaken in a tube with a 1/4” cylindrical ceramic bead (Qbiogene, Inc.) in a FastPrep FP 120 (Qbiogene, Inc.) for three cycles of 15 seconds each to increase cell lysing.

I used five chloroplast microsatellite markers developed for *Q. petraea* (Mattuschka) Liebl. and *Q. robur* L. (Deguilloux et al., 2003) to reveal chloroplast haplotypes. Seven nuclear microsatellite markers, five developed from *Quercus rubra* L. (Aldrich et al., 2002, 2003a) and two from *Quercus petraea* (Steinkellner et al., 1997), were used to amplify microsatellite loci in *Q. garryana* (see Section 4.6 and Table 4.2). I performed PCR for both chloroplast and nuclear markers in a total volume of 25 μl containing 5 U GoTaq Flexi (Promega Corporation), 1X colorless GoTaq Flexi buffer, 2.5 mM MgCl₂, 200 nM each dNTP (Fermentas, Inc.), and 5 pM forward and reverse
### TABLE 4.2

**PROPORTION OF HETEROZYGOTES EXPECTED (HE) AND OBSERVED (HO) PER LOCUS AND SAMPLING SITE**

<table>
<thead>
<tr>
<th>Sample site</th>
<th>N</th>
<th>ssrQpZAG 9 He/Ho</th>
<th>ssrQpZAG 0C19 He/Ho</th>
<th>quru-GA-0G12 He/Ho</th>
<th>quru-AC-0M05 He/Ho</th>
<th>quru-GA-1G13 He/Ho</th>
<th>quru-GA-1M17 He/Ho</th>
<th>Overall Mean He/Ho</th>
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<td></td>
<td></td>
<td></td>
<td>He/Ho</td>
<td></td>
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<tr>
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<td>0.82/0.69</td>
<td>0.73/0.56</td>
<td>0.58/0.50</td>
<td>0.62/0.56</td>
<td>0.41/0.44</td>
<td>0.18/0.19</td>
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<td>0.79/0.84</td>
<td>0.58/0.47</td>
<td>0.70/0.68</td>
<td>0.68/0.47</td>
<td>0.15/0.16</td>
<td>0.47/0.47</td>
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<td>0.60/0.53</td>
<td>0.75/0.65</td>
<td>0.47/0.59</td>
<td>0.48/0.29</td>
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<td>0.67/0.82</td>
<td>0.56/0.53</td>
<td><strong>0.74/0.35</strong></td>
<td>0.53/0.59</td>
<td>0.40/0.35</td>
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<td>0.56/0.75</td>
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<td>0.40/0.38</td>
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<td>0.64/0.45</td>
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<td>0.70/0.67</td>
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<td>0.76/0.60</td>
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<tr>
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<td>0.37/0.44</td>
<td>0.81/0.81</td>
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<td><strong>0.51/0.41</strong></td>
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<td>0.38/0.47</td>
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<tr>
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<td><strong>0.45/0.31</strong></td>
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<tr>
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<td>0.64/0.82</td>
<td>0.55/0.35/0.12</td>
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<td>15</td>
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<td>0.69/0.80</td>
<td>0.40/0.47</td>
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<td>0.74/0.80</td>
<td>0.39/0.33</td>
<td>0.56/0.47</td>
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</tbody>
</table>

**NOTE:** Italicised pairs show a statistically significant departure from Hardy-Weinberg equilibrium at 0.01<P<0.05. Bold face type indicates significant deviation from equilibrium at P<0.01. Loci at sampling sites that Micro-checker suggested as having null alleles are marked with their calculated null allele frequency (r) as determined following Brookfield (1996).
primer. The PCR protocol consisted of a 2 min initial denaturing step at 95°C followed by 35 cycles of 30 sec at 95°C, 30 sec at marker-specific annealing temperatures (Table 4.2), and 30 sec at 72°C, then a 10 min final elongation at 72°C. Microsatellite fragment lengths were detected using forward primers labelled with WellRED dye (Sigma-Proligo) on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc.). For each marker, I ran samples once on plates with positive and negative controls, to determine if the fragment lengths were accurate and free of contamination. If amplified fragments were difficult to score, PCR was performed again for the given sample and marker combination. Samples that did not amplify or remained too difficult to accurately score were discarded. Over all repeated sample and marker combinations, the genotyping error rate was 0.5%.

4.2.4. Statistical analyses

I used Arlequin 3.1 (Excoffier et al., 2005) to examine population substructure through an analysis of molecular variance (AMOVA) (10,000 permutations) for the chloroplast microsatellite data. Also, I determined within-population diversity ($H_S$), overall diversity ($H_T$), and population differentiation ($G_{ST}$) using the program cpSSR (http://www.pierroton.inra.fr/genetics/lab0/Software/PermutCpSSR/index.html) following Pons & Petit (1995, 1996).

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

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

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

4.3. Results

In five chloroplast microsatellites, I detected 2-3 alleles at each marker and a total of six haplotypes over 22 sampling locations (Fig. 4.2; Table 4.3). Three of the haplotypes were common and found at multiple locations; the remaining three were found at only one sample site each (Fig. 4.2; Table 4.4). Within-population diversity ($H_S$) was low (0.08 ± 0.03 SE), overall diversity ($H_T$) was higher (0.67 ± 0.06 SE), and population differentiation ($G_{ST}$) was high (0.88 ± 0.05 SE; Fig. 4.2; Table 4.5). At 16 out of the 22 chloroplast sampling sites, all individuals within the population shared a single chloroplast haplotype.

The seven nuclear microsatellite loci ranged from 6 (quru-GA-1M17) to 17 alleles (ssrQpZAG 9) (Table 4.6). For the 22 sites genotyped with nuclear microsatellite markers, the overall allelic richness ranged from 27 (sites 16 & 18) to 43 (site 3) alleles (Fig. 4.3). The allelic richness decreased significantly with increasing latitude ($R^2=0.63$, $P<0.001$) (Fig. 4.3). This reduction in the number of alleles at the range periphery was due to a lack of alleles that are rare in the species core. Forty-two of the 61 total alleles were found across the entire study region.
Figure 4.2. Chloroplast haplotypes from 22 sampling sites. Haplotypes A=white, C=light grey, and F=black are the predominant haplotypes over the study region. Haplotypes B (one individual at site 22 only), D (two individuals at site 1 only), and E (17 individuals at site 1 only) are less common and are marked on the map.
TABLE 4.3

HAPLOTYPES DESCRIBED BY THE AMPLIFIED FRAGMENT LENGTH OF EACH OF THE FIVE REPEAT LOCI

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>μcd4</th>
<th>μcd5</th>
<th>μdt1</th>
<th>μdt3</th>
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<tr>
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<td>75</td>
<td>83</td>
<td>126</td>
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<tr>
<td>F</td>
<td>94</td>
<td>74</td>
<td>84</td>
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TABLE 4.4

DISTRIBUTION TABLE OF HAPLOTYPES BY SAMPLE SITE

<table>
<thead>
<tr>
<th>Sample site</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>18</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>21</td>
<td>16</td>
<td>1</td>
<td></td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>18</td>
<td>1</td>
<td></td>
<td></td>
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<td>19</td>
</tr>
<tr>
<td>∑</td>
<td>192</td>
<td>1</td>
<td>81</td>
<td>2</td>
<td>15</td>
<td>87</td>
<td>378</td>
</tr>
</tbody>
</table>

NOTE: Sites 1-22 correspond to those in Fig. 4.1.
TABLE 4.5

AMOVA FOR BOTH NUCLEAR AND CHLOROPLAST MICROSATTELITE DATA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>$F_{ST}$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHLOROPLAST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>21</td>
<td>104.7</td>
<td>0.287</td>
<td>86.07</td>
<td>0.861</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Within populations</td>
<td>356</td>
<td>16.6</td>
<td>0.047</td>
<td>13.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>377</td>
<td>121.3</td>
<td>0.334</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NUCLEAR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>21</td>
<td>113.3</td>
<td>0.109</td>
<td>4.94</td>
<td>0.049</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Within populations</td>
<td>646</td>
<td>1352.7</td>
<td>2.094</td>
<td>95.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>667</td>
<td>1466.0</td>
<td>2.203</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Significance of variance components assessed with 10,000 permutations.
### TABLE 4.6

**CHARACTERIZATION OF POLYMORPHIC MICROSATellite LOCI FOR QUERCUS GARRYANA FROM MARKERS DEVELOPED FOR Q. PETRAEA (NUCLEAR=SSRPZAG AND CHLOROPLAST=M) AND Q. RUBRA (NUCLEAR=QURU)**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences (5’→3’)</th>
<th>Repeat motif</th>
<th>Ta (°C)</th>
<th>Size range (B.P.)</th>
<th>A</th>
<th>Citation</th>
<th>GenBank accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssrQPZAG 9</td>
<td>F: gcaattacagctagctgg R: gtcctgacagtccctatg</td>
<td>(AG)_{12}</td>
<td>56</td>
<td>243-276</td>
<td>17</td>
<td>Steinkellner et al., 1997</td>
<td>N/A</td>
</tr>
<tr>
<td>ssrQPZAG 36</td>
<td>F: gataaatttggaatttaagag R: acttgttggttgactctagtag</td>
<td>(AG)_{19}</td>
<td>50</td>
<td>204-222</td>
<td>9</td>
<td>Steinkellner et al., 1997</td>
<td>N/A</td>
</tr>
<tr>
<td>quru-GA-0C19</td>
<td>F: ttagcttaacagtctg R: cggcttgctgggctg</td>
<td>(GA)_{18}</td>
<td>58</td>
<td>211-229</td>
<td>8</td>
<td>Aldrich et al., 2002</td>
<td>AF523853</td>
</tr>
<tr>
<td>quru-AC-0G12</td>
<td>F: aaccttcttaagtacta R: cagcaaaatgtttagttaga</td>
<td>(AC)_{13}</td>
<td>54</td>
<td>208-223</td>
<td>7</td>
<td></td>
<td>EF377339</td>
</tr>
<tr>
<td>quru-GA-0M05</td>
<td>F: ctcaagtaacagccttaa R: cttgtgacagttcattac</td>
<td>(GA)_{20}</td>
<td>58</td>
<td>188-208</td>
<td>7</td>
<td>Aldrich et al., 2002</td>
<td>AF523856</td>
</tr>
<tr>
<td>quru-GA-1G13</td>
<td>F: aaaaatcagacctgagctaatg R: gatttaacagctactg</td>
<td>(GA)_{14}</td>
<td>48, 58</td>
<td>166-180</td>
<td>7</td>
<td>Aldrich et al., 2002</td>
<td>AF523862</td>
</tr>
<tr>
<td>quru-GA-1M17</td>
<td>F: gtttacctgtcggggagt R: ttacctttctgccctagtaa</td>
<td>(GA)_{19}</td>
<td>60</td>
<td>113-127</td>
<td>6</td>
<td>Aldrich et al., 2003</td>
<td>N/A</td>
</tr>
<tr>
<td>μcd4</td>
<td>F: tatttttttttttttca R: ttccccatagagttgtat</td>
<td>(T)_{12}</td>
<td>50</td>
<td>94-96</td>
<td>3</td>
<td>Deguilloux et al., 2003</td>
<td>AJ489832</td>
</tr>
<tr>
<td>μcd5</td>
<td>F: ccccgcatctgctactg R: taataaaggagatacataa</td>
<td>(A)_{8}</td>
<td>50</td>
<td>74-75</td>
<td>2</td>
<td>Deguilloux et al., 2003</td>
<td>AJ489833</td>
</tr>
<tr>
<td>μdt1</td>
<td>F: atcttaactaagctggaa R: tcctaaacactgtgatcc</td>
<td>(A)_{11}</td>
<td>50</td>
<td>82-84</td>
<td>3</td>
<td>Deguilloux et al., 2003</td>
<td>AJ489837</td>
</tr>
<tr>
<td>μdt3</td>
<td>F: tggtagtaactctgtaggt R: aggttaaatcttctgaat</td>
<td>(A)_{11}</td>
<td>50</td>
<td>124-126</td>
<td>3</td>
<td>Deguilloux et al., 2003</td>
<td>AJ489838</td>
</tr>
<tr>
<td>μdt4</td>
<td>F: gataataaaagctagtaaat R: cggaaagtctctacatctg</td>
<td>(A)_{9}</td>
<td>50</td>
<td>146-148</td>
<td>3</td>
<td>Deguilloux et al., 2003</td>
<td>AJ489839</td>
</tr>
</tbody>
</table>

**NOTE:** Ta, annealing temperature; A, number of alleles.
Figure 4.3. Correlation between latitude and allele richness combined over all seven nuclear microsatellite loci. $R^2=0.63$, $P<0.001$. 
Of the remaining 19 alleles, 11 were unique to southern Oregon. I found five alleles that were not represented in southern Oregon samples, two alleles farther north in both mainland and peripheral island locations, two alleles farther north confined to the mainland, and one allele limited to the peripheral island populations. An additional three alleles were found in all regions except the peripheral islands. Pairwise $F_{ST}$ values ranged from 0.000 to 0.184 among all 22 sampling sites, with a mean $F_{ST}$ of 0.049 ($P<0.0001$).

Observed heterozygosity from each sample site averaged over all loci ranged from 0.44 to 0.66 (Table 4.7). Significant departures from HW equilibrium ($P<0.05$) occurred in at least one sampling site for each marker. Markers quru-0G12 and quru-1M17 showed significant departures at one sampling site each; ssrQpZAG 36 and ssrQpZAG 9 showed significant departures at two sampling sites; quru-1G13 showed departures at three sites; quru-0C19 showed departures at four sites; and quru-0M05 showed departures at six sites (Table 4.2). Departures from equilibrium caused by homozygote excess were commonly found to be due to null alleles. Micro-checker analyses showed that short allele dominance or scoring errors due to stutter are not implicated in the markers. Instead, I found that marker quru-0M05 exhibited evidence for null alleles at six of the 22 sampling sites, and markers quru-0C19 and ssrQpZAG 9 exhibited null alleles at two sites each (Table 4.2). At the site and marker combinations showing null alleles, frequencies ranged from 0.11 to 0.29 (Table 4.2). Site 8 showed evidence for null alleles in two markers, but the other eight sites with null alleles, had them only in one marker each. The presence of null alleles has the potential to bias results, but because of their low number (10 of the 154 marker and site combinations
<table>
<thead>
<tr>
<th>Sample site</th>
<th>N</th>
<th>Mean He/Ho</th>
<th>Mean $A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>0.61/0.55</td>
<td>6.000</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>0.61/0.58</td>
<td>6.000</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>0.67/0.64</td>
<td>6.143</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>0.66/0.61</td>
<td>5.857</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.58/0.49</td>
<td>5.143</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0.64/0.57</td>
<td>4.571</td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>0.64/0.66</td>
<td>4.857</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>0.61/0.44</td>
<td>5.571</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>0.67/0.62</td>
<td>5.429</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>0.61/0.59</td>
<td>5.286</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>0.57/0.56</td>
<td>4.000</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>0.64/0.58</td>
<td>5.143</td>
</tr>
<tr>
<td>13</td>
<td>15</td>
<td>0.57/0.50</td>
<td>4.143</td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>0.55/0.55</td>
<td>4.143</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>0.58/0.51</td>
<td>4.857</td>
</tr>
<tr>
<td>16</td>
<td>12</td>
<td>0.56/0.51</td>
<td>3.857</td>
</tr>
<tr>
<td>17</td>
<td>12</td>
<td>0.54/0.48</td>
<td>4.000</td>
</tr>
<tr>
<td>18</td>
<td>12</td>
<td>0.53/0.44</td>
<td>3.857</td>
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<tr>
<td>19</td>
<td>17</td>
<td>0.59/0.56</td>
<td>5.000</td>
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<tr>
<td>20</td>
<td>16</td>
<td>0.58/0.49</td>
<td>4.571</td>
</tr>
<tr>
<td>21</td>
<td>17</td>
<td>0.54/0.52</td>
<td>4.286</td>
</tr>
<tr>
<td>22</td>
<td>15</td>
<td>0.56/0.57</td>
<td>4.000</td>
</tr>
</tbody>
</table>

NOTE: Sample site numbers refer to sites shown in Fig. 4.1. Mean $A$ is the number of alleles averaged over all seven loci. Mean He and Ho per locus are shown in Table 4.2.
show a tendency for null alleles with frequency >0.1) and haphazard distribution across markers and sample sites in this study, I interpret the results without modification. Departures from HW equilibrium at markers and sites not exhibiting null alleles could be due to the relatively small sample number per location. It is not likely that departures from equilibrium are the result of inbreeding.

Bayesian analyses in Structure indicated the data are most likely when k = 2 (P>0.999) (Fig. 4.4). In the two-cluster case, all populations north of approximately 48 degrees north latitude grouped together. This northern cluster also contained one Oregon population (7) and one southern Washington population (12) (Fig. 4.4). All other populations group together in a single southern cluster. For the AMOVA given two groups, significant levels of variation were explained within sampling site, within regions among sampling sites, and between the two regions (Table 4.8). Yet, over 90 percent of the genetic variation was captured within sampling sites, indicating that all sites had a high level of genetic diversity (Table 4.8). Very little (~6%) of the nuclear diversity is explained among populations. The pattern driving the differences between regions was due, in part, to lower allelic diversity at the range periphery (Fig. 4.3). A weaker, but similar pattern was observed for levels of heterozygosity (not shown).

Geographic distances were significantly correlated with both chloroplast (Mantel test: 10,000 permutations, rM=0.402, P=0.0012) and nuclear genetic distance (Mantel test: 10,000 permutations, rM=0.622, P=0.0001). Mantel correlograms showed a significant positive genetic and geographic relationship within the first 200 km for both chloroplast and nuclear data (Fig. 4.5). Likewise for both marker systems, a significant negative correlation was detected between 700 and 900 km (Fig. 4.5). The only
Figure 4.4. Degree of admixture per individual across a latitudinal gradient (site 1 to 22; refer to Fig. 4.1). Model k =2 with admixture (shown as assignment probabilities for the proportion grey to white per sample). Sampling locations with black arrows (7 & 12) denote those sites that are located in the species core but have a greater proportion of the individuals assigned to the northern cluster.
<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>$F_{ST}$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between regions</td>
<td>1</td>
<td>26.7</td>
<td>0.068</td>
<td>3.03</td>
<td>0.030</td>
<td>0.0001</td>
</tr>
<tr>
<td>Among populations</td>
<td>20</td>
<td>86.6</td>
<td>0.074</td>
<td>3.30</td>
<td>0.034</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>within region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within populations</td>
<td>646</td>
<td>1352.7</td>
<td>2.094</td>
<td>93.67</td>
<td>0.063</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>667</td>
<td>1466.0</td>
<td>2.236</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Significance of variance components assessed with 10,000 permutations.
Figure 4.5. Mantel correlograms from chloroplast (top) and nuclear (bottom) data. Filled squares indicate significant correlations between genetic and geographic distance at $P<0.05$. Points above the zero line are positively correlated, and those below the line are negatively correlated.
discrepancy in the relationship with geographic distance between the two genomes is at
the 600 to 700 km range. The nuclear data show a negative correlation with geographic
distance, whereas the chloroplast data show a positive correlation. This positive
correlation with chloroplast data is driven by a shared haplotype from a site in Southern
Oregon (haplotype 1, site 5) with sites on Vancouver Island. From the data I estimated
pollen to flow at a rate of ~120 times that of seed dispersal.

4.4. Discussion

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

The peripheral island samples are homogeneous for chloroplast haplotypes with two exceptions. At the second-most northern site (site 21), one individual possessed a unique haplotype. The Bayesian assignment test groups this individual with the others in its sample site, indicating that it might be an in situ mutation. At the most northern site (site 22), however, another single individual had a haplotype found only in more southerly mainland populations. The nuclear assignment test for this individual groups it with the core cluster with a higher probability than with the northern cluster (Fig. 4.4). I postulate that this tree is a more recent colonist to this site and could have been planted by humans since this location is managed as a community park of a rural residential development.

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

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

Populations in the periphery are recognizably distinct from core populations but are similar to each other. These data support the supposition that colonization to the peripheral islands came from few mainland colonists and from one or few source populations. The apparent low-level of cross-colonization by seed among glacial source populations, given the age of these populations, provides evidence that effective seed dispersal in this species is limited. High nuclear diversity and little genetic differentiation due to high levels of pollen flow within the peripheral study region, however, is not
unexpected given that oaks persisted very near the ice sheet in unglaciated peripheral sites throughout the glacial period (e.g., site H in Table 4.1).

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

It is important to recognize that low population differentiation determined from nuclear markers and high differentiation from chloroplast markers can occur even in situations of low pollen flow for tree species. Firstly, the effective population size of the chloroplast genome is one-quarter that of the nuclear genome (Muir et al., 2004). In addition, founding populations may no longer be small at the time of seed reproduction. For example, Austerlitz et al. (2000) modeled tree colonization and found that when the time to first reproduction is taken into account (often ~50 years for oaks), founder effects were reduced by the accumulation of migrants over time, forming a large founding population with much of the genetic diversity of the source. In this study system, if
founding events on the islands were common, we might expect to see more mainland haplotypes represented on the islands (i.e., those found at sites 9-12 and 17 in Fig. 4.2). We do not. Therefore, founding events were likely rare, and pollen flow is the likely mechanism for high nuclear diversity within peripheral populations. In addition, it has been shown with stochastic modeling that in situations with minimal seed dispersal, pollen flow has a strong homogenizing effect among populations (Austerlitz & Garnier-Géré, 2003).

There is low chloroplast haplotype richness across the entire study region, even with sample sizes larger than what is typical for chloroplast haplotyping. I found very little within-population diversity and high levels of differentiation (in agreement with other studies) with ~four times the number of samples per site than in many previous studies (Dumolin-Lapègue et al., 1997; Romero-Severson et al., 2003; Deguilloux et al., 2004; Grivet et al., 2006). I observed 14 total alleles across the 5 loci, indicating that chloroplast mutation rates are high enough to detect population-level differences.

4.5. Conclusions

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

4.6. Appendix

The research described in this chapter used microsatellite markers developed in other oak species. In order to determine appropriate markers for use in \textit{Quercus garryana} Dougl. ex Hook., I tested 12 \textit{Q. petraea} (Mattuschka) Liebl. and 10 \textit{Q. rubra} L. markers on \textit{Q. garryana} samples, based on initial reports that some of these markers might be useful in other \textit{Quercus} species (Steinkellner et al. 1997, Aldrich et al. 2002, 2003a). I also examined primer sequences developed for \textit{Q. rubra} that were not previously published. If a marker amplified successfully in an initial screen of at least seven \textit{Q. garryana} individuals from geographically segregated populations, it was retained for further testing. Twelve of the 22 tested microsatellites amplified well in initial screening and PCR conditions were further optimized to obtain high quality markers on a large number of samples. Seven of the 12 amplicons were determined to be useful in \textit{Q. garryana}. All amplicons have similar size ranges to those of the species in which they were developed. For \textit{Q. garryana}, ssrQpZAG 9 alleles encompass roughly the same 30 bp allele range, but the amplicons are 60 bp larger than for \textit{Q. petraea} (Table 4.6). Four of the 12 markers that passed the initial screen were monomorphic (ssrQpZAG 1/2 and ssrQpZAG 110) or produced patterns not easily scored (\textit{quru-TCAC-0K18} and ssrQpZAG 16). An additional marker (ssrQpZAG 1/5) showed low levels of
heterozygosity with the likelihood of null alleles for many of the sample sites used in the study. These five markers were not used.
5.1. Introduction

Plants are facing and will continue to face increases in atmospheric CO$_2$ and shifts in temperatures and precipitation (IPCC, 2007). Our understanding of how populations and species respond to atmospheric and climatic changes is based upon the study of few, often classic study systems (see e.g., the *Abutilon/Amaranthus* system of F.A. Bazzaz). By necessity, ecologists frequently extrapolate their findings to other, unstudied species with similar characteristics to increase generality. Though it would be absurd to think that every species could be studied for its responses to global change, extreme care must be given when generalizing results. Knowing the hierarchical level (e.g., population, species, family) at which generalizing leads to inaccurate prediction is critical for understanding plant ecology and managing biodiversity.

Previous research has examined the response of contrasting species to elevated CO$_2$ and climatic change, such as C$_3$ vs. C$_4$ plants and trees vs. shrubs vs. herbs vs. grasses (Carter & Peterson, 1983; Oechel & Strain, 1985; Poorter, 1993; Ainsworth & Long, 2005; Cleland et al., 2006; Housman et al., 2006). Different responses among
these groups are attributed to differences in physiological processing of CO2 (in the case of C3 vs. C4 plants) or to different baseline growth rates for species with different growth habit. It has been known for a long time, however, that species do not always respond to elevated CO2 in ways predicted by their physiology or photosynthetic pathway (Garbutt et al., 1990). Of greatest interest in this study are species-specific responses within a functional group and among closely related taxa to examine the possibility that shared phylogeny and physiology confer shared responses to altered conditions of global change.

There is evidence that populations within species respond differently to elevated CO2 and increased temperature (Garbutt & Bazzaz, 1984; Wulff & Alexander, 1985; Bazzaz et al., 1995; Thomas et al., 1999; Ziska et al., 2005; Stinson et al., 2006). These population-level differences must be carefully examined for the direction and amount of difference to the given treatments, as subtle but statistically significant differences within populations may still provide the same qualitative result. For example, Garbutt and Bazzaz (1984) found that in all cases of floral measurement and biomass, population identity resulted in significantly different responses regardless of CO2 treatment or in an interaction between population and CO2 for genetically distinct populations of Phlox drummondii. For the majority of the responses, however, elevated CO2 resulted in similar qualitative results across populations such as floral birth rate, maximum number of births, and maximum floral display. The quantitative differences were such that certain populations responded better at the middle CO2 treatment than at the high CO2 treatment, but all populations responded in the same direction to elevated CO2. Even more subtle were the interactions among CO2 increases and Bromus tectorum populations collected from three different elevations (Ziska et al., 2005). Biomass of all three
populations increased from the lowest to the highest CO₂ concentrations tested. Stinson et al. (2006) found that *Ambrosia artemisiifolia* plants were larger with increased branching toward the top of the plants in elevated CO₂, but that this pattern was stronger for subordinate plants than for dominant individuals. The quantitative differences found by Stinson et al. may result in qualitative differences in community structure and competition, but their directions of change are not qualitatively different with respect to elevated CO₂. Other population-level differences have been pronounced. For example, Bazzaz et al. (1995) found significant interactions between CO₂ treatment and genotypes of *Abutilon theophrasti* and *Betula alleghaniensis* that resulted in qualitative differences. Certain genotypes grew larger under elevated CO₂, whereas others were smaller. In addition, Wulff and Alexander (1985) found that the combined effects of elevated CO₂ and temperature had different qualitative influences on growth responses depending on half-sib family identity of *Plantago lanceolata*.

Given such population-level findings, it seems reasonable to hypothesize that closely related species may qualitatively differ in their growth responses to changing CO₂ and climate and that species could show differential responses greater than among populations. Few researchers have pursued studies that examine differences among closely related species, however. Poorter (1993) and Cleland et al. (2006) conducted studies that included congeners for comparison, but the main focus of these studies was the similarities and differences among functional groups, not congeneric species. This study uses three related species that co-occur and have similar life history traits to determine if, and to what degree, similar species vary in their response to global change. On the one hand, related, similar taxa may be expected to perform similarly if selective
forces have acted to conserve similar traits (niche conservatism). On the other hand, competition for the same resources may have allowed for evolution of different responses in similar, related taxa (overdispersion) (see Cavender-Bares et al., 2004; 2006). The similarity in outcome of responses to a changing climate will depend upon what taxonomic scale climate forces act.

I investigated three species within the same genus that grow in the open habitats of the oak-savanna ecosystem of the Pacific Northwest of North America (Pojar & MacKinnon, 1994). *Lomatium utriculatum* (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose, *L. nudicaule* (Pursh) J.M. Coult. & Rose, and *L. dissectum* (Nutt.) Mathias & Constance co-occur but differ in their abundance, particularly at the northern edge of their geographic range on Vancouver Island, British Columbia, Canada (*L. utriculatum* > *L. nudicaule* > *L. dissectum*) (Douglas et al., 1998; unpublished data). All three species are adapted to seasonal patterns of precipitation typical of Mediterranean and sub-Mediterranean climates. The climate in the region where the species co-occur is characterized by mild, wet winters and springs and a dry summer season. In their areas of historical occupancy, climate change is expected to warm winter and summer temperatures and increase the seasonality of precipitation patterns (Christensen et al., 2007). The three species germinate in late winter in nature (February through March) and older plants begin to re-emerge in winter (January through March) and put out a large flush of vegetation in spring (April-May). The plants flower in April and May, though seasonally anomalous earlier flowers may be observed in *L. utriculatum* given a warm early season (personal observation). They attract generalist insect pollinators. Fruits develop in May and ripen in June for *L. utriculatum* and *L. dissectum* and in July (and
even August toward the north) for *L. nudicaule*. There is no known specialized dispersal mechanism for *Lomatium* seeds, though Thompson (1985) suggested they are wind dispersed. By July 1, most *L. utriculatum* and *L. dissectum* plants have senesced, avoiding the driest time of year and sequestering photosynthate in large taproots. *Lomatium nudicaule* has much the same life history, but its waxy glaucous cuticle reduces evaporation and the species does not senesce until July or August in most years (personal observation).

Climate models predict that mean temperatures in the region occupied by these species will increase by as much as 4°C, with lesser increases closer to the Pacific coast. Precipitation is expected to increase in the winter months and decrease in the summer months (Christensen et al., 2007). Increased temperature will increase evaporation/transpiration. This combination of higher temperatures and drier summer conditions may result in the shortening of the growing season, particularly if developmental cues are determined by changing light regimes (which will not be altered under human-induced climate change). Currently, water is not limiting during germination and seeding growth as these species establish during periods of high soil moisture (unpublished data). It is possible that water will become more limiting during the establishment period if growing conditions become less favorable.

Using these three species, I tested the hypothesis that closely related taxa with similar life history characteristics would respond to elements of climate change in different ways. Because of the importance of the seedling stage to individual establishment and because of the importance of considering multiple, interacting factors of global change, I focused on the responses of seedlings to elevated CO₂ and
temperature. I expected that for all three species, elevated temperature would enhance growth as simulated spring-time temperatures are low and increases should result in faster physiological processes without negative consequences of heat stress. I also expected that during early growth, elevated CO₂ would enhance photosynthesis for all species. The only expected difference \textit{a priori} that I anticipated was for \textit{L. nudicaule} to be better at retaining water due to its glaucous cuticle and its extend growth into the dry season in nature. As seedlings germinate in a wet portion of the year, I did not examine altered precipitation in this experiment. The experiments were conducted in growth chambers under non-competitive environments with equal and abundant watering treatments.

5.2. Materials and Methods

Seeds of the three \textit{Lomatium} species were grown in four Conviron growth chambers (CMP5090). Each chamber had a different combination of two treatments: CO₂ and temperature. One chamber was set to ambient levels of CO₂ and an ambient temperature regime; a second chamber was programmed for ambient CO₂ and elevated temperature; a third chamber for elevated CO₂ and ambient temperature; and a fourth for elevated CO₂ and elevated temperature.

Ambient CO₂ concentrations were current room conditions occupied by the growth chambers (~400 ppm). Elevated concentrations were double-ambient (800 ppm) and represent values at the low end of the range of 730-1020 ppm predicted by 2100 under the A2 emissions scenario of the IPCC (2007). The ambient CO₂ chambers fluctuated from a daily high of ~450 ppm at night when plants were not actively
photosynthesizing to a daily low of ~350 ppm during peak light. The elevated CO₂ chambers held constant at 800 ppm with a standard deviation of 15 ppm.

The ambient temperature treatment was set to historical spring-time conditions in Salem, Oregon, a location represented by species co-occurrence and the source of seed used in this study (Heritage Seedlings Co.). Ambient temperatures were calculated as the average maximum and average minimum temperatures occurring during the growing season of March through May over the years 1970 to 2000, resulting in a maximum temperature of 16.5°C and a minimum of 4.5°C (Western Regional Climate Center < www.wrcc.dri.edu>). By 2100, the increase in global mean is expected to approach 4°C based upon A1B, A2, and A1FI emissions scenarios (IPCC, 2007). Thus, the elevated temperature chambers were set 4°C higher than the ambient chambers, with a maximum of 20.5°C and a minimum of 8.5°C. Minimum and maximum temperatures were held steady for six hours each, and temperatures were ramped over six hours to reach these values. The light regime in each chamber began with a half-hour of incandescent light only (dawn), followed by a 12-hour incandescent and fluorescent light combination (450-550 µmol/m²/s), followed by a half-hour incandescent light again (dusk), and then 11 hours of dark.

Before planting, seeds were cold-moist stratified in vermiculite at 4°C for 2 weeks for *L. utriculatum*, 8 weeks for *L. nudicaule*, and 12 weeks for *L. dissectum* as was previously determined to be optimal for each species (unpublished data). Four hundred seeds of each species were planted in each chamber. Seeds were placed in single-species mixtures of ten seeds per 6” round pot, totaling 1,200 seeds in 120 pots per chamber or 4,800 seeds in 480 pots over the entire experiment. A Fafard Complete potting mix
(0.09-0.04-0.06) was used as the nutritive soil substrate in all plantings. All pots were kept evenly moist over the course of the experiment so that water was not limiting plant growth.

Four weeks passed before initial harvesting of samples. This amount of time allowed the seeds to germinate and for the majority of seedlings to have at least one true leaf. To determine growth rates of each species, a subset of 15-20 plants per species and per treatment combination was removed in the evening once a week for four weeks (weeks 5-8 of the experiment). Due to high levels of germination in *L. dissectum*, seedlings were sampled for an additional five weeks (weeks 9-13). *Lomatium nudicaule* had moderate germination and was sampled for an additional four weeks (weeks 9-12), but sample sizes during the additional sampling events ranged from 5-10 plants per treatment combination. At each sampling time, a seedling was randomly selected using a random number table from pots containing the largest number of seedlings (i.e., crowded pots). For *L. dissectum*, the largest species and also the species with highest germination, pots contained 1-10 individuals with a median of 5. This strategy of seedling removal had the advantage of minimizing within-pot competition as pots with 10 individuals were sampled first when the plants were small and all pots contained only one individual during the final two sampling events.

Whole plants were harvested with as much of the fine roots as possible. Seedlings were weighed fresh then dried and weighed again to determine proportion water content ([wet mass-dry mass]/dry mass) and dry biomass. Dry biomass was used as the primary growth metric as it is the most fundamental measure of growth through CO₂ assimilation. Biomass accumulation (growth) rate was also used in analysis because
it enabled direct species comparisons as the species differ with respect to absolute size. The water content of plants also was analyzed as an index of water-use efficiency (i.e., milligrams of CO$_2$ fixed per gram of water transpired).

Systat 12 was used to conduct a two-factor ANOVA of dry biomass to test for differences in growth caused by the treatments within each species. ANOVAs were conducted for each species at week 8 and also at week 12 for *L. nudicaule* and week 13 for *L. dissectum*. Rate of biomass accumulation (growth rate) was measured as the percent change in biomass. Mean biomass from week 5 was subtracted from biomass data collected during week 8, and the difference was then divided by biomass from week 5. These values were then multiplied by 100. Water content at week 8 was determined by subtracting the dry mass from the wet mass and dividing by the wet mass. ANOVAs including species, CO$_2$, and temperature as main effects were run to test the factors important in determining biomass accumulation rates and plant water content. To conform to the assumptions of normality and homogeneity of variance, data for growth rate were square root transformed. Because some of the values were negative, a constant was added to each value before the square root was taken. Data for water content were inverse transformed, but to keep data points in their original order it was necessary to multiply all proportional values by -1, add 1 to create positive values, and then take the inverse.

5.3. Results

In week 8 of the experiment, *L. dissectum* showed significantly increased biomass due to elevated CO$_2$ ($F_{1,66}=4.63; P=0.035$) and elevated temperature ($F_{1,66}=7.39; P=0.008$) (Fig.
5.1a). The interaction term was not significant. Likewise, L. utriculatum showed increased biomass in elevated CO2 ($F_{1,76}=9.95; P=0.002$) and elevated temperature ($F_{1,76}=17.98; P<0.001$) without a significant interaction among treatments (Fig. 5.1c). In contrast, L. nudicaule did not produce more biomass due to the elevated treatments ($F_{1,76}=1.56; P=0.215$ for CO2; $F_{1,76}=3.62; P=0.061$ for temperature) (Fig. 5.1b), though the trend for increased growth from elevated temperature approached significant. For L. dissectum and L. nudicaule, the pattern observed in week 8 held until the end of the experiment, except that trends for increased growth due to elevated treatments were lost in L. nudicaule (week 13 for L. dissectum: $F_{1,66}=38.76; P<0.001$ for CO2; $F_{1,66}=9.02; P=0.004$ for temperature; week 12 for L. nudicaule: $F_{1,29}=0.35; P=0.56$ for CO2; $F_{1,29}=0.55; P=0.46$ for temperature; again, no interaction terms were significant).

For biomass accumulation rate, species was the only significant main effect (Table 5.1). Lomatium utriculatum accumulated biomass more quickly than L. dissectum, and L. dissectum accumulated biomass more quickly than L. nudicaule (Fig. 5.2). I also observed a significant interaction between CO2 and temperature (Table 5.1). At ambient temperatures, plants grown in elevated CO2 tended to accumulate biomass at a faster rate, but at elevated temperatures the elevated CO2 treatment resulted in equal or slower growth rates (Fig. 5.2).

Water content significantly differed between species and treatments (Table 5.2). Lomatium utriculatum proportionally held the most water, followed by L. nudicaule and L. dissectum (Fig. 5.3). Elevated temperature resulted in proportionally less water held for each species, and elevated CO2 resulted in proportionally more water (Fig. 5.3). A
Figure 5.1. Weekly growth as measured by dry biomass for (a) *L. dissectum*, (b) *L. nudicaule*, and (c) *L. utriculatum*. Filled gray circles are ambient CO₂ and ambient temperature, hollow black circles are elevated CO₂ and ambient temperature, filled gray squares are ambient CO₂ and elevated temperature, and hollow black squares are elevated CO₂ and temperature. Error bars are 95% confidence intervals.
### TABLE 5.1

PERCENT CHANGE IN BIOMASS (GROWTH RATE) FROM WEEK 5 TO WEEK 8
OF THE EXPERIMENT ANALYZED WITH A THREE-FACTOR ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III SS</th>
<th>df</th>
<th>Mean Squares</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>6066.1</td>
<td>2</td>
<td>3033.0</td>
<td>77.3</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>CO2</td>
<td>37.0</td>
<td>1</td>
<td>37.0</td>
<td>0.945</td>
<td>0.332</td>
</tr>
<tr>
<td>Temperature</td>
<td>127.7</td>
<td>1</td>
<td>127.7</td>
<td>3.3</td>
<td>0.073</td>
</tr>
<tr>
<td>Species x CO2</td>
<td>101.4</td>
<td>2</td>
<td>50.7</td>
<td>1.3</td>
<td>0.277</td>
</tr>
<tr>
<td>Species x Temperature</td>
<td>113.2</td>
<td>2</td>
<td>56.6</td>
<td>1.4</td>
<td>0.238</td>
</tr>
<tr>
<td>CO2 x Temperature</td>
<td>188.1</td>
<td>1</td>
<td>188.1</td>
<td>4.8</td>
<td>(0.030)</td>
</tr>
<tr>
<td>Species x CO2 x Temperature</td>
<td>4.1</td>
<td>2</td>
<td>2.1</td>
<td>0.1</td>
<td>0.949</td>
</tr>
<tr>
<td>Error</td>
<td>8510.1</td>
<td>217</td>
<td>39.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** \(n=229\). A Tukey post-hoc test shows that \(L.\ utriculatum > L.\ dissectum > L.\ nudicaule\) with \(P<0.001\) for each pairwise comparison. Data were square-root transformed. Significant \(P\)-values of \(<0.05\) are shown in bold font.
Figure 5.2. Percent change in biomass (growth rate) from week 5 to week 8 of the experiment for each species in each treatment combination. Data were square-root transformed. Error bars are 95% confidence intervals. See also Table 5.1.
### TABLE 5.2

WATER CONTENT IN PLANTS ANALYZED WITH A THREE-FACTOR ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III SS</th>
<th>df</th>
<th>Mean Squares</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>52.8</td>
<td>2</td>
<td>26.4</td>
<td>71.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CO₂</td>
<td>6.0</td>
<td>1</td>
<td>6.0</td>
<td>16.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>28.4</td>
<td>1</td>
<td>28.4</td>
<td>76.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species x CO₂</td>
<td>2.4</td>
<td>2</td>
<td>1.2</td>
<td>3.2</td>
<td>0.041</td>
</tr>
<tr>
<td>Species x Temperature</td>
<td>1.5</td>
<td>2</td>
<td>0.8</td>
<td>2.1</td>
<td>0.128</td>
</tr>
<tr>
<td>CO₂ x Temperature</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.642</td>
</tr>
<tr>
<td>Species x CO₂ x Temperature</td>
<td>0.4</td>
<td>2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.561</td>
</tr>
<tr>
<td>Error</td>
<td>80.7</td>
<td>217</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** (n=229). Tukey post-hoc tests show that *L. utriculatum* > *L. nudicaule* > *L. dissectum*; elevated CO₂ > ambient CO₂; ambient temp > elevated temp; P<0.001 for all pairwise comparisons. Data were reflect inverse transformed. Significant P-values of <0.05 are shown in bold font.
Figure 5.3. Water content of harvested plants at week 8 of the experiment for each species at each treatment combination. Data were reflect inverse transformed. Error bars are 95% confidence intervals. See also Table 5.2.
significant interaction between species and CO$_2$ also was observed as *L. utriculatum* was not as responsive to elevated CO$_2$ as the other two species (Fig. 5.3).

5.4. Discussion

I investigated the seedling growth of three related and co-occurring plant species to determine if they differed in their response to elevated CO$_2$ and elevated temperature. Previous research suggests that plants do vary in their response to various factors of global change (see e.g., Morse & Bazzaz, 1994; Grünzweig & Körner, 2003) but no one has contrasted multiple, closely-related taxa to determine if species-specific reactions predominate in spite of similar life histories and shared phylogeny. Previous work examining populations within a species (Garbutt & Bazzaz, 1984; Wulff & Alexander, 1985; Bazzaz et al., 1995; Thomas et al., 1999; Ziska et al., 2005; Stinson et al., 2006), however, suggest that individualistic responses to global change are likely, even among relatives.

I found that *L. dissectum* and *L. utriculatum* grew larger under the elevated CO$_2$ and elevated temperature treatments. *Lomatium nudicaule*, in contrast, did not respond to either treatment. In week 8, there was a non-significant trend toward increased growth due to elevated temperature, but this trend was lost in measurements over the subsequent four weeks. This difference in response is qualitatively important and contrasts with some population-level findings that differed in the quantity but not the quality of the response (but see Wulff & Alexander 1985; Bazzaz et al., 1995; Thomas et al., 1999 for qualitative differences of certain characteristics). Thus, a key conclusion of this study is that co-occurring, congeneric species that share many life history traits can have species-
specific responses to elevated CO₂ and temperature. Using the rate at which biomass was accumulated to make direct statistical comparison among species (due to differential sizes), I found that the species accumulated biomass at different rates no matter the CO₂ or temperature treatment. The apparent discrepancy in response using biomass versus growth rate occurred either because germination happened earlier for *L. dissectum* and *L. utriculatum* in the elevated CO₂ and temperature treatments, or growth rates were only faster for the first four (unsampled) weeks of growth. Studying five epigeal species, Tischler et al. (2000) found biomass increases due to elevated CO₂ within as few as the first three days of growth from seed. In general, biomass increases in plants due to elevated CO₂ is a common and expected result (Bazzaz, 1990; Ainsworth & Long, 2005; but see Bazzaz & Garbutt, 1988). I did not find increased biomass with elevated CO₂ or temperature treatments for *L. nudicaule*, indicating that seedlings are not responsive to these factors, which I attribute to a lower growth rate than was found for its congeners.

Though the CO₂ x temperature interaction is not what is expected based upon theoretical prediction, it has been shown by other researchers who attributed the finding to differences in above and below ground biomass or the balance between whole-plant respiration and photosynthesis (Coleman & Bazzaz, 1992; Lilley et al., 2001; Hely & Roxburgh, 2005). I measured whole-plant biomass, however, and obtained a similar interaction. It would not be surprising that an increased temperature did not increase growth rates under elevated CO₂ if a high enough temperature to make CO₂ assimilation more efficient was not reached (Morison & Lawlor, 1999). Therefore, I conclude that the elevated temperature treatment was not sufficiently high to result in increased carbon fixing rates under elevated CO₂.
For *L. dissectum* and *L. nudicaule*, water content increased with elevated CO₂, and increased water-use efficiency is expected (see Drake et al., 1997; Wullschleger et al., 2002; Hely & Roxburgh, 2005; Housman et al., 2006). Increased temperature decreases water-use efficiency, but increased CO₂ can mitigate this effect (Qaderi et al., 2006). I found, however, that for *L. utriculatum* water content was not increased by elevated CO₂, even though it held more water than the other species. My expectation of highest water holding capacity by *L. nudicaule* was not supported, but because the seedlings were not water stressed, water retaining adaptations of the epidermis were likely not fully utilized.

This study has several limitations that restrict generalization to field conditions. To isolate the effects of increased temperature and increased atmospheric CO₂, other conditions were held constant such as watering regime and soil nutrients and at levels high enough to facilitate plant growth. Previous research has shown that the growth-enhancing effects of CO₂ are minimized by low soil fertility (Grünzweig & Körner, 2003; Maestre et al. 2007) and drought conditions (Housman et al., 2006). Evidence also suggests that nutrient or water limitation causes plants to allocate proportionally more photosynthate to their roots than they allocate to their shoots. This strategy could be beneficial for *Lomatium* spp. under climate change (Bazazz, 1990 and citations within) if summer drought intensifies or begins earlier as it is predicted to do (Chirstensen et al., 2007). In addition, species grown alone and those grown in competitive assemblages can show different responses to elevated CO₂ and temperature. For example, Bazzaz and Garbutt (1988) showed that competitive interactions sometimes eliminated growth enhancement from elevated CO₂. Alternatively, Wray and Strain (1987) showed that
growth responses to CO₂ were enhanced when species were grown in competition relative to being grown alone. Finally, this experiment does not measure the effect of extreme events, which are likely to increase under climatic change in a field setting. Nonetheless, the study design is suitable for the purposes of exploring basic species differences, my primary research goal.

I investigated seedling growth because this stage is vital to the recruitment of new individuals and for population establishment. Mature plants also often have energy stores to survive unfavorable conditions, but seedlings do not. Previous research suggests that reproductive phenology and output can be hastened and increased by elevated CO₂ and temperature, but this is not universal (Oechel and Strain, 1985; Garbutt et al., 1990; He et al., 2005; Springer & Ward, 2007). Longer-term studies over multiple years and life stages are needed in these species to reveal if such effects exist and differ among taxa.

Though I extrapolate with caution for the reasons listed above, the results suggest that there could be changes in the abundances of these species as the atmosphere and the climate change. I predict that L. nudicaule is unlikely to increase in the future, and if this species is affected by changes such as the intensification of summer drought, it could decline. In contrast, L. dissectum responded favorably to elevated CO₂ and temperature when water was not limiting, potentially indicating that its recruitment could increase in the future. This potentially is good news as L. dissectum currently is rare, particularly at the northern portion of its geographic distribution. Lomatium utriculatum, the most abundant of the three species, exhibited the fastest growth rates and was the most responsive to increased CO₂ and temperature. Thus, it also may have the potential to increase under climate change.
CHAPTER 6

CONCLUSION

Ecology often aims to glean general principles by determining responses from a few taxa. This research shows that a comparative approach allows for determining which outcomes may be generalizable and which may be species- or trait-specific. In this research I focused on plant species which share a northern range boundary in the Garry oak ecosystem in the Pacific Northwest of North America. Here I highlight key conclusions from my work and describe how it advances the field of global change biology.

All three *Lomatium* species are limited by dispersal within their current ranges, unable to reach isolated habitat patches beyond the species range (Chapter 2). This finding is important for two reasons: 1) it challenges the long-held paradigm that climate is the principal driver for the poleward edge of species’ distributions and 2) it shows that similar species, even those that vary greatly in their natural abundances may share range-limiting factors. This second point is important for making generalization to unstudied taxa. Other species associated with Garry oak habitats are likely limited in their current ranges, not by abiotic or biotic constraints, but by limited suitable habitat at their current range limit and inadequate dispersal to colonize those sites.
Even though the ranges of all three *Lomatium* species currently appear to be constrained by dispersal limitation, **the *Lomatium* genetic data suggest that abundance is important in determining the ability of long-distance seed dispersal, given species with similar dispersal mechanisms** (Chapter 3). In the three *Lomatium* species studied, haplotype richness, within-population diversity, and population differentiation follow the predicted pattern of more limited seed dispersal from less abundant species. The most abundant species, *L. utriculatum*, has the greatest haplotype richness of the three species studied, and it has lower population differentiation than *L. nudicaule*, indicating more common long-distance seed dispersal events. The rare species, *L. dissectum*, only exhibited a single haplotype making it uninformative for most comparisons except haplotype richness, for which it followed the expected pattern within the abundance gradient. In addition, in the field experiment (Chapter 2), *L. dissectum* displayed the poorest survivorship of the three species in accord with its low abundance. Therefore, the data support the hypothesis that patterns of migration within the range periphery of the study species are related to abundance. **An important trait-based generalization that comes out of this study is that species abundance is a demographic character that has large consequences for dispersal.** I conclude that species with low abundance will likely be limited in their abilities for future ranges relative to more abundant species because they will be producing fewer emigrants and may suffer poorer survivorship once dispersed. In other words, if plant populations are going to migrate due to current anthropogenic climate change, more abundant species may have greater success than rare taxa in shifting their geographic ranges.
Generalizations about range changes in oaks as a taxonomic group seem to be relatively universal, no matter the historical landscape conditions. The research on genetic diversity patterns in *Q. garryana* (Chapter 4) shows that even in a post-glacial range different from those elsewhere in eastern North America and Europe, oaks as a group display a pattern of restricted seed dispersal and widespread pollen flow. I examined the genetic structure of *Quercus garryana* Dougl. ex Hook. to infer post-glacial patterns of seed dispersal and pollen flow and found that the pattern of haplotype loss at the northern periphery is similar to that observed for other oak species that migrated great distances from glacial refugia. However, this was unexpected in this system as refugial oak populations remained nearby the current post-glacial range even during the last glacial maximum. The observed pattern suggests restricted seed dispersal events from mainland to peripheral islands. Using nuclear markers, I found high within-population diversity and population differentiation only over large spatial scales, suggesting that pollen flow is relatively high among populations. This result is also in accord with previous oak population genetic research. For *Q. garryana* at its northern range limit, regionally high pollen flow counteracts the effects of drift due to founder effects. As the current climate changes, a poleward geographic range shift may be possible for *Q. garryana* if seed dispersal limitation is overcome.

In contrast to broad generalization, this research also shows that closely related, co-occurring species have the potential to differ in their responses to their environment. In the field experiment (Chapter 2) the three *Lomatium* species differed in their levels of germination depending upon where they were planted and if vegetation was removed. These species-specific responses dampened upon re-emergence, but
Lomatium nudicaule still showed improved survivorship when vegetation was removed within the range, though the other two species did not. These results indicate that L. nudicaule may not be as strong a seedling competitor as its congeners. Increased temperatures and levels of atmospheric CO₂ from the growth chamber experiment (Chapter 5) resulted in qualitatively different responses from the species, in which L. nudicaule did not grow larger under the elevated treatments, though the other two species did. These differences in response cannot be attributed to abundance differences or any obvious life-history differences. Thus, they emphasize the difficulty in generalizing certain responses to unstudied taxa.

Limitations to poleward range expansion may play an important role in the future distribution of species. To overcome dispersal barriers, humans may need to accelerate the migration process through purposeful translocation outside a species’ range (McLachlan et al., 2007; Hoegh-Guldberg et al., 2008). The environmental conditions at the range limit may be already suitable or may become suitable more rapidly than native populations can track the changes at their northern limits. This research provides evidence that an assisted migration strategy may be viable for species that are dispersal limited near their geographic range boundaries. Specifically, seed additions outside the species’ range may be a strategy for overcoming dispersal limitation and assisting in the poleward migration of species. Seed additions alone, which overcome dispersal and recruitment limitation, may be a relatively inexpensive and effective management strategy for conserving species under climate change. Further, oak evidence suggests that long-distance pollen flow to the new populations is likely to restore regional genetic diversity. Protected areas outside the historical distribution of target species could be
used as reserves. Experimental populations would have to be monitored after seed additions to determine if growth and survivorship of the planted individuals results in a reproductive, self-sustaining population.

If we do not purposefully move dispersal-limited species of conservation interest to new areas, that space may become filled with species that disperse easily and have a high probability of establishment. Many invasive species fall into this group, for example (Hellmann et al., 2008). Seed additions work to allow establishment of otherwise competitive species that are dispersal and/or recruitment limited (Tilman, 1997). Seed additions of target species may be more relevant now than ever before because evidence is accumulating that biological invasions are aided by environmental change associated with human activities (Dukes & Mooney, 1999; MacDougall & Turkington, 2005; Hellmann et al., 2008). Therefore, active invasive species management along with purposeful additions of target species may improve “native” habitat under anthropogenic climate change.

Basic and applied research in the fields of ecology and biogeography is more important now than ever because of the threats to global biodiversity. Due in large part to current, rapid climate change, scientific interest in population dynamics at range margins is likely to continue to grow. More species will be studied for their survivorship and dispersal at their range margins, and increasingly sophisticated models will be developed to predict general patterns of biotic response to observed and anticipated changes. I look forward to a scientific career that contributes to this understanding so that conservation practices can be improved.
LITERATURE CITED


Karron, J.D. (1987) A comparison of levels of genetic polymorphism and self-
compatibility in geographically restricted and widespread plant congeners. *Evolutionary Ecology*, 1, 47-58.

Karron, J.D. (1997) Genetic consequences of different patterns of distribution and
abundance. *The Biology of Rarity: Causes and Consequences of Rare-Common

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release

Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and

herbivory on three non-native versus three native woody plants. *Forest Ecology
and Management*, 255, 92-98.

*Trends in Ecology and Evolution*, 16, 199-204.


postglacial gene flow among refugia. *Proceedings of the National Academy of
Sciences USA*, 99, 14590-14594.

Lilley, J.M., Bolger, T.P. & Gifford, R.M. (2001) Productivity of *Trifolium subteraneum*
and *Phalaris aquatica* under warmer, high CO₂ conditions. *New Phytologist*, 150,
371-383.


