GENETIC VARIATION, POPULATION STRUCTURE AND MATING SYSTEM IN
BIGLEAF MAPLE (*ACER MACROPHYLLUM* PURSH)

by

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Ing. For. University of Pinar del Rio, 1993.

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA

May, 2005

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ABSTRACT

Ecological characteristics and life history traits of long lived woody plants influence their levels of genetic variation. To embark upon sound management, utilization and conservation of plant species, a thorough understanding of genetic processes affecting their persistence is essential. In this thesis, I studied genetic diversity, population structure, and mating system as well as compared genetic diversity and inferred differences in genetic processes in continuous versus fragmented populations of bigleaf maple (*Acer macrophyllum* Pursh). Bigleaf maple is one of the most abundant hardwood species in the Pacific Northwest and its native range extends from latitude 33° N to 51° N along the Pacific coast of North America.

Genetic diversity, estimated using isozyme markers, revealed a mean expected heterozygosity (H_E) of 0.152 similar to other North American angiosperm trees. The level of population differentiation was moderately low ($F_{ST} = 0.054$), indicating extensive gene flow among populations. Estimated outcrossing rates in two populations were high (95%) but significantly less than one, with no biparental inbreeding evident. A relatively high level of correlated matings was found, consistent with 2-5 effective pollen donors per tree, indicating low adult density and limited pollinator dispersal.

Seedling and adult populations possess similar levels of genetic variation regardless of whether populations are fragmented or continuous. However, seedling cohorts have higher levels of inbreeding than adult cohorts, on average, in both continuous and fragmented populations. Analysis of spatial genetic structure indicates non-random distribution of genotypes in all three fragmented populations and one of the three continuous populations. I found a significant positive autocorrelation ($\rho_{ij} = 0.20$) among individuals located up to 100 m apart in all three

fragmented populations and among individuals located at approximately 100-200 m apart ($p_{ij} = 0.14$) in one of three continuous populations.

Finally, for quantitative traits, provenances and families within provenances showed significant genetic variation for height growth and bud flush traits, but not for diameter growth. Individual heritabilities for all traits were generally low to moderate (0.15-0.21), and family heritability was higher only for bud flush. Comparison of Q_{ST} and F_{ST} in this study (mean $Q_{ST} = 0.17 > \text{mean } F_{ST} = 0.09$) suggests the involvement of selection for different phenotypes in different populations of bigleaf maple.

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ACKNOWLEDGEMENTS

I would first like to acknowledge with deep appreciation the Department of Foreign Affairs and International Trade for the Award of Canadian Commonwealth Scholarship through the International Council for Canadian Studies. Funding for research was made available initially from the BC Ministry of Forests, Research Branch through Dr. Cheng Ying and complimented by a Natural Sciences and Engineering Research Council (NSERC) grant to Dr. Kermit Ritland. This study would not have been completed without the much needed fellowship and additional funding for research provided to me by my co-supervisor Dr. Sally Aitken through the Centre for Forest Gene Conservation via the Forest Genetics Council of BC from the Forest investment Account of BC and the NSERC Industry Junior Chair in Population Genetics.

I would like to thank my co-supervisors Drs Sally Aitken and Kermit Ritland and committee member Dr. Jeannette Whitton for their guidance, support and constructive comments. Special thanks go to Dr. Sally Aitken who spent an extra time on my draft, challenging me to write concisely and encouraging me to think critically and realistically. My sincere thanks also go to Dr. Carol Ritland for her initial involvement in my committee, for providing fresh perspective on my research during the initial stages, planning my field trips and supervising my lab work. To Dr. Cheng Ying, thanks very much for serving on my committee, for the guidance and numerous fruitful comments and suggestions on my research, for your friendship and fatherly advice and for providing the quantitative data and encouraging me to work on bigleaf maple. To Mr. Don Pigott for sharing your expertise with me and the great help in collection of samples in the field.

I wish to also thank the following:

- My colleagues, Cherdsak Liewlaksaneeyanawin, Charles Chen, Yanik Berube and Hugh Wellman.
- Drs. Tongli Wang, Andreas Hamann for their help and advice and Pia Smets for being so opened to discussing issues beyond academics.
- My wife **Yanela** and children **Leandro** and **Neina** for their enormous sacrifices and support throughout the duration of my program.
- To my mother, brothers and sister and my extended family members for their encouragement and advice.

DEDICATION

To the memory of my late father- Mba Iddi (Gushei-Naa)

"After great pain a formal feeling comes." Emily Dickinson

Chapter One

GENERAL INTRODUCTION

Forests are declining in most regions world-wide, and this has caused grave concern among scientists and policy makers throughout our world. Much of the world's biodiversity is harbored in forests, particularly tropical forests which are estimated to contain up to 70 percent of the world's species (Groom 1994). However, over the last two centuries, the exponential growth of human populations coupled with growth of cities, industrialization and agriculture has led to widespread destruction and degradation of many forested and other natural systems (FAO 1997). Approximately half of the world's forest area has been cleared or degraded since the beginning of the Holocene (Groombridge and Jenkins 2002). Currently, about 30 percent of the world's land area is covered with forest (FAO 2001). Many of these forests are partially converted to agricultural or urban use, resulting in the loss of some unique characteristics that were previously present.

The main goal of population genetics is to understand the origin, distribution and maintenance of genetic diversity, which is the raw material for evolutionary change (Ledig 1992; Hartl and Clark 1997). In small populations in particular, genes undergo genetic drift and as a consequence genetic diversity is randomly and continuously lost (Vitalis and Couvet 2001). Moreover, genetic drift in small populations can be more important than selection in determining the fate of new alleles (Whitlock 2000). In subdivided populations, the maintenance of neutral alleles depends on the relative strength of local genetic drift and the extent of gene flow as a homogenizing force (Slatkin 1995).

Changes in genetic structure and levels of diversity in subdivided or small populations of forest tree species will depend on several factors, such as the magnitude and frequency of forest destruction and degree of isolation among fragmented forests (Bawa 1994). Forest management practices also affect genetic

diversity (Savolainen and Karrkainen 1992; El-Kassaby and Namkoong 1994). Harvesting can lead to a reduction in stand density, which may result in increased levels of inbreeding and a decline in genetic diversity (Murawski and Hamrick 1992; Buchert et al. 1997). Knowledge of mating systems, the levels and distribution of genetic variation, and factors influencing its maintenance is necessary for effective forest management and conservation programs (Eriksson et al. 1995).

In recent years, there has been an accumulation of data concerning the patterns of genetic variation in many North American coniferous tree species. However, relatively little information is available about genetic variation in temperate woody angiosperms. This situation is particularly true for intolerant, early successional and shrubby trees, the majority of which are non-commercial and suffer some degree of habitat fragmentation.

To improve our understanding of the genetic structure of forest trees, it is necessary to broaden the scope of study to include angiosperms with different mating systems, pollination vectors, patterns of dispersal, and evolutionary histories. Life history and ecological factors that would promote genetic diversity of woody early successional species, like *Acer macrophyllum* (bigleaf maple), are likely to be similar to those of relatively long-lived species.

Thesis overview

In order to embark on any useful conservation program for any species, knowledge of how genetic variation is partitioned among and within populations is the first necessary step. The main goals of chapter two are: 1) to review the literature on the biology of *Acer macrophyllum*; 2) to review the basic ideas that shape our thinking about genetic diversity and mating patterns in small and subdivided populations; 3) to review the role of evolutionary forces in explaining genetic differentiation for neutral genetic markers and quantitative traits; and 4) to address genetic consequences of forest fragmentation and spatial genetic structure in natural populations. In chapter three I use isozyme markers to

investigate the genetic variation, population structure and mating system in bigleaf maple. This is an important basic step to understanding the population genetics of this species. I hypothesized that fragmentation may lead to erosion of genetic variation. In chapter four this is tested by comparing seedling and mature cohorts in fragmented and non-fragmented populations. I also examine the extent of spatial structuring within stands of bigleaf maple trees and examine how structuring is affected by fragmentation. In chapter five I study the quantitative variation in height, diameter and bud flush traits and compare quantitative genetic differentiation among populations and genetic differentiation at neutral loci. I then conclude by summarizing the major findings in chapter six, providing specific recommendations for management and suggesting areas for future research that will enhance our knowledge for management and conservation of bigleaf maple genetic resources for present and future generations.

Chapter two

LITERATURE REVIEW

BIOLOGY AND SILVICS OF BIGLEAF MAPLE (*Acer macrophyllum* Pursh)

The maple family, Aceraceae, includes two genera, *Dipteronia* and *Acer*. *Dipteronia* contains only two species of small trees both native to central China. *Acer* contains about 148 species of small trees and shrubs that are widely scattered throughout the Northern Hemisphere but are most abundant in the eastern Himalayan Mountains and in central China (Peterson et. al 1999). Thirteen species of maples are indigenous to the North America, ten of which are native to Canada (Farrar 1995). Three of Canada's ten maple species are native to British Columbia: *Acer macrophyllum* Pursh (bigleaf maple); *Acer glabrum* Torr.var. *dauglasii* (Hook.) Dipple (Douglas or Rocky Mountain maple); and *Acer circinatum* Pursh (vine maple).

While substantial information is available on the silvics, management and genetics of maple species in eastern North America, the extent to which this information is applicable to bigleaf maple is unknown (Peterson et al. 1999). Some plant biogeographers initially suggested that because of the isolating effect of Pleistocene continental ice sheets on plant distributions (Ritchie 1987), bigleaf male is actually more closely related to some of the Asian and European maples than to the maples in eastern North America based on taxonomic features (Elias 1980). This conclusion was further supported by molecular phylogenetic studies conducted on *Acer* by Ackerley and Donoghue (1998).

The native range of bigleaf maple extends from latitude 33° N to 51° N., mostly within 300 km of the Pacific Coast (Fig 1.1) and it is the Pacific Northwest's second most abundant species of hardwood after red alder (Niemic et al. 1995). Bigleaf maple grows over a wide range of temperature and moisture conditions, from the cool, moist maritime climate of Coastal British Columbia to the warm, dry climate of

southern California. It often occurs on coarse gravel soil in mixed stands with *Alnus rubra* (red alder), *Populus trichocarpa* (black cottonwood), *Thuja plicata* (western red cedar), *Pseudotsuga menziesii* (Douglas-fir) and *Tsuga heterophylla* (western hemlock) (Farrar 1995).

Bigleaf maple is able to produce flowers and seeds as early as 10 years after germinating from seed on open and high productivity sites. Seed crops are produced every year, and can be prodigious, especially in open-grown trees. Flowers of bigleaf maple are relatively small but insect-pollinated. It is polygamous and both staminate and perfect flowers are mixed in the same dense cylindrical racemes (Minore and Zasada 1990). The fruit is a double samara with slightly divergent wings and a hairy seed case. The flowering period is usually from early April to May. Fruit ripens by September or October and seed dispersal occurs from October through January (Ruth and Muerle 1958). Seed dispersal is primarily by wind and gravity but dispersal by some small mammals (mice, wood rats, and squirrels) and birds has been reported (Fowels 1965). Seeds are not dormant and germinate soon after dispersal.

Bigleaf maple is moderately shade tolerant and an excellent shade tree. Its wood is known to have good properties for use as furniture but it is neither as strong nor as hard as sugar maple (Kerbes 1968). The wood of bigleaf maple is very popular in the piano industry, where it is the most preferred species for piano frames. It also has several industrial and domestic uses such as decorative face veneer, container materials, moulding, hardwood flooring, kitchen utensils, pallets, turnery and hardwood plywood, as well as for firewood (Kerbes 1968; Niemiec et al. 1995).

GENETIC VARIATION AND STRUCTURE IN NATURAL POPULATIONS

Ecological characteristics and life history of plant species influence levels of genetic variation. Important species characteristics associated with levels of variation include taxonomic status, regional distribution, geographic range, life form, mode of reproduction, seed dispersal mechanism, and successional status (Hamrick and Godt

1989). Plant breeding systems are a primary determinant of genetic structure in plant populations, because they alter the probability of random mating among individuals within a population. Outcrossing mechanisms reduce the rate of inbreeding and therefore maintain genetic variability within populations (Richards 1986). Selfing tends to decrease genetic variation within populations and promote genetic differentiation among populations (Barrett and Kohn 1991). Inbreeding can occur through either selfing or consanguineous matings (Ritland 1985). Most trees are predominantly outcrossing but their reliance on either wind pollination or a wide variety of biotic pollination agents generates considerable variation in outcrossing rates and mating patterns among individuals (Aizen and Feisinger 1994; Hamrick et al. 1991).

Gene flow occurs through seed and pollen dispersal, and decreases the level of genetic differentiation among populations (Hamrick and Godt 1989). Seed dispersal has a greater homogenizing effect than pollen dispersal because seed transmits both maternal and paternal genes whereas pollen transmits only paternally inherited genes (Nason and Hamrick 1997). Within a given geographical region, seemingly large, contiguous populations often consist of subpopulations that are linked along temporal and spatial scales. This network of populations is defined as meta-population (Hanski 1997). Non-random association of genotypes within meta-populations creates further structuring at relatively small scales (Muona et al. 1991).

Effects of population size on genetic variation

Small populations undergo processes predicted by genetic drift theory and population structure models (Tempelton et al. 1991). Kimura and Crow (1964) demonstrated that for a diploid population the expected heterozygosity (H_e) at equilibrium is a direct function of mutation and effective population size:

$$H_e = \frac{4N_e\mu}{1 + 4N_e\mu}$$

where N_e is the effective population size and μ is the mutation rate. Thus the larger the population, the higher the heterozygosity that can be maintained, all else being equal. For example, *Drosophila* populations and mammal species exhibit heterozygosities of 12% and 5-6%, respectively for allozyme loci with corresponding value of $N_e \mu$ of 0.035 and 0.015 (Ohta 1992). A meta-analysis of genetic diversity in common and rare plants in the same genus concludes, however, that historically large populations that have recently become fragmented may still harbor significant genetic diversity despite current small population size. However, in most cases the predicted correlation between genetic diversity and population size holds (Gitzendanner and Soltis 2000).

The complexity of random effects of genetic drift on allele frequencies in finite populations is summarized by Kimura (1955) and Wright (1951) in which after one generation of random mating, a population with initial heterozygosity (H_0) would be expected to decrease by a proportion of $\frac{1}{2N_e}$ on average such that in generation t the expected value of expected heterozygosity (Hartl and Clark 1989) is:

$$H_e = \left(1 - \frac{1}{2N_e}\right)^t H_0$$

Decline in population size due to deforestation or fragmentation in already subdivided populations further increases the probability of loss of alleles and enhances the decline in heterozygosity due to genetic drift. However, such effects do not derive only from direct reduction in effective population size, because the magnitude of genetic drift can be predicted as a simple function of census population size only when the population characteristics meet the assumptions of the Fisher-

Wright drift model (Caballero 1994). Usually this is not the case (Hartl and Clark 1989) and N_e tends to be considerably smaller than the census population size N (Frankham 1995). Several factors are responsible, including unbalanced sex ratio, unequal fecundity among individuals and population size fluctuations (Falconer 1989; Futuyma 1986; Hartl and Clark 1989; Yeh 2000). Aldrich and Hamrick (1998) found that reproduction of the tree *Symphonia globulifera* in a 38.5 ha circular plot was dominated by numerous small groups of remnant pasture land trees which experienced a post-fragmentation increase in fecundity leading to a secondary constriction of the fragmentation bottleneck. Similarly in New Zealand mistletoe (*Perexia tetrapetala*) pollination and seed set was more than four-fold higher in isolated than continuous forest (Kelly et al. 2000).

Effects of population size on mating systems

The mating system of plant species is an important biological characteristic because it is a key determinant of genetic variation, genetic structure and evolutionary potential of plant populations (Clegg 1980; Brown 1990). Modes of pollination, population size and density, and plant and floral architecture are all likely to influence mating systems (Clegg 1980). Plant mating systems are characterized by 1) proportion of outcrossing versus selfing; 2) consanguineous matings, and 3) the level of correlated paternity, defined as the proportion of full-sib pairs among outcrossed maternal progenies (Ritland 1989a).

Reductions in population size and increases in the degree of isolation and fragmentation of populations can lead to increases in inbreeding (e.g. Farris and Mitton 1984; Murawski et al. 1994; Rajimann et al. 1994). These effects may be of particular significance in woody angiosperms because population sizes and densities can be significantly reduced as a consequence of forest harvesting practices and other land use practices. In small populations, elevated levels of inbreeding are expected (Barrett and Kohn 1991). Under these conditions, selection can purge early-

acting lethal and semi-lethal recessive alleles from populations as they become exposed as homozygotes (Lande and Schemske 1985; Charlesworth and Charlesworth 1987). However, mutations of mild deleterious effects may become fixed in a process called mutational meltdown (Lynch 1985).

The level of correlated paternity defines the probability that a seed tree draws two male gametes from the same pollen donor. This can be regarded as the inverse of effective pollination neighbourhood size, analogous to Wright's neighbourhood size, when considering only the dispersal variance of male gametes (Austerlitz and Smouse 2001). The level of correlated paternity, together with selfing rate, will determine the degree of departure from random mating and the significance of genetic drift under the isolation-by-distance model (Ritland 1989a). Correlated mating may also influence patterns of selection and competition among siblings (Karron and Marshall 1990). Among the major factors that may enhance correlated paternity in wind-pollinated plants are pollen limitation (Surles et al. 1990), spatially restricted pollen dispersal (Smouse and Sork 2004), asynchronous floral phenology, unequal male fecundity (Ericksson and Adams 1989; Burczyk and Prat 1997), and low conspecific density (Smouse and Sork 2004).

Reductions in population size may directly affect the mating system for three reasons. First, in small populations the number of local compatible mates is reduced, which even under random mating will increase the likelihood of correlated mating and self-fertilization (Surles et al. 1990). Second, total pollen availability will decrease in small plant populations, which may result in reduced seed set or increased selfing (Larson and Barrett 2000). The impact of pollen limitation in wind pollinated species, however, remains unclear (Koenig and Ashley 2003). Third, as a consequence of the typically leptokurtic shape of the pollen dispersal curve in plants (Levin and Kerster 1974), pollen pool diversity around individual trees in small populations may be reduced by the absence of a broad spectrum of long-distance pollen donors (Adams 1992; Ellstrand 1992). Evidence from experiments on conifer species suggests that both the quantity and diversity of available pollen in small stands may be significantly

lower than in large populations (Sarvas 1962; Koski 1970, 1973). Little is known, however, about the precise consequences of this potential pollen pool impoverishment for the mating system of particular species.

Most studies dealing with the consequences of small population size on plant mating systems have focused on the outcrossing rate and reproductive output of insect pollinated species, in which the interaction between the spatial structure of populations and the pollen foraging behaviour of pollinators poses an additional challenge (Levin and Kerster 1974; van Treuren et al. 1993; Hauser and Loeschcke 1994; Kennington and James 1997; Routley et al. 1999). Although no significant effects of small population size were detected in some of these studies, a general trend towards increased selfing and reduced seed set has been observed as population sizes decrease.

EFFECTS OF FRAGMENTATION ON GENETIC VARIATION IN PLANT POPULATIONS

Habitat disturbances causing forest fragmentation can impact the genetic structure of species. Fragmentation can disrupt the pre-existing genetic structure of populations, alter genetic processes and result in a net loss and redistribution of genetic diversity. Differentiation among populations will tend to increase as once contiguous populations become subdivided into small, isolated fragments (Barrett and Kohn 1991). The first genetic consequence of a reduction in population size is the loss of rare alleles, and over time a concomitant decrease in heterozygosity will occur through genetic drift (Barrett and Kohn 1991).

Additionally, fragmentation can impact plant-pollinator and plant-seed disperser interactions (Aizen and Feisinger 1994). A shift in landscape pattern is expected to result in altered animal foraging behaviour (Dirzo and Miranda 1991). Generally, alterations of pollinator behaviour will tend to limit pollen dispersal, increase the level

of inbreeding within populations, reduce the rate of inter-population pollen dispersal and therefore increase among-population differentiation (Bawa 1990). Similarly, a change in foraging behaviour of seed dispersers could reduce seed flow among existing populations, and also decrease colonizing events, thus reducing the establishment of new populations (Aizen and Feisinger 1994).

Recently, two broad approaches for detecting effects of forest fragmentation on genetic variation have been used: (i) comparison of fragmented and unfragmented (continuous) populations, and (ii) analysis of relationships between measures of genetic diversity and indices of fragmentation (e.g. population size, isolation, or different age cohorts). Studies of these sorts have produced diverse results. In several cases, important genetic effects have been detected. Fragmentation has been associated with a decline in allelic richness in a number of cases. For example, in 17 fragmented populations of the perennial *Swainsona recta*, Buza et al. (2000) reported a significant reduction in the presence of rare alleles in small populations. Similar relationships were reported by van Treuren et al. (1991) for *Salvia pratensis* and *Scabiosa columbaria* and Prober and Brown (1994) for *Eucalyptus albens*. Young et al. (1999) found reduced allelic diversity in small populations of *Rutidosia leptorrhynchoides*, a perennial and self-incompatible species. Further, they argued that the association of decreased genetic diversity with low seed production was a consequence of parallel reductions in the number of alleles present at loci controlling self-incompatibility (SI). Erosion of allelic richness at SI loci in small populations has also been found in the rare lakeside daisy *Hymenoxys acaulis* (DeMauro 1993).

Gene flow between fragments might restore lost alleles very quickly but only when lost alleles are still present in the post-fragmentation metapopulation as a whole. For, instance, in a study of *Acer macrophyllum* (sugar maple) in Canada, Young et al. (1993) compared genetic variation in eight patchy populations with variation in another eight continuous control populations. They assumed that genetic variation in the large control populations represented variation in the pre-fragmentation population, and that the present patchy populations were derived from once continuous populations. A

comparison of genetic diversity parameters between fragmented and control populations found genetic diversity (as measured by percentage polymorphic loci, allelic diversity and heterozygosity) was not significantly lower in the fragmented populations, nor was there any increase in inbreeding. However, the total number of alleles was six fewer in the fragmented populations, which was attributed to possible founder effects.

As outlined above, not all measures of genetic diversity are expected to be sensitive to founder effects. However, declines in values of expected heterozygosity and allelic richness have been reported. For example, Prober and Brown (1994) demonstrated that small populations (< 500 reproductive individuals) of *Eucalyptus albens* that were less than 250 m from a larger population had a higher allelic richness than more isolated small populations. These results are crucial as they point out a threshold up to which gene flow from a large population can maintain genetic diversity, but beyond which genetic diversity can decline. Similarly, a significant reduction in genetic diversity and increased genetic differentiation was documented in fragmented relative to continuous populations of the tropical tree *Pithecellobium elegans* (Hall et al. 1996). A relatively high correlation between population size and genetic diversity was also reported by Raijmann et al. (1994) for *Gentiana pneumonanthe*. However in a number of other studies on relatively recently fragmented populations, there were no clear relationships between genetic diversity and population size (van Treuren et al. 1991; Foré et al. 1992; Young et al. 1993, 2000; Buza et al. 2000).

Evidence for more rapid genetic erosion in small isolated populations than in less isolated populations was reported by Dayanandan et al. (1999). They found that genetic distance between adult and seedling cohorts in fragmented populations of *Carapa guianensis* in Costa Rica was greatest in the most isolated population, which was also the only one in which allelic diversity was lower in the adult cohorts. The results from these studies seem to suggest that species with similar life history characteristics such as those mentioned above may be particularly vulnerable to the

consequences of fragmentation since they typically exist at low densities and are predominantly outcrossed (Hamrick and Godt 1989; O'Malley and Bawa 1987).

Effects of fragmentation on spatial genetic structure

Spatial genetic structure is the non-random distribution of genetic variation among sexually reproducing individuals (McCauley 1997). The spatial distribution of genotypes within plant populations is influenced by many ecological and evolutionary processes such as limited seed and pollen dispersal (Wright, 1943; Schoen and Latta, 1989; Bacilieri et al. 1994), adult density (Knowles et al. 1992; Hamrick et al. 1993; Hamrick and Nason, 1996; Vekemans and Hardy 2004), colonization and disturbance history (Epperson and Chung, 2001; Parker et al. 2001), spatial and temporal patterns of seedling establishment (Ellstrand, 1992; Schnabel and Hamrick, 1995; Parker et al. 2001), differential selection and micro-environmental selection (Linhart et al. 1981; Slatkin and Arter, 1991) and forest fragmentation (Doligez and Joly 1997). Of these factors, probably the most widely studied influence on spatial genetic structure is pattern of gene dispersal (Hamrick et al. 1993; Ennos 1994; Hamrick and Nason 1996).

Kalisz et al. (2001) described general scenarios of seed and pollen dispersal under which genetic structure could develop: (i) If at the scale of investigation, seed dispersal is localized while pollen disperses long distances or randomly, spatial clustering of full and half-sibs will result in the development of significant spatial structure in the absence of inbreeding (e.g., Peakall and Beattie, 1996; Kalisz et al. 2001). (ii) If pollen dispersal is also restricted, this will result in inbreeding thereby reinforcing the buildup of more intense genetic structure (Wright 1943; Barbujani 1987). (iii) In contrast, if seeds are widely and independently dispersed then regardless of whether pollen disperses long or short distances, seed dispersal will effectively randomize the spatial distribution of genetic variation within populations (e.g. Dewey and Heywood 1988; Loiselle et al. 1995).

In most studies of spatial genetic structure of tree species with wind dispersed seeds, whether animal or wind pollinated, many authors have reported either weak or no spatial genetic structure (e.g., *Acer saccharum* (Perry and Knowles 1991; Young et al. 1993), *Quercus* spp. (Streiff et al. 1998), *Psychotria officinalis* (Loiselle et al. 1995); *Carapa procera* (Doligez and Joly 1996); *Pinus* spp (Parker et al. 2001; Epperson et al. 2003); *Vitellaria paradoxa* (Kelly et al. 2004)). Their results explained spatial genetic structure by overlapping seed shadows and extensive gene flow via pollen. Woody insect-pollinated species with seeds widely dispersed by birds also show weak genetic structure (Dewey and Heywood 1988, Chung et al. 2000). A lack of spatial structure was found for other species by Sokal and Oden (1978), Doligez and Joly (1997), and Chung et al. (2000). They attributed their results to extensive gene flow, wide seed dispersal, self incompatibility and dispersal agents.

In conclusion, fragmentation effects on population genetics of forest tree populations are complex and difficult to predict. Theoretical considerations in particular are perhaps more useful in understanding empirical results rather than predicting them (Young et al. 1996). However it is worth noting that both theoretical and empirical studies suggest that fragmentation can exert some effects on genetics of fragmented populations.

MOLECULAR AND QUANTITATIVE VARIATION

Forest trees are long-lived, sessile organisms that are exposed to large temporal fluctuations in their environmental conditions. Consequently, the demands placed on the adaptability of trees are extremely high compared to other organisms. To fulfill these demands, forest tree species need to maintain large amounts of genetic variation for the preservation of adaptability and survival to subsequent generations (Muller-Starck and Gregorius 1985). On the other hand, the existence of populations of healthy plants with little or no detectable genetic variation shows that long-term

survival is possible. Without variability, however, such species will be unable to adapt to new environmental conditions. Acquisition of sufficient information on the extent and pattern of genetic diversity, population differentiation across species ranges, and the ecological and genetic relationship among individuals and among populations, are essential for establishing guidelines on conservation and utilization of genetic resources (Eriksson et al. 1995). The need to understand genetic structure stems from the necessity to answer the essential question of whether one population or many different populations will be an effective collection of all the important alleles for a particular species (Bradshaw and Stettler 1995). The answer to this question is critical for the efficient management of natural forests or for any effort to restore deforested habitats by reintroduction (Namkoong 1988).

Hereditary basis of differentiation in morphology and development have been demonstrated in studies of intraspecific variation in quantitative traits beginning over two centuries ago (see review by Langlet 1971). However, because patterns of geographic variation within species are influenced by different selective pressures, barriers to gene flow and genetic drift, the maintenance of genetic variation in natural populations thus become very complex (Grant and Linhart 1996).

The importance of genetic drift and gene flow as evolutionary forces in natural populations has been thoroughly addressed in studies using molecular markers. However, using such markers to determine genetic structure has several limitations in providing information that could be useful to define conservation strategies (Lynch 1996). Despite these limitations, molecular markers have been proposed as an indirect indicator of quantitative genetic variation available for adaptation (Petit et al. 1998). However Waldmann and Anderson (1998) indicated three major shortcomings of this approach:

- (i) The higher mutation rates of quantitative trait characters suggests that the recovery times after a bottleneck will be shorter for polygenic variation than for single locus polymorphism.

- (ii) When non-additive variance is high, the expected loss of additive variance caused by genetic drift follows a different pattern than the reduction in single-locus heterozygosity.
- (iii) The effect of small population size on genetic variation is expected to differ for monogenic and polygenic characters owing to selection having different effects on these types of characters.

In view of these differences, planning conservation efforts based exclusively on marker gene loci may be misleading. For this reason quantitative genetic analysis is an important complement in studies of species (Lynch 1996; Storfer 1996).

Comparisons of divergence in neutral genetic markers (as measured by F_{ST}) and polygenically-controlled quantitative traits (as measured by Q_{ST} ; Wright 1951) allow for an assessment of the relative importance of natural selection and genetic drift as a cause of population differentiation in quantitative traits (Spitze 1993; Prout and Barker 1993; Long and Singh 1995; Podolsky and Hartsford 1995; Bonin et al. 1996; Yang et al. 1996; Waldmann and Anderson 1998; Lynch et al. 1999; González-Martínez et al. 2002; Merilä and Crnokrak 2001; McKay and Latta 2002; Howe et al. 2003). Higher divergence in quantitative traits than in neutral markers ($Q_{ST} > F_{ST}$) is indicative of directional selection favouring different genotypes in different populations, whereas the opposite ($Q_{ST} < F_{ST}$) suggests that the same genotypes are favoured in different populations, i.e. stabilizing selection. However, if the two measures do not differ significantly ($Q_{ST} = F_{ST}$), then patterns of variation for both neutral markers and quantitative traits are both assumed to reflect only the actions of genetic drift and gene flow (Merilä and Crnokrak 2001).

Comparative studies of quantitative trait and neutral marker divergence are relevant from a conservation genetics perspective as management decisions often rest on population genetic analyses conducted with neutral molecular markers (e.g. Moritz 1994; Haig 1998; Reed and Frankham 2001). For instance, operational definitions of evolutionarily significant units (ESUs) are based on divergence in neutral or nearly neutral markers (Moritz 1994; Moritz et al. 1995). However, quantitative

characters are more likely to be related to fitness and therefore to population persistence (Lynch 1996; Storfer 1996). Nevertheless, the question of whether the levels of variation and the degree of population differentiation are correlated between neutral genetic markers and genetic variation in quantitative traits remains controversial (Pfender et al. 2000; Merilä and Crnokrak 2001; Reed and Frankham 2001; Latta and McKay 2002). In a meta-analysis of 18 studies reporting Q_{ST} and F_{ST} values for 20 species, Merilä and Crnokrak (2001) did indeed find a positive correlation between the two divergence indices across different studies (see reviews in Crnokrak and Merilä 2002; Latta and McKay 2002; McKay and Latta 2002; Howe et al. 2003). However, studies comparing the predictive power of neutral markers as indicators of divergence in quantitative traits among populations within species are still lacking (but see Steinger et al. 2002), as are studies examining the sensitivity of Q_{ST} estimates to genotype-environment interactions.

Figure 2.1. Native range of *Acer macrophyllum* (Bigleaf maple).

Chapter three

GENETIC VARIATION, POPULATION STRUCTURE AND MATING SYSTEM IN BIGLEAF MAPLE (*Acer macrophyllum* Pursh)¹

INTRODUCTION

Studies of the genetic variation and population structure of woody angiosperms with molecular genetic markers have shown that they possess relatively high levels of genetic variation within populations, but little among population differentiation (Hamrick et al. 1992; Loveless 1992). Genetic variation enables species to survive and adapt to changing environments, therefore, information on genetic variation of forest tree species is fundamental to management and conservation (Eriksson et al. 1995). The most important determinants of genetic variation are natural selection, mutation, genetic drift, migration and the mating system (Hartl and Clark 1997). However, human activities such as deforestation, air pollution and forest fragmentation can modify the direction and amplitude of these evolutionary forces and alter genetic variation of natural forest resources (Lande 1988). Measurement and characterization of this variation, particularly in relation to human activities, are important first steps towards developing strategies to preserve the genetic variation of native forest tree species (Hamrick et al. 1991).

Bigleaf maple (*Acer macrophyllum* Pursh) occurs along the Pacific coast of North America, in populations of scattered individuals or as small groves, in association with both conifers and broad-leaved trees. Its flowers are polygamous and both staminate and perfect flowers are mixed in the same dense cylindrical, racemes (Minore and Zasada 1990). Pollination is primarily by insects (Minore and Zasada

¹ A version of this chapter has been published. **Mohammed N. Iddrisu and Kermit Ritland**. Genetic variation, population structure and mating system in bigleaf maple (*Acer macrophyllum* Pursh). Can. J. Bot. **82**: 1817–1825 (2004).

1990). It is an early successional species with consistent seed production. The seeds are double samaras with slightly divergent wings and can be disseminated by wind for long distances. Its scattered distribution over the coastal Pacific Northwest and southwestern British Columbia in rural areas makes it a prime woodlot species (Minore and Zasada 1990). In the past, bigleaf maple populations have suffered much habitat disturbance due to agricultural practices, and the marketing of bigleaf maple wood products may in the long run lead to an accelerated loss of its genetic resources. This could decrease the opportunities for the genetic improvement and conservation of bigleaf maple. However, there has been little study of the genetic structure of bigleaf maple, and this is needed to develop a management strategy designed to maintain stable, productive and sustainable forest populations of this species.

The objectives of this study were to: (1) determine the amount and distribution of genetic variation among bigleaf maple (*Acer macrophyllum*) populations, (2) estimate the mating system in two populations from the Lower Mainland of British Columbia, and (3) recommend a strategy for the management and conservation of bigleaf maple genetic resources. I hypothesize that bigleaf maple is predominantly an outcrossing species with extensive gene flow, and therefore, has much genetic variation within populations but little population differentiation.

MATERIALS AND METHODS

Seeds from eight populations representing the range-wide distribution of *Acer macrophyllum* were collected. Population locations are given in Fig. 3.1. Seeds were collected from 36 adult trees in Jericho and 40 trees in Fraser populations respectively. In each of these populations, seed progenies (progeny arrays) with about 30 seeds per mother tree were collected and used for estimating outcrossing rates. For the rest of the populations sampled in the southern portion of the species range,

seeds were collected from 20 adult trees in each population with the exception of Artic where only 14 adult trees were sampled. The Artic population was at the western edge of the species distribution in Oregon and no additional trees could be found after travelling 3 km westward towards the coast. Individual sampled trees were spaced approximately 30 meters apart as minimum. As much as possible, seeds were collected before the first rainfall. If they were damp from fog and dew or collected after the first rains, they were dried indoors at room temperature until seeds and paper sacks felt dry, then stored in large plastic garbage bags with holes bored for ventilation at 2-4 °C until germination.

Isozyme assay

Seeds were de-winged and germinated in Petri dishes on filter papers moistened with distilled water, then kept in a 4°C refrigerator for 5-8 d before seed dissection and enzyme extraction. Individual cotyledons with emerging radicles were excised and placed in separate wells in microtiter plates for enzyme extraction. The freshly excised material was ground in 2-3 drops of extraction buffer: 0.283g germanium dioxide, 25mL water, 0.0917g diethyldithiocarbonic acid, 0.1g sodium bisulfate, (0.16M) 2.67mL phosphate buffer at pH 7, 2.67mL DMSO, 17mL 2-phenoxythenol and 0.66mL β -mercapthoethanol. The extracts were absorbed onto filter paper wicks (3 x 13 mm), loaded onto 12% starch gels. Gels were cooled overnight to 4°C before loading samples. Sample wicks were removed after half an hour of electrophoresis. The voltage was then set and run from 5 to 7 hours.

Recipes for histochemical staining solutions followed Murphy et al. (1996). Buffer systems used were: lithium borate pH 8.3, 80 mA (Ridgeway et al. 1970); and morpholine citrate pH 8.0, 50 mA (Clayton and Tretiak 1972). In all, 33 enzyme systems were initially screened for polymorphism and 10 putative allozyme loci for 6 enzymes were resolved clearly and consistently, thus selected for analysis. These were glutamic dehydrogenase (*GDH*; 1 locus), phosphoglucose isomerase (*PGI*; 2 loci), leucine aminopetidase (*LAP*; 2 locus), isocitic dehydrogenase (*IDH*; 1 locus),

aspartate aminotransferase (AAT; 2 loci), and 6-phosphogluconate dehydrogenase (6-PGD; 2 loci). For enzyme systems with multiple loci, the most anodal migrating locus (fastest locus) was assigned as 1 and other loci were assigned increasing numbers with decreasing migrating distance. At each locus, the most common allele was arbitrarily designated as 1 and the others 2, and so on.

Data analysis

Estimates of the following quantities were obtained: allele frequencies, mean number of alleles per locus (A), percentage polymorphic loci (% P) at 99% criterion, observed heterozygosity (H_O) and expected heterozygosity ($H_E = 1 - \sum p_i^2$, where p_i the frequency of the i th allele). This analysis was performed with BIOSYS-2 (William C. Black IV, Department of Microbiology, Colorado State University), a modified version of the BIOSYS-1 program by Swofford and Selander (1981).

Wright's F_{ST} (Wright 1965) was computed for individual loci of the eight populations. Nei's (1978) genetic distance (D) was computed between all pairs of populations. A dendrogram of genetic relationships among populations was constructed from these distances using the unweighted pair group method (UPGMA, Sneath and Sokal 1973), and standard error bars calculated with Ritland's (1989b) genetic distance and clustering program (GDD). Dendrograms plotted using these procedures help to visualize the genetic relationship among populations. In each population, departures of genotypic frequencies from Hardy-Weinberg expectations were characterized by estimating Wright's inbreeding coefficient as $F = 1 - H_O/H_E$. Genepop (Raymond and Rousset 1995) was used to estimate P -values from exact test of departure from Hardy-Weinberg equilibrium using the Markov chain method with 1000 iterations (Guo and Thompson, 1992).

Linear regression was performed to study the relationship between the expected heterozygosity and latitude, heterozygosity and elevation, and between genetic distance and geographic distance using S-PLUS statistical software (S-PLUS 6, Insightful, Corp. 2001). In addition, isolation by distance was tested using

Rousset's method (1997), which involves a linear regression of pairwise $F_{ST} / (1-F_{ST})$ on the natural logarithm of geographic distances between populations. Significance was tested statistically using the Mantel test (Mantel 1967) in the program IBD version 1.4 (Bohonak 2002). This test assesses whether the pairwise geographic distance matrix correlates with the pairwise genetic distance matrix.

The degree of genetic isolation (gene flow) was estimated by Nm , the number of migrants per generation. Nm was estimated by two methods, by the relationship between F_{ST} and Nm and by the method of private alleles. From Wright (1951): $Nm = (1-F_{ST})/4F_{ST}$, where F_{ST} is the proportion of the total genetic diversity among populations. I used Genepop (Raymond and Rousset, 1995) to estimate Nm based on the private alleles (unique alleles found in only one population) method developed by Slatkin (1985), using the frequency and distribution of rare alleles among populations.

Mating system parameters were estimated using the mixed mating model of Ritland and Jain (1981), as implemented in MLTR (Ritland 1990). Single-locus (t_s) and multi-locus (t_m) estimates of population outcrossing rates were estimated for the two populations (Jericho and Fraser) in which progeny arrays were sampled. Maternal genotypes were inferred from progeny arrays for these two populations following Brown and Allard (1970) and used to estimate genetic diversity parameters for the northern range of the species distribution. Multilocus outcrossing rates (t_m) were compared with mean single-locus (t_s) rates to detect any selfing due to biparental inbreeding ($t_m - t_s$). The correlation of outcrossed paternity (r_p) was estimated following Ritland's (1989a) sibling pair model. These parameters and the average single-locus inbreeding coefficient of maternal parents (F) were also estimated via the MLTR program (Ritland 1990).

RESULTS

Allele frequency distribution

Seedling genotypes were scored for a total of 10 loci in two enzyme systems. Some loci were apparently monomorphic but were inconsistent in resolution and were excluded from the analysis (*SKD-1*, *SKD-2*, *MDH-2*). Others were variable but could not be used for the analysis because of overstaining of one locus on top of another, e.g. *PGM-1*, or because of very faint banding patterns that could not be properly interpreted, e.g. *G-6P-1*, *G-6PDH*, *ME-1*, *ACO-1*, *ACO-2* and *UGUT-1*.

Allele frequencies for each population are given in Table 3.1. A total of 24 alleles were detected in this study. One locus (*LAP-2*) was monomorphic across all populations sampled. Of the nine polymorphic loci, 1 locus (*AAT-2*) was polymorphic in only one population, 2 loci (*6PG-2*, *GDH*) were polymorphic in two populations, and one other locus (*LAP-1*) was polymorphic in six populations. Two loci (*6PG-1*, *PGI-1*) were polymorphic in seven populations and the remaining 3 (*AAT-1*, *IDH*, *PGI-2*) were polymorphic across all populations. Two of the eight populations had a total of 3 private alleles (i.e. alleles found in only one population). These private alleles were unique to the two northern populations (Jericho, *AAT-2-2*, *AAT-2-3*; Fraser, *IDH-1-3*). Variable loci exhibited just 2 alleles per locus with the exception of Jericho and Fraser populations that exhibited 3 alleles at several loci (Table 3.1).

Genetic diversity

Genetic variation statistics are summarized for all populations in Table 3.2. Alleles per locus averaged 1.71, and ranged from 1.5 (Artic) to 2.2 (Jericho). On average, 61.2% of the loci were polymorphic, ranging from 50% (Artic) to 80% (Jericho). The expected heterozygosity within populations ranged from 0.102 (Jericho) to 0.189 (Artic), and averaged 0.152 (Table 3.2). A significant negative relationship was found between expected heterozygosity and latitude ($R^2 = 0.71$; $p \leq 0.05$). An unexpected relationship was also found between expected heterozygosity and alleles

per locus ($R^2 = 0.89$; $p \leq 0.05$), and expected heterozygosity and percent polymorphic loci ($R^2 = 0.53$; $p \leq 0.05$).

Genetic structure

Observed heterozygosities varied from 0.108 to 0.160, with an average of 0.118. The mean observed heterozygosity was 22% lower than the expected heterozygosity (0.152). Hardy-Weinberg equilibrium was rejected for three of the eight populations ($P < 0.05$), which showed a deficiency of heterozygotes. Observed heterozygosities were found to be slightly lower than the expected values within most populations. This heterozygote deficiency is reflected in the mean inbreeding coefficient ($F = 0.166$). The sample from Siletz had the highest inbreeding coefficient of 0.334, while Jericho had a slight excess of heterozygosity ($F = -0.050$) (Table 3.2). The mean fixation index (F_{IS} ; Wright 1951) across loci and populations was 0.193, suggesting *Acér macrophyllum* populations have some degree of inbreeding.

The proportion of genetic variation due to differences among populations was $F_{ST} = 0.054$ (Table 3.3), indicating that 94.6% of the genetic variation resides within populations. The relatively low value indicates little population differentiation which may be due to high levels of gene flow, as estimated by the indirect method ($N_m = 4.39$) and for the private alleles method ($N_m = 4.10$).

Nei's genetic distance between populations was low averaging 0.011 (SD=0.005) and ranging from 0.001 to 0.042. The dendrogram of genetic relationship among the eight populations is shown in Fig. 3.2. Significant clusters of populations occur when the length of the shaded portion (thicker bar) is less than half that of (thinner bar); the method for determining the error is based upon the among-locus variance of genetic distance between clades (Ritland 1989b). The most genetically distinct population is Artic, which is separated from the rest of the populations (Fig. 3.2). Several statistically significant groups are evident, but the cluster between Oakville and Helmick was not statistically significant, as a large geographic distance also exists between the 2 populations (228 km). The similarity between the Jericho

and Fraser populations is obvious in that they both share the highest value of allelic diversity, lowest level of heterozygosity and with little inbreeding as compared to the other populations. The reason for their similarity could be that they share the most common recent ancestor. No significant correlation was found between genetic distances and geographic distances (Mantel test, $r = 0.36$, one tailed $p=0.059$).

Mating system

Estimated multi-locus outcrossing rates for the two populations were high (Table 3.4). Single-locus outcrossing rates estimate (t_s) ranged from 0.939 to 0.942, with an average of 0.94 for the two populations. Multi-locus estimates of outcrossing rates (t_m) ranged from 0.941 to 0.950 and averaged 0.945 for the two populations. Single-locus and multi-locus outcrossing estimates for all populations differed significantly from unity ($t=1$). Differences in multi-locus and single-locus (t_m-t_s) can indicate biparental inbreeding, however the difference in this case was essentially zero, indicating no biparental inbreeding (Table 3.4).

Individual tree outcrossing rate estimates were heterogeneous in the two populations. Both populations exhibited predominant outcrossing, with a large portion of trees having outcrossing rates equal to or greater than 0.90. Maternal inbreeding coefficients (F) were low and did not differ significantly from zero in either population. The correlation of outcrossed paternity r_p (probability that sibs shared the same father) for both populations ranged from 0.234 to 0.544 and averaged 0.389. This value is high, indicative of few effective pollen donors ($N_{ep} = 1/r_p = 2.57$, see Ritland 1989a).

DISCUSSION

Genetic variation

Compared with the genetic diversity found in other woody angiosperms, *Acer macrophyllum* has a higher percentage of polymorphic loci ($P=61.2\%$) and expected heterozygosity ($H_E=0.152$) than average. However, the number of alleles per locus in bigleaf maple ($A=1.71$) is slightly less than that in woody species, on average, and slightly higher than the mean in all woody angiosperm (Table 3.5). Differences in the amount of genetic variation among populations, particularly differences in expected heterozygosity relative to polymorphism, may reflect the action of different genetic processes. Jericho and Fraser were the two populations with the lowest levels of heterozygosity, and higher polymorphism, with no evidence of deviation from random mating. In this case it seems more likely that the low heterozygosity may be a reflection of low overall population genetic variation. This is possibly the result of genetic drift or selection in some parts of the northern range of this species. The most genetically distinct population is Artic, which is separated from the rest of the populations (Fig.3.2). This separation in my view is anomalous in that there is no clear environmental explanation as to why it should be different. Isozymically, it differs from other populations in having the highest expected heterozygosity (0.189), yet it is the only population which is invariant at the *PGI-1* locus, thereby having the lowest proportion of polymorphism. In addition, the sample size of 14 individuals is small compared to the rest of the populations which could partially be responsible for its genetic distinctiveness.

Population genetic structure and gene flow

Differentiation among populations, as measured by Nei's genetic distance (D), averaged 0.011 for the eight populations of bigleaf maple in this study. This value is similar to that observed in 22 populations of *Alnus crispa* ($D = 0.012$, Bousquet et al. 1987) but higher than those observed for *Acer saccharum* ($D = 0.003$, Perry and

Knowles 1989; $D = 0.007$, Young et al. 1993). The value of genetic distance (0.011) in this study is probably due to large geographic distances. The mean geographic distance between pairs of population was 250 km and the largest distance was 562 km suggesting absence of isolation by distance across the species range. Most of the sites sampled were similar in terms of climate and edaphic factors. These factors may promote low differentiation among populations. This result is supported by a positive but non-significant correlation between genetic and geographic distances. Similar results have been found for *Acer saccharum* (Young et al. 1993) and *Populus tremuloides* (Hyun et al. 1987).

The level of population differentiation in bigleaf maple, $F_{ST} = 0.054$, is similar to that reported for *Acer saccharum* ($F_{ST} = 0.049$, Young et al. 1993; $F_{ST} = 0.033$, Perry and Knowles 1989), *Alnus crispa* ($F_{ST} = 0.051$, Bousquet et al. 1987). These low values reflect extensive gene flow via pollen or seed, or recent colonization (Huh 1999). Species with more pollen or seed movement should have less genetic differentiation than species with restricted gene flow. In support of these predictions, Foré et al. (1992) observed an average seed dispersal distance of up to 100 metres for sugar maple (*Acer saccharum*). Common life history traits such as allogamy, wind dispersal of seed, high reproductive capacity, longevity and successional behaviour could account for the low differentiation observed.

The apparent lack of association between geographic and genetic distances somewhat indicated a tendency towards isolation by distance but not statistically significant. Similar findings have been found for *Alnus rubra* (Xie et al. 2002) that has similar patchy distribution to *Acer macrophyllum* and also occupies a similar geographic range. Relationships between genetic and geographic distances has been observed for several tree species (e.g. *Camellia japonica*, Wendel and Parks 1985; *Tsuga mertensiana*, Ally et al. 2000; *Pseudotsuga menziesii*, Yeh and O'Malley 1980), indicating that for these species isolation by distance may be an important factor in population differentiation. However, most studies that have demonstrated such

significant associations sample a larger number of populations or involve species that cover larger longitudinal or latitudinal distribution than the current study.

The observed geographic separation between the two British Columbia populations (Jericho and Fraser) and the rest of the populations may be of recent origin as hypothesized by Pielou (1991); it is therefore reasonable to suggest that strong gene flow through pollen and seed have overcome the effects of genetic drift so that physically separated small patchy bigleaf maple populations within each geographic region share a more or less continuous common gene pool as those continuously distributed. For instance, Siletz is widely separated geographically (> 500 km) from Jericho and Fraser but is genetically similar.

Mating system

High outcrossing rates were found in the two populations sampled using progeny arrays (Table 3.4). These outcrossing estimates are similar to other temperate angiosperm tree species, e.g. *Fagus sylvatica* (mean $t = 0.96$, Rossi et al. 1996), *Alnus crispa* ($t = 0.95$, Bousquet et al. 1987), *Quercus lobata* ($t = 0.96$, Sork et al. 2001), and *Eucalyptus urophylla* ($t = 0.91$, House and Bell 1996). Although *Acer macrophyllum* is pollinated by insects, wind pollination can not be ruled out. Wind-pollination has been reported for other North American *Acer* species that were originally thought to be only insect pollinated (e.g. *Acer grandidentatum*, Barker et al. 1982; and *Acer saccharum*, Gabriel and Garrett 1984). Interestingly, six of eight populations of bigleaf maple had an excess of homozygotes, and the estimated F was 0.166. These results again suggest that some inbreeding and selfing occurs in most populations (Table 3.1). Assuming inbreeding equilibrium and assuming all inbreeding is due to selfing, this level of inbreeding can be explained by a selfing rate of $2F / (1 + F) = 0.28$.

Some inbreeding in bigleaf maple may result from geitonogamous pollinations by bumblebees (*Bombus* spp.), through positive assortative mating (i.e., preferential mating among similar genotypes, Sullivan 1983), or through mating among relatives.

Acer macrophyllum has perfect as well as staminate flowers and the pollination mechanism is mainly entomophilous (Minore and Zasada 1990). The movement of pollinators among adjacent flowers within the crown or between adjacent crowns of related neighbours could cause inbreeding and selfing (González-Astorga and Núñez Farfán 2001).

I found no significant difference between multi-locus (t_m) and single-locus (t_s) estimates of outcrossing rates in either bigleaf maple populations, indicating an absence of consanguineous mating. This result is in agreement with the study by Sork et al. (2001) for *Quercus lobata*, which occurs in open landscape and is patchily distributed, and for *Stemmadenia donnell-smithii* by James et al. (1998). Biparental inbreeding has been reported for some woody species, including western larch (El-Kassaby and Jaquish 1998), *Fagus sylvatica* (Rossi et al. 1996) and *Eucalyptus marginata* (Millar et al. 2000).

Two significant results I obtained were that “correlated matings” (the fraction of outcrossed sibling pairs that share the same father) were high, especially for the Fraser population ($r_p = 0.544$, Table 3.5). This correlation is influenced by two factors: (i) multiple deposits of pollen from a single male parent, or (ii) repeated mating among a relatively small number of neighbours nearer to one another. A value near one-half implies that only a few pollen donors (1-2) must have sired the majority of seeds within each tree, while the value of 0.234 for the Jericho population indicates 4-5 effective pollen donors per tree. The lower number of effective pollen donors at Fraser could be due to features of this habitat, compared to Jericho. Fraser is a roadside population which may be expected to show reduced outcrossing rates due to disturbances. However, the outcrossing rates were similar between the two populations, suggesting that high outcrossing was being maintained in the relatively disturbed Fraser population due to insect or pollen movement along a corridor of trees (Chase et al. 1996).

The other significant result was that, despite the evidence of moderate inbreeding ($F_{IS} > 0$) in this species, I found high levels of outcrossing ($t = 0.95$).

Allozyme-based estimates of outcrossing rates based upon seed progeny could give upwardly biased estimates of outcrossing if selfing reduces the germination capacity, or if filled seeds are used for estimating outcrossing rates (e.g., the effects of embryonic lethals due to selfing on seed development and the formation of empty seeds are not accounted for; Rajora et al. 2000, 2002). In this study, I used entirely germinated and filled seeds since these yielded interpretable enzyme bands. However, this may have upwardly biased estimates of outcrossing. Self-fertilization has been found to adversely affect both embryo development and seed germination in conifers (Sorensen 1969). This also holds for some species in the maple family; for example, Gabriel (1962) examined the interior of carpels of sugar maple seeds (*Acer saccharum*), and suggested that the low seed set after selfing may be related primarily to post-zygotic abortion. In addition, Gabriel (1962) noted a reduction in viability of selfed seeds compared to outcrossed seeds, e.g., inbreeding depression.

IMPLICATIONS FOR MANAGEMENT AND CONSERVATION

The observed genetic differentiation among bigleaf maple populations probably reflects the combined effects of ecological, evolutionary and biogeographic factors such as pollen and seed dispersal mechanisms. Considering the relatively large geographic scale in this study, my results indicate lower than expected levels of differentiation among populations of bigleaf maple, in light of the fact that most populations in this study have trees patchily distributed, and that trees are pollinated by insects. This study also found that bigleaf maple is predominantly outcrossing ($t = 0.95$) and lacks biparental inbreeding. High outcrossing is related to its floral biology and characteristics, e.g., protogyny. Perhaps the most interesting finding was the small numbers of effective pollen donors, which probably reflects the relatively low density of populations coupled with limited pollinator movement. Correlated paternity results in an increased genetic relatedness of progeny, and a decreased genetic

diversity of individual tree progenies, which may limit local adaptive responses (James et al. 1998).

Understanding genetic diversity and population genetic structure is not only crucial in the conservation of species under threat of extinction, but it is also essential for the maintenance of healthy populations and the breeding of widespread indigenous tree species (Millar and Westfall 1992). The genetic variation and population genetic structure revealed in this study are instructive for making conservation plans and developing breeding strategies. The estimate of $F_{ST} = 0.054$ in this study indicated up to about 95% of the total genetic diversity resides within populations. Therefore for such a predominantly outcrossing species ($t_m = 0.95$) with insect pollination and seed dispersal by wind, it may be advisable to sample fewer populations but more individuals per population for breeding purposes.

Measures of genetic diversity based on number of alleles (allelic richness) are important, especially in the field of conservation genetics. Since one goal of a conservation program is to maintain as many alleles as possible, choices of populations to conserve *in situ* should be based on allelic richness of the population (Marshall and Brown 1975). In view of this, I suggest that although a few *in situ* populations would contain most of the existing genetic variation in the species, it would be essential to also consider the populations from the northern range; thus, Jericho and Fraser would be favored. Although inbreeding *per se* does not lead to loss of alleles nor alter their frequencies in a population, it leads to increased in homozygosity, and thus may decrease the mean fitness of the population. To this end, populations displaying extensive inbreeding would not be desirable for future *in situ* gene conservation.

The lack of correlation between genetic and geographical distances in the species distribution suggests that when sampling, we may not necessarily need to sample sites evenly across the species range. Although bigleaf maple is widely distributed without any current threat of extinction, effective *in situ* conservation and reasonable management of its populations in the wild will promote and enhance its

adaptability to changing environments, and also sustain its gene pool for future genetic improvement.

Table 3.1. Distribution of allele frequencies at 10 loci in eight natural mature populations of bigleaf maple (*Acer macrophyllum*).

Locus	Allele	Populations							
		JERC	FRAS	ARTC	CASC	ELBE	HELM	OAKV	SILE
AAT-1	1	0.942	0.958	0.857	0.900	0.895	0.789	0.833	0.917
	2	0.058	0.042	0.143	0.100	0.105	0.211	0.167	0.083
AAT-2	1	0.904	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH	1	0.923	0.854	0.786	0.850	0.850	0.850	0.850	0.850
	2	0.077	0.125	0.214	0.150	0.150	0.150	0.150	0.150
	3	0.00	0.021	0.000	0.000	0.000	0.000	0.000	0.000
6PG-1	1	0.981	1.000	0.786	0.825	0.825	0.800	0.875	0.850
	2	0.019	0.000	0.214	0.175	0.175	0.200	0.125	0.125
6PG-2	1	0.885	0.875	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.038	0.063	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.077	0.062	0.000	0.000	0.000	0.000	0.000	0.000
PGI-1	1	0.962	0.896	1.000	0.816	0.853	0.789	0.875	0.825
	2	0.019	0.042	0.000	0.184	0.147	0.211	0.125	0.175
	3	0.019	0.063	0.000	0.000	0.000	0.000	0.000	0.000
PGI-2	1	0.885	0.938	0.714	0.825	0.775	0.875	0.875	0.800
	2	0.077	0.021	0.286	0.175	0.225	0.125	0.125	0.200
	3	0.038	0.042	0.000	0.000	0.000	0.000	0.000	0.000
GDH	1	0.981	0.917	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.019	0.083	0.000	0.000	0.000	0.000	0.000	0.000
LAP-1	1	1.000	1.000	0.538	0.825	0.781	0.850	0.850	0.800
	2	0.000	0.000	0.462	0.175	0.219	0.150	0.150	0.200
LAP-2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Note: JERC = Jericho; FRAS = Fraser; ARTC = Artic; CASC = Cascadia; ELBE = Elbe; HELM= Helmick; OAKV = Oakville; SILE = Siletz.

Table 3.2. Summary of genetic diversity within eight mature natural populations of bigleaf maple (*Acer macrophyllum*) based on 10 allozyme loci.

POPULATION	<i>N</i>	<i>A</i>	% <i>P</i>	<i>H_O</i>	<i>H_E</i>	<i>F</i>
1. Jericho	40	2.2 (0.2)	80.0	0.108 (0.029)	0.102 (0.026)	-0.050 ^{NS}
2. Fraser	36	2.0 (0.3)	60.0	0.112 (0.036)	0.105 (0.033)	-0.086 ^{NS}
3. Artic	14	1.5 (0.2)	50.0	0.160 (0.060)	0.189 (0.066)	0.105 ^{NS}
4. Cascadia	20	1.6 (0.2)	60.0	0.121 (0.037)	0.164 (0.046)	0.222 ^{NS}
5. Elbe	20	1.6 (0.2)	60.0	0.109 (0.035)	0.172 (0.049)	0.285*
6. Helmick	20	1.6 (0.2)	60.0	0.118 (0.038)	0.176 (0.049)	0.332*
7. Oakville	20	1.6 (0.2)	60.0	0.117 (0.033)	0.148 (0.041)	0.186 ^{NS}
8. Siletz	20	1.6 (0.2)	60.0	0.102 (0.031)	0.163 (0.047)	0.334*
Mean	23.75	1.71	61.2	0.118	0.152	0.166*

Note: *N*, sample size; *A*, average number of alleles per locus; %*P*, percent polymorphic loci; *H_O*, observed and heterozygosity; *H_E*, expected heterozygosity; *F*, inbreeding coefficient; and numbers in parenthesis, standard errors. Exact test of departure from Hardy-Weinberg equilibrium * *P* < 0.05,

^{NS} not significant after sequential Bonferroni correction (Rice 1989)

Table 3.3. Total gene diversity (H_T), genetic diversity within populations (H_S), expected heterozygosity (H_O), alleles per locus (N_A), fixation index over the total populations (F_{IT}), fixation index within population (F_{IS}), and genetic differentiation among populations (F_{ST}) for eight mature natural populations of bigleaf maple (*Acer macrophyllum*) at nine polymorphic loci.

Locus	H_T	H_S	H_O	N_A	F_{IS}	F_{IT}	F_{ST}
AAT-1	0.202	0.202	0.169	2.00	0.116	0.143	0.030
AAT-2	0.024	0.023	0.024	3.00	-0.088	-0.013	0.067
IDH	0.254	0.258	0.222	3.00	0.125	0.134	0.009
6PG-1	0.231	0.226	0.134	2.00	0.396	0.431	0.057
6PG-2	0.059	0.056	0.060	3.00	-0.101	-0.029	0.065
PGI-1	0.219	0.213	0.194	3.00	0.060	0.105	0.048
PGI-2	0.279	0.276	0.180	3.00	0.308	0.335	0.039
GDH	0.025	0.024	0.026	2.00	-0.076	-0.015	0.056
LAP-1	0.277	0.252	0.171	2.00	0.300	0.390	0.128
Mean	0.174	0.170	0.131	2.55	0.193	0.236	0.054

Table 3.4. Estimates of multi-locus outcrossing rates (t_m), single locus outcrossing rates (t_s), biparental inbreeding ($t_m - t_s$), parental inbreeding coefficients (F) and correlation of paternity among siblings (r_p).

Population	t_m	t_s	$t_m - t_s$	F	r_p
Jericho	0.941 (0.057)	0.939 (0.008)	0.002 (0.052)	0.052 (0.010)	0.234 (0.053)
Fraser	0.950 (0.067)	0.942 (0.054)	0.008 (0.034)	0.053 (0.009)	0.544 (0.067)
Mean	0.945	0.940	0.005	0.052	0.389

Note: Standard errors in parentheses.

Table 3.5. Comparison of within-population genetic diversity for *Acer macrophyllum* with average values for all plants, woody species, woody angiosperms, and for *Acer* species.

Categories	A	% P	H_E	G_{ST}/F_{ST}	Reference
All plant species	1.52	34.6	0.113	0.228	Hamrick et al. 1992
Woody species	1.76	49.3	0.148	0.084	Hamrick et al. 1992
Woody angiosperms	1.68	45.1	0.143	0.102	Hamrick et al. 1992
<i>Acer saccharum</i>	2.80	87.5	0.150	0.012	Foré et al. 1992
<i>Acer saccharum</i>	1.95	38.2	0.110	0.033	Perry and Knowles 1989
<i>Acer saccharum</i>	2.03	53.7	0.109	0.049	Young et al. 1993
<i>Acer platanoides</i>	1.92	53.9	0.128	0.120	Rusanen et al. 2000
<i>Acer platanoides</i>	2.0	54.5	0.132	0.009	Rusanen et al. 2003
<i>Acer campestre</i>	3.15	100	0.287	-	Bendixen 2001
<i>Acer macrophyllum</i>	1.71	61.2	0.152	0.054	This study

Note: See tables 3.2 and 3.3 for definition of variables.

Figure 3.1. Geographic locations of eight *Acer macrophyllum* mature natural populations.

Figure 3.2. UPGMA cluster analysis of Nei's genetic distances between eight mature populations of *Acer macrophyllum*.

Chapter four

EFFECTS OF FOREST FRAGMENTATION ON GENETIC VARIATION AND SPATIAL GENETIC STRUCTURE IN NATURAL POPULATIONS OF BIGLEAF MAPLE (*Acer macrophyllum* Pursh)

INTRODUCTION

Worldwide, human development is rapidly encroaching upon and subdividing many remaining natural areas. Fragmentation of the landscape produces remnant vegetation patches, surrounded by a matrix of different vegetation types or unvegetated land uses. Habitat fragmentation, the breaking up of continuous forest into smaller patches, can reduce population size and increase population isolation (Young et al. 1993; Andren 1994). It also reduces the availability of suitable colonization sites for the establishment of new populations (Wilcox and Murphy 1985). Studies of natural plant populations have shown that population size is an important factor in determining the amount of genetic variation maintained within sexually mature populations and the distribution of this variation among individuals (Sampson et al. 1988).

Habitat fragmentation may erode genetic diversity and increase population differentiation, affecting population viability in the short or long term (Young et al. 1996). These effects are due mainly to increased genetic drift and inbreeding in habitat fragments with small census sizes and reduced gene flow between fragments (Young et al. 1996). The subdivision caused by fragmentation will promote local population differentiation if gene flow barriers are established and subpopulations diverge due to genetic drift (Bacles et al. 2004). In addition to isolation, the genetic

structure of natural populations prior to fragmentation may determine how large the impact of fragmentation or habitat loss would be. For example, if fragments are large enough to maintain the genetic structure of the original population, differentiation among fragments may be less marked. On the other hand, if fragments are small and scattered, they will be more likely to contain a biased sample of the original genetic variation, and differentiation will be further promoted if isolation persists (Nason and Hamrick 1997). The impact of fragmentation varies among organisms, depending on the effects of fragmentation on reproduction, dispersal and gene flow, and the original distribution of genetic diversity (Young 1996).

Another potential consequence of fragmentation is a change in mating system. In plant populations in particular, inbreeding can increase due to either increased self-pollination or through an increased probability of mating between individuals sharing recent common ancestry (Raijmann et al. 1994; Young et al. 1996).

Results available from empirical studies of the effects of habitat fragmentation on several angiosperm tree species from the tropics have indicated that fragmentation can have significant genetic consequences. Reduced population size and increased isolation associated with habitat fragmentation may cause a reduction in genetic variation, increased population differentiation between habitat fragments (Wilcove 1987; Templeton et al. 1990; Chase et al. 1996; White et al. 1999), and increased inbreeding (Lee et al. 2000; Fuchs et al. 2003) as predicted by theory. However, there is also growing empirical evidence for enhanced gene flow between isolated trees in forest fragments (Young et al. 1993). It appears that the effects of habitat fragmentation on the genetic behavior of tree species are more varied and complex than first thought (Young 1996; Aldrich and Hamrick 1998).

Populations of long-lived woody perennials seem to be resistant to changes in genetic diversity due to long generation times, overlapping generations and high levels of gene flow (White et al. 1999; Merwe et al. 2000). However, genetic losses are more observable in seedling cohorts than in adult cohorts because seedlings reflect the

genetic effects of reduced present-day levels of gene flow and population size (Lee et al. 2000).

Bigleaf maple is a conspicuous species in the temperate coastal rainforests of the Pacific Northwest. It grows in a variety of soils throughout its range and it is usually a small to medium-sized tree. The trees are usually scattered or in small groves in association with conifers and other broad-leaved species. As this species is commonly found in remnant forests surrounded by pasture lands and agricultural fields, it is likely that forest fragmentation has divided formerly larger *Acer macrophyllum* populations into smaller and isolated patches in some parts of its range. Accordingly, *Acer macrophyllum* populations have experienced reductions in effective population size and spatial extent, which may have significantly reduced genetic variation and altered population genetic structure. It is therefore hypothesized that forest patch populations (fragments) of *Acer macrophyllum* will have less genetic variation and increased levels of inbreeding than more continuous populations. In this study, I compared genetic diversity, genetic structure and inbreeding level in seedling and adult cohorts from both fragmented and continuous populations. In addition I used computer simulations to forecast the decline in genetic variation due to forest fragmentation.

The specific questions addressed are:

1. Does genetic diversity differ between adult bigleaf maple populations of trees in fragmented and continuous forests?
2. Is inbreeding different in seedling than that in adult cohorts?
3. Is there spatial genetic structure in bigleaf maple populations?
4. If spatial genetic structure exists, does it differ between continuous and fragmented populations?

If *Acer macrophyllum* genetic variation is being affected by fragmentation, we would expect: a) less overall genetic diversity in fragmented forests than in continuous

forests; b) lower inbreeding in continuous than in fragmented landscapes; c) stronger spatial genetic structure in fragmented than in continuous populations.

MATERIALS AND METHODS

Populations and sampling

Six study populations were located on Vancouver Island (Fig 4.1). These comprised three populations that occurred in areas in which forests have been fragmented over the past 150 years due to agriculture and urban development, and three in relatively continuous forests. The intent of the sample design was to have a control (continuous populations) against which the genetic effects of fragmentation could be tested. Populations sampled occurred between 20 and 150 m in elevation. For each population and at each sampling site, several East-West transects were established, each approximately 100 m wide. Along these transects, 50 seedlings were collected from the forest floor from each of the six populations, and terminal buds of lateral shoots were collected from each of 50 adult trees in each population. As much as possible, seedling and mature trees were sampled at least 30 m apart to avoid sampling closely related individuals. The total area from which tree samples were collected varied widely due to the patchy nature and variable density of populations, ranging from 79 ha at Rosewall Creek to 312 ha for Nitinat (Table 4.1).

After collection, seedling and bud samples were wrapped in aluminum foil, labeled and frozen in liquid nitrogen. Upon return to University of British Columbia, samples were immediately transferred into a -20°C freezer for the seedlings and to a -80°C freezer for bud samples until electrophoresis.

Electrophoresis

Horizontal starch gel electrophoresis was used to obtain allozyme data for seedlings and adults for the same 10 loci described in chapter three, with the exception of LAP-1, which was monomorphic across all adult populations and was not included in this analysis. The extraction buffer used to grind emerging leaf tissues from seedlings and bud tissues from adult trees was the same as described in chapter three.

Data Analysis

Standard genetic diversity parameters (allele frequencies, average number of alleles per locus (A), observed heterozygosity (H_O), and expected heterozygosity (H_E) were estimated for both seedling and adult cohorts in all populations. This analysis was performed with BIOSYS-2 (W.C. Black IV, Department of Microbiology, Colorado State University), a modified version of the BIOSYS-1 program, by Swofford and Selander (1981). Departures in genotype frequencies from Hardy-Weinberg expectations were tested using a Markov chain method following the algorithm of Guo and Thompson (1992). Exact tests for these departures were conducted at each of the variable loci, and linkage disequilibrium was tested between pairs of variable loci. The inbreeding coefficient F_{IS} (Wright 1951) was estimated following Weir and Cockerham (1984). All calculations and tests above were performed using Genepop (version 3.1d) (Raymond and Rousset, 1995).

I also used BOTTLENECK (version 1.2.02) described by Cornuet and Luikart (1996) to test for historical reductions in population size. If a population has been through a bottleneck it should show a signature of reduced allelic richness compared to expected heterozygosity, as rare alleles are lost faster than heterozygosity decreases. After a bottleneck, the expected heterozygosity (H_E) computed from allele frequencies for a sample of genes should be larger than the heterozygosity expected

(H_{eq}) based on the number of alleles in the same sample, assuming the population is at mutation-drift equilibrium (Cornuet and Luikart, 1996). Isozymes are expected to conform to the infinite allele model (IAM), where each new mutation gives rise to a new allele different from all existing ones (Kimura and Crow, 1964), thus data were analyzed under this model. To test for a deficiency or excess in H_E , the Wilcoxon signed-ranks test was used as it has more power than the sign-test and can be used effectively with fewer loci (Cornuet and Luikart, 1996; Piry et al. 1999).

Genetic structure

The genetic structure of populations was assessed according to Wright's (1965) F -statistics following Weir and Cockerham (1984). These fixation indices were used to measure deviations from Hardy-Weinberg equilibrium attributable to individuals within local populations (F_{IS}), variation among populations (F_{ST}) and variation among individuals relative to all populations pooled (F_{IT}). The significance of these parameters was tested based on 1800 permutations of alleles among individuals within samples, genotypes among samples, and alleles among samples, respectively. Means and standard errors were obtained by jackknifing over loci. A bootstrap confidence interval (CI) of 95% was considered significant when confidence intervals did not overlap zero. These calculations were made using the program FSTAT (Goudet, 2000).

Spatial autocorrelation analysis of genetic variation

Spatial genetic structure within populations was assessed using Cockerham's (1969) estimates between all possible pairs of individuals at different inter-tree distances in each population for the adult cohort. This method provides a powerful test of spatial genetic structure (Hardy and Vekemans 2002). The coancestry coefficient

(ρ_{ij}) has been used in a number of studies recently (e.g., Loiselle et al. 1995; Peakall and Beattie, 1996; Burke et al. 2000; Kalisz et al. 2001; Parker et al. 2001).

The parameter ρ_{ij} was estimated for each distance class using the software program Spatial Pattern Analysis of Genetic Diversity (SPAGeDi) 1.1 (Hardy and Vekemans 2002). The software uses the estimator described by Loiselle et al. (1995) as follows:

$$\rho_{ij} = \frac{\sum_{ij}(p_i - p)(p_j - p)}{kp(1 - p)} + \frac{2}{(8k + 1)^{0.5} - 1}$$

where p_i and p_j are the frequencies of homologous alleles at a locus for individuals i and j ; p is the mean frequency for that allele; and $k = n(n - 1) / 2$, the number of possible pairs between n individuals located in each distance class. The second term in the equation adjusts for bias associated with a finite sample size and results in ρ_{ij} having an expected value of zero for a population in Hardy-Weinberg equilibrium. The results were combined across loci to estimate coancestry by weighting the values for each locus by its polymorphic index, $\sum p_i (1 - p_i)$. For a population in Hardy-Weinberg equilibrium, the coancestry between individuals is a measure of the inbreeding coefficient of their hypothetical offspring with expected values of 0.25 for pairs of full-sibs, 0.125 for half-sibs, and 0.0625 for first cousins.

Individual tree locations were identified by a coordinate grid system using a hand-held Global Positioning System instrument (GPS Garmin Model 12XL). Each tree was mapped on a North-South and East-West (x,y respectively) grid using GPS data to construct inter-tree distance matrices for spatial autocorrelation analysis. With this procedure, each scored genotype was assigned to its corresponding spatial location within each population.

Eleven to fourteen distance classes were used for the spatial autocorrelation analysis. All populations had the following intervals: 0 – 50 m, 50 – 100 m and nine 100 m interval up to 1000 m for all populations. Rosewall had two additional distance classes (1000 – 1200 m and 1200 – 1500 m), and Maple Bay, Yellow Point, and Nitinat an additional four classes; (1000 – 1200 m, 1200 – 1500 m, 1500 – 2000 m, 2000 – 2500 m). Distance classes were chosen so that each contained at least 30 pairwise comparisons. This analysis tests whether pairs of trees within a specified distance interval exhibit the same alleles more often than expected by chance under a random spatial distribution (Hardy and Vekemans 2002). Mean estimates of coancestry were obtained over all pairs of individuals for the distance classes described above. When $\rho_{ij} = 0$, there is no genetic correlation between the frequencies of alleles in individuals at the spatial scale of interest; when $\rho_{ij} > 0$, individuals in a given distance class are more closely related than expected by chance; and conversely, when $\rho_{ij} < 0$, individuals within a given distance class are less related than expected by chance. Estimates of coancestry were tested for significance with a randomization procedure that generated populations with a random spatial distribution of genotypes (i.e. no spatial structure). In each plot, intact multilocus genotypes were randomly drawn, with replacement, from the sampled data and assigned to points occupied by plants; new ρ_{ij} values were then calculated. This randomization procedure was repeated 499 times for each plot, giving (together with the originally sampled data) 500 ρ_{ij} values, from which 95% confidence intervals were constructed. A ρ_{ij} estimate falling outside this confidence limit is considered significant. If genetic structure exists, then we expect a pattern of significant values at shorter distance classes becoming non-significant or negative with increasing distance. Finally, to test whether the slope (b) of the correlograms obtained for ρ_{ij} was statistically significant, ρ_{ij} estimates were permuted (999 times) with respect to the upper bound (m) of each distance class under the null hypothesis $b=0$.

Simulations

I used the simulation program BOTTLESIM (Kuo and Janzen 2003) to estimate the current levels of genetic variation in fragmented and continuous populations, forecast their future genetic diversity levels and make recommendations with respect to sustainable population size. The program allows specification of an arbitrary population size and projects the decline genetic diversity due to genetic drift based on the actual allele frequencies estimated from the genotypic data input.

In order to project the most realistic projections of decline in genetic variation for Bigleaf maple, I used the over-lapping generation model of the program. Other parameters during the simulation process were set as follows: degree of generation overlap = 100 (i.e. all individuals start with random age value that is within the longevity limit), monoecy with random mating and selfing reproductive system, expected longevity = 125 years, age of reproductive maturation = 10 years (Minore and Zasada 1990), number of years simulated = 250 years, effective population sizes $N_E = 50$ and $N_E = 100$ respectively for both continuous and fragmented populations, and number of iterations = 1000.

I compared the empirical data to the simulation results in order to determine whether the levels of genetic variation found in fragmented populations will be lower than those in continuous populations. If the fragmented populations show levels of genetic variation that are lower relative to continuous populations, then the empirical data are consistent with the hypothesis that fragmentation affects *Acer macrophyllum* populations. In contrast, if there is no decrease in values of A_O and H_E respectively in fragmented populations relative to continuous populations, then the empirical data are consistent with the hypothesis that fragmentation has not affected populations of *Acer macrophyllum*.

RESULTS

Allele frequencies

Eight of the nine loci were polymorphic in at least one of the populations examined for both seedlings and adults. In all populations sampled, GDH was monomorphic for both adults and seedlings. Two to three alleles were detected for each polymorphic locus, with a total of 18 alleles observed for adults in continuous populations and 20 alleles in fragmented populations (Table 4.2 a). In seedlings, a total of 19 alleles were observed in continuous populations and 18 in fragmented populations (Table 4. 2 b). The majority of the alleles were common and distributed widely across most populations, but a few rare alleles were private, unique to one population, or found only in couple of populations. The distribution of allele frequencies was the typical U-shaped, with most alleles either rare or nearing fixation (Fig 4.2a-b). Exact test for linkage disequilibrium did not yield any significant values for seedlings or adults in any populations, indicating independence of loci used in this study.

Genetic diversity

In all six populations, both seedling and adult cohorts possessed similar levels of genetic variation regardless of whether populations were fragmented or continuous (Table 4.3). The mean number of alleles per locus for adults averaged 1.66 in continuous populations and 1.60 in fragmented populations, these estimates were 1.73 and 1.66, respectively, in seedlings. Expected heterozygosity was slightly higher in adults in fragmented (0.134) than continuous populations (0.120), but slightly lower for seedlings (0.130 versus 0.140). Seedlings in continuous populations had a slightly higher proportion of polymorphic loci than seedlings in fragmented populations or in the adult cohorts (Table 4.3).

Levels of Inbreeding

Within all adult populations, genotypic frequencies showed significant departures from Hardy-Weinberg expectations ($P < 0.05$) with an excess of homozygotes. In the seedling cohort, one continuous population (Elk Falls) and one fragmented population (Maple Bay) did not deviate significantly from Hardy-Weinberg expectations. F_{IS} varied considerably among both loci and populations, with overall values of 0.20 for adults and 0.28 for seedlings in continuous populations, and 0.25 in adults and 0.37 for seedlings in fragmented populations, suggesting substantial levels of inbreeding (Table 4.3).

Bottleneck test

There was no significant bottleneck signature in any of the populations. Recently bottlenecked populations should show a mode shift of distribution in allele frequencies so that alleles in low frequency classes (< 0.1) become less abundant than intermediate and high frequency classes. The bottleneck program did not show any significant mode shift, thus, all allele frequency distributions were U-shaped (Fig 4.2a-b), moreover, the Wilcoxon test detected more heterozygosity than expected under mutation-drift equilibrium (Table 4.4).

Genetic structure

Eight polymorphic loci were consistently scored, of which PGI-2, LAP-2, and IDH had high gene diversities (H_T) in excess of 20%, suggesting adequate variation for appreciable genetic structure (Table 4.5 a-b). F_{IS} and F_{IT} estimates were positive and significantly greater than zero, suggesting a deficit of heterozygotes. However, individual loci showed a great deal of variation in their fixation indices. For instance,

AAT-1, 6PG-2, PGI-1, and PGI-2, show significant excess of heterozygotes, while AAT-2, IDH, and LAP-2 show a significant deficiency, in a manner consistent with inbreeding (Table 4.5 a-b).

There was a low but significant amount of genetic differentiation among adult populations both in the continuous and the fragmented forests, with a mean F_{ST} across loci of 0.015 (95% CI = 0.005 – 0.035) for continuous populations (Table 4.5 a) and 0.031 (95% CI = 0.010 – 0.056) for fragmented populations (Table 4.5 b). Pairwise F_{ST} estimates among populations (Table 4.6) were also low in all cases suggesting extensive gene flow between these populations or a recent common ancestral population (Table 4.6).

Spatial genetic structure

Analysis of spatial genetic structure revealed significant, positive spatial genetic structure in four of the six populations. All fragmented populations (Fig 4.3 d-f) had significant, positive multilocus coancestry (p_{ij}) coefficients at inter-tree distances of up to 100 m distance for Rosewall and Maple Bay, and up to 200 m for Yellow Point. For instance, in the 50-100 m distance class for these fragmented populations, p_{ij} ranged from 0.13 to 0.30, averaging 0.22. This estimate suggests that in fragmented populations of *Acer macrophyllum*, trees sampled up to approximately 100 m apart are likely to be nearly as similar as full-sibs. Beyond the 50 – 100 m distance class, genetic structuring remained significant ($\alpha = 0.05$) but less pronounced up to approximately 600 m, then became non-significant or negative up to 2500 m. Of the continuous populations, only Elk Falls revealed significant spatial genetic structure, with a positive coancestry coefficient ($p_{ij} = 0.14$) for only the 100 – 200 m distance class. The overall slopes of the correlograms for all three fragmented populations and for Elk Falls were negative and significant ($P < 0.05$), indicating spatial genetic structure for these populations.

Simulations

Estimates for simulations of the observed number of alleles (A_0) and expected heterozygosity (H_E) likely to be retained over a 250-year period compared to the current levels are summarized in Table 4.7. The observed number of alleles decline slightly faster than expected heterozygosity consistent with theoretical predictions (Nei et al. 1975; Chakraborty 1980). However the decline is much faster when $N_E = 50$ than when $N_E = 100$ (Table 4.7). Based on actual allele frequencies, over 90% of expected heterozygosity would be retained for both fragmented and continuous populations over two generations (after 250 years) irrespective of whether $N_E = 50$ or $N_E = 100$ (Table 4.7).

DISCUSSION

Effects of fragmentation on genetic variation and inbreeding

Habitat fragmentation can cause a loss of population genetic variation in two ways. First, a transient reduction in population size could result in a substantial loss of alleles (Frankel and Soulé 1981; Young et al. 1996). The extent to which this occurs is dependent on the extent and pattern of forest loss, and its coincidence with any fine-scale genetic structure. An immediate loss of heterozygosity would, however, only be evident if the population size was greatly reduced. Second, subsequent to this initial allelic loss, fragmented populations that remain small and isolated for several generations will continue to lose alleles due to genetic drift, further reducing levels of genetic variation within the stands (Barrett and Kohn 1991; Ellstrand and Elam 1993).

Heterozygosity is mostly affected by intermediate-frequency alleles (Taggart et al. 1990), whereas rare alleles are the most likely to be lost in small or fragmented populations and high frequency alleles are likely to become fixed. Nearly all of the differences observed in number of alleles in this study were caused by low frequency alleles (<0.10). (LAP-2, 2 being the one exception).

There are three likely explanation for the maintenance of genetic variation in fragmented populations: (i) There have been insufficient generations since fragmentation for detectable loss of diversity through genetic drift and inbreeding or for mutation and genetic drift to generate differences among populations; (ii) Despite fragmentation, effective population size (N_e) remains large so that initial loss of heterozygosity is very small, since the proportionate reduction in expected

heterozygosity $\Delta H_e = \frac{1}{2N_e}$. Even though populations are fragmented, they could still

have hundreds or even thousands of individuals (depending on the neighborhood size); (iii) Gene flow is sufficient and there was no isolation by distance in fragmented population.

I hypothesized that one effect of fragmentation would be decreased heterozygosity and polymorphism in seedlings compared to adults in fragmented populations, but did not detect evidenced of this. On the contrary, polymorphism was higher in seedlings than adults in both continuous and fragmented populations, and most alleles found in seedlings were common and widespread across all populations, just as in adults, suggesting high gene flow. González-Astorga and Núñez-Farfán (2001) found low frequency alleles in seedlings of *Brongniartia vazquezii* a monoecious, animal pollinated shrub in Central Mexico, which were not found in adult populations and attributed this to gene flow. There is no reduction of genetic variation in fragmented compared to continuous populations in either adults or seedlings, indicating there may be substantial gene flow among fragmented populations or that fragmented populations have large effective population sizes (Levin and Kerster 1974; Young et al. 1996).

Similar studies conducted by Young et al. (1993) on *Acer saccharum* also found no reduced genetic variation in fragmented populations compared to continuous populations. Combined with higher mean levels of polymorphism in fragmented populations, this indicated increased gene flow may be a consequence of fragmentation. Similarly, Foré et al. (1992) compared inter-population genetic divergence between pre-fragmentation and post-fragmentation seedling and adult cohorts in 15 *A. saccharum* populations in Ohio, USA. In their study, genetic divergence in post-fragmentation cohorts was less than half that of continuous populations, indicating a reduction in genetic differentiation since fragmentation, and suggesting increased inter-population gene flow. It appears therefore that maple species may be resilient to fragmentation.

The results obtained from this study are somewhat in contrast to similar studies conducted on some angiosperm trees species in the tropics that showed some effects of fragmentation on the overall genetic structure. (e.g. *Swietenia humilis*; White et al. 1999; and *Spondias mombin*, (Nason and Hamrick 1997)). The authors attributed their results mainly to the demographic and reproductive characteristics of these species in the tropics. For instance, many tropical trees occur at low densities, are pollinated by animals, have high outcrossing rates, and have breeding systems that involve complex mechanisms of self-incompatibility (Bawa 1990; Hamrick and Murawski 1990).

Inbreeding in adults versus seedlings

In this study a high proportion of the allozyme loci were not in Hardy-Weinberg equilibrium, with significant inbreeding (F_{IS}) (Table 4.3). Deviations from Hardy-Weinberg expectations due to nonrandom mating within fragmented populations of either adults or seedlings would be expected across all loci. However, the high values of F_{IS} were not consistent for individual loci or within a particular cohort or population type. For instance, adults in all populations had significant inbreeding, but seedlings in the Elk Falls and Maple Bay populations did not differ from Hardy-Weinberg equilibrium. Two genetically unlinked loci (6PG-1 and LAP-2) contributed to the overall high estimates of F_{IS} in both adults and seedlings. For, instance if these two loci are removed from seedlings in Rosewall, F_{IS} estimated drops from 0.57 to 0.39, reducing the inbreeding estimate by 33%. There are two possible explanations for the high F_{IS} observed in this study. First, if population sub-structuring has been ignored in sampling, the inbreeding coefficient would be overestimated, i.e., the patchy distribution of related individuals may generate a Wahlund effect (Barbujani 1987). Second, there may be a significant amount of inbreeding occurring in these populations (see chapter three). Fragmentation, coupled with localized pollinator movement and seed dispersal, may have resulted in higher correlated paternity or

selfing for these sampled populations compared to continuous populations, causing a deficiency of heterozygotes. As suggested by Shea (1990), inbreeding could also result from differential selection pressures resulting from micro-environmental variations favoring related individuals. While the evidence of inbreeding was shown in both continuous and fragmented populations for both adults and seedlings, seedlings had higher levels of inbreeding compared to their adult cohorts in four of the six populations (all but Elk Falls and Maple Bay). Positive values of F_{IS} at the seedling stage may be due to partial selfing. In *Shorea leprosula*, an insected pollinated dipterocarp with the highest isozyme heterozygosity ($H_E = 0.40$) ever recorded in long lived plant species (Lee et al. 2000), a higher inbreeding level found for seedlings in natural populations compared to adults was attributed to selection against homozygotes between the seedling and adults stages. However in *Eucalyptus regnans*, elevated inbreeding found in natural populations compared to seedlings in a seed orchard was explained by spatial genetic structure (Muona et al. 1990). This also could be the case with *A. macrophyllum*, in which trees in natural populations sometimes exist in clumps. Inbreeding may also be due to mating between relatives in these clumps.

Population structure

Mean F_{ST} estimates indicate weak but significant population differentiation in this species, among both fragmented ($F_{ST} = 0.031$) and continuous ($F_{ST} = 0.015$) populations (Table 5.5 a, b). This is consistent with earlier findings of low inter-population differentiation in a range-wide genetic study of this species (chapter three), and suggests that stands sampled on Vancouver Island, BC, may essentially form single large population with weak within-population structure.

Spatial genetic structure

The degree of spatial genetic clustering within a population is determined by a variety of genetic and demographic factors including population size, micro-environmental selection, seed and pollen dispersal, plant density, temporal variation in population reproductive rates, patterns of competition-induced mortality and other sources of mortality, spatial scale of gap formation and possibly other details of the regeneration process (Frankel et al. 1995). In particular, the magnitude and spatial scale of genetic structure is strongly influenced by seed dispersal mechanisms and adult density that characterize individual species (Hamrick et al. 1993; Hamrick and Nason 1996; Doligez and Joly 1997; Kalisz et al. 2001).

Spatial genetic structure in this study revealed apparent differences between continuous and fragmented populations. The distribution of genotypes in all fragmented populations was non-random with significant positive values for coancestry (ρ_{ij}) up to 100 m or 200 m whereas in two of the three continuous populations, genotypes were distributed randomly (non-significant coancestry estimates) with weak or no spatial genetic structure. The observed (ρ_{ij}) values for two of the continuous populations are much less than that expected for full or half-sibs at all distances, suggesting overlapping of seed shadows from maternal parents. The strong spatial genetic structure observed in fragmented populations could be due to the high degree of seed production favoring regeneration of seedlings in the neighborhood of the mother trees as seeds of bigleaf maple are dispersed by wind and gravity. In contrast, trees of bigleaf maple that grow in continuous forests develop narrow a crown that is supported by a stem free of branches for more than half of its total height due to strong competition for light (Minore and Zasada 1990). This habit may lead to low seed production or a smaller seed shadow thereby reducing the number of siblings that are likely to develop around the neighborhood of the mother trees (Kelly et al. 2004). The higher species richness (number of species per hectare) of the forest ecosystem in continuous forests and the habitat of dispersal agents may

also lead to a reduction in spatial genetic structure in continuous populations. For example, some small mammals such as mice, wood rats, squirrels and birds as reported by Fowels (1965), can collect fruits from different bigleaf maple trees and disperse them in the forest.

Hamrick et al. (1993) and Hamrick and Nason (1996) suggested that plant species with high adult densities have weaker fine-scale genetic structure than species with lower densities. During sampling, continuous populations appeared to have higher species densities than fragmented populations. This study is consistent with the findings of Gapare and Aitken (2005) for *Picea sitchensis* in which core populations with higher densities had no spatial structure, while peripheral populations with lower density had strong spatial genetic structure up to 500 m. In addition, Vekemans and Hardy (2004) re-analyzed data for six species and compared spatial genetic structure for differing population densities with species classified as low or high density. In each of the six pairwise comparisons, populations with low densities consistently revealed spatial genetic structure. These findings suggest that relatively high population density in continuous forests compared to fragmented populations could have a strong influence on spatial genetic structure.

The lack of spatial genetic structure observed in the two continuous populations (Nitinat and Port Alberni) is similar to that observed for a number of other tree species (e.g. *Pinus contorta* (Epperson and Allard 1989); *Picea mariana* (Knowles 1991); *Pinus banksiana* (Xie and Knowles 1991); and *Neolitsea sericea* (Chung et al. 2000)). In contrast, in some tree species with restricted seed dispersal, significant spatial genetic structure was detected. For, example *Quercus rubra* (Sork et al. 1993); and *Quercus petraeae* (Streiff et al. 1998) exhibit spatial genetic structure at short spatial scales which was attributed to large, gravity dispersed seed, as pollen movement by wind is known to be extensive in those species. Strong spatial genetic structure was also found in tree species featuring restricted dispersal of both seed and pollen due to

pollination by small insects and seed dispersal by gravity, for example, in *Eurya emarginata* (Chung et al. 2000).

The high values of F_{IS} (Table 4.3) indicate that some populations of bigleaf maple experience appreciable inbreeding. In the four populations where genetic structure was evident, aggregation of genotypes most likely resulted from limited seed dispersal. Similar studies conducted recently by Kevin et al. (2004) in two *Shorea* spp. attributed spatial genetic structure to limited seed dispersal.

Computer simulations of fragmentation effects

The results obtained from the computer simulations suggest that fragmented populations would maintained well over 90% of their genetic variation over a 250-year period even with an effective population size of $N_E = 50$. When the effective population size is increased to $N_E = 100$ in the simulation both continuous and fragmented populations on average retained over 97% of expected heterozygosity. These results suggest that an effective population size of at least 100 can effectively minimize the decline of genetic diversity for bigleaf maple populations. Overall the simulation projections corroborates my earlier findings about maintenance of genetic variation in fragmented populations and might further help explain why fragmented populations maintain the same levels of genetic variation as the continuous populations.

It has been suggested that fragmentation represents a significant threat to the long-term survival of many plant species (Templeton et al. 1990; Young et al. 1996). In addition, it has been argued that erosion in genetic variation is one of the important consequences that fragmentation may have on plants species that remain in the smaller patches due to genetic drift, reduction in gene flow, and elevated inbreeding (Templeton et al. 1990; Young and Merriam 1994). In this thesis, using both empirical data and simulation projections, I have shown that fragmentation has not led to overall reduction in genetic variation nor elevated levels in inbreeding at this time in *Acer*

macrophyllum and is not likely to in the near future unless populations are very small and no gene flow occurs.

Lastly, I argued that I did not detect any significant impacts of fragmentation on the overall genetic variation on bigleaf maple populations. This argument must be taken with caution in view of the fact that with only three fragmented and three continuous populations, statistical power was weak. In a power test conducted using the SAS Analyst module (SAS Inc. 1994) with both a one- and two- tailed test with $\alpha = 0.05$, statistical power ranged from 6% to 12.5%. However, no trends were seen in overall genetic variation between continuous and fragmented populations, so the lack of significant differences did not seem to be a function of the power of the statistical tests.

Table 4.1. Summary of population information for adult trees and seedlings of *Acer macrophyllum*.

Forest type	Population	Size of plot or forest area (ha)	No. of trees analysed	No. of seedlings analysed
Continuous	Nitinat	312	40	50
	Elk Falls	102	50	50
	P. Alberni	112	50	50
Fragmented	Maple Bay	170	46	50
	Rosewall	79	45	50
	Yellow Pt.	278	50	50
Total			281	300

Table 4.2 a. Allele frequencies for nine loci for adults in continuous and fragmented populations of *Acer macrophyllum*.

Locus	Alleles	Continuous			Fragmented		
		NIT	ELF	PAL	MBY	RWC	YPT
AAT-1	1	0.990	1.000	1.000	0.957	0.978	0.969
	2	0.010	0.000	0.000	0.043	0.022	0.031
AAT-2	1	0.857	0.733	0.900	0.910	0.845	0.944
	2	0.143	0.244	0.100	0.090	0.131	0.056
	4	0.000	0.022	0.000	0.000	0.024	0.000
IDH	1	0.900	0.809	0.840	0.716	0.784	0.864
	2	0.100	0.191	0.160	0.261	0.216	0.136
	4	0.000	0.000	0.000	0.023	0.000	0.000
6PG-1	1	0.944	1.000	0.920	1.000	0.901	1.000
	2	0.056	0.000	0.008	0.000	0.099	0.000
6PG-2	1	0.860	0.990	0.920	1.000	0.922	1.000
	2	0.140	0.010	0.080	0.000	0.078	0.000
PGI-1	1	1.000	0.970	1.000	1.000	1.000	0.938
	2	0.000	0.030	0.000	0.000	0.000	0.063
PGI-2	1	0.850	0.818	0.859	0.744	0.833	0.878
	2	0.000	0.000	0.000	0.023	0.000	0.000
	3	0.150	0.182	0.141	0.233	0.167	0.122
GDH	1	1.000	1.000	1.000	1.000	1.000	1.000
LAP-2	1	0.949	1.000	0.910	0.837	0.956	0.739
	2	0.051	0.000	0.090	0.163	0.044	0.261

Note: Populations abbreviations. NIT= Nitinat; ELF = Elk Falls; PAL = Port Alberni; MBY = Maple Bay; RWC = Rosewall Creek; YPT = Yellow Point.

Table 4.2 b. Allele frequencies for nine loci studied for seedlings in continuous and fragmented populations of *Acer macrophyllum*.

Locus	Alleles	Continuous			Fragmented		
		NIT	ELF	PAL	MBY	RWC	YPT
AAT-1	1	0.900	1.000	1.000	0.925	1.000	1.000
	2	0.100	0.000	0.000	0.075	0.000	0.000
AAT-2	1	0.906	0.768	0.904	0.780	0.917	0.936
	2	0.094	0.232	0.096	0.220	0.083	0.064
IDH-1	1	0.833	0.727	0.800	0.739	0.833	0.811
	2	0.167	0.273	0.200	0.261	0.167	0.189
6PG-1	1	0.851	1.000	0.978	1.000	0.851	0.978
	2	0.149	0.000	0.022	0.000	0.149	0.022
6PG-2	1	0.956	0.929	0.917	0.952	0.978	0.929
	2	0.044	0.071	0.083	0.048	0.022	0.071
PGI-1	1	1.000	0.928	1.000	1.000	1.000	0.901
	2	0.000	0.072	0.000	0.000	0.000	0.099
PGI-2	1	0.775	0.761	0.807	0.784	0.788	0.818
	2	0.050	0.102	0.023	0.102	0.038	0.023
	3	0.175	0.136	0.170	0.114	0.175	0.159
GDH	1	1.000	1.000	1.000	1.000	1.000	1.000
LAP-2	1	0.939	1.000	0.837	1.000	0.959	0.867
	2	0.061	0.000	0.153	0.000	0.041	0.133
	3	0.000	0.000	0.010	0.000	0.000	0.000

Table 4.3. Genetic diversity estimates for adults and seedlings in continuous and fragmented populations of *Acer macrophyllum*.

Populations	N	A	%P	Ho	He	F_{IS}
<i>Continuous</i>						
Nitinat						
Adults	50	1.6	60	0.101±0.037	0.116±0.037	0.17*
Seedlings	47	1.8	70	0.091±0.042	0.142±0.0436	0.39*
Elk Falls						
Adults	49	1.6	60	0.103±0.048	0.122±0.053	0.25*
Seedlings	47	1.6	50	0.124±0.048	0.144±0.056	0.100 ^{NS}
P. Alberni						
Adults	50	1.5	50	0.083±0.030	0.113±0.036	0.17*
Seedlings	47	1.9	70	0.090±0.035	0.134±0.043	0.36*
Mean						
Adults		1.6	56.6	0.095	0.120	0.20
seedlings		1.8	63.3	0.102	0.140	0.28
<i>Fragmented</i>						
Maple Bay						
Adults	44	1.7	50	0.104±0.046	0.146±0.055	0.34*
Seedlings	47	1.6	50	0.109±0.044	0.133±0.054	0.13 ^{NS}
Rosewall						
Adults	44	1.7	60	0.095±0.036	0.130±0.043	0.20*
Seedlings	47	1.8	70	0.076±0.037	0.130±0.042	0.57*
Yellow Pt						
Adults	48	1.6	60	0.095±0.029	0.126±0.042	0.22*
Seedlings	47	1.8	70	0.074±0.032	0.128±0.039	0.41*
Mean						
Adults		1.7	56.6	0.098	0.134	0.25
seedlings		1.7	63.3	0.086	0.130	0.37

Table 4.4. Wilcoxon signed ranked test for recent bottleneck (Cornuet and Luikart 1996) in *Acer macrophyllum* populations under the Infinite Alleles Model.

Population	Number of loci with H_E excess	Wilcoxon test	
	Exp	$H_E > H_{eq}$ P	$H_E < H_{eq}$ P
Nitinat	3.11	0.9609	0.0546
Elk Falls	2.86	0.5781	0.5000
P. Alberni	2.47	0.7187	0.3437
Maple Bay	3.62	0.3711	0.6796
Rosewall Creek	2.79	0.9453	0.0781
Yellow Point	2.99	0.6562	0.4218

Note: Exp = expected number of loci with a heterozygosity excess; H_E = expected Heterozygosity; H_{eq} = heterozygosity expected at mutation drift-equilibrium.

Table 4.5. Genetic diversity statistics for the eight polymorphic isozyme loci for continuous populations (a) and fragmented populations (b).

(a)

Locus	H_T	H_S	F_{IS}	F_{IT}	F_{ST}
AAT-1	0.008	0.008	-0.002	0.001	0.003
AAT-2	0.279	0.272	0.229	0.258	0.038
IDH	0.269	0.271	0.626	0.622	-0.010
6PG-1	0.111	0.108	0.075	0.098	0.015
6PG-2	0.133	0.130	-0.100	-0.054	0.042
PGI-1	0.020	0.020	-0.020	-0.001	0.019
PGI-2	0.268	0.268	-0.179	-0.186	-0.006
LAP-2	0.215	0.212	0.392	0.402	0.016
Mean	0.170	0.168	0.140	0.149	0.015
SE			0.175	0.171	0.012
95%CI			(-0.077, 0.480)	(-0.079, 0.486)	(-0.005, 0.035)

(b)

Locus	H_T	H_S	F_{IS}	F_{IT}	F_{ST}
AAT-1	0.063	0.063	-0.025	-0.032	-0.007
AAT-2	0.183	0.182	0.517	0.519	0.006
IDH	0.337	0.339	0.595	0.600	0.010
6PG-1	0.165	0.154	0.267	0.281	0.045
6PG-2	0.051	0.048	-0.073	0.000	0.068
PGI-1	0.032	0.032	-0.056	-0.002	0.051
PGI-2	0.283	0.283	-0.167	-0.147	0.017
LAP-2	0.270	0.257	0.377	0.421	0.071
Mean	0.174	0.169	0.178	0.204	0.031
SE			0.185	0.179	0.014
95%CI			(-0.070, 0.507)	(0.041, 0.518)	(0.010, 0.056)

Table 4.6. Pairwise F_{ST} between adult fragmented (MBY, RWC YPT) and continuous (NIT, ELF, PAL) populations of *Acer macrophyllum*.

	NIT	ELF	PAL	MBY	RWC	YPT
NIT	--					
ELF	0.024	--				
PAL	0.009	0.020	--			
MBY	0.031	0.016	0.018	--		
RWC	0.004	0.017	0.005	0.013	--	
YPT	0.034	0.029	0.023	0.023	0.047	--

Note in parenthesis: Populations abbreviations as in Table 4.2a.

Table. 4.7. Percentage of allozyme diversity retained over 250-year period based on computer simulations BOTTLESIM (Kuo and Janzen 2003) for adult populations of *Acer macrophyllum* in fragmented and continuous forests assuming 125-year generation length.

Populations	$N_E = 50$		$N_E = 100$	
	A_O	H_E	A_O	H_E
<i>Continuous</i>				
Nitinat	92.78	94.01	96.15	97.53
Elk Falls	85.88	95.14	88.57	97.23
P. Alburni	92.67	93.71	96.46	98.00
<i>Fragmented</i>				
Maple Bay	91.61	95.32	94.95	97.36
Rosewall	91.41	93.77	95.46	97.81
Yellow Pt.	89.60	93.77	96.69	97.08

Figure 4.1. Geographic locations of bigleaf maple populations sampled on Vancouver Island.

Figure 4.2. Distribution of allele frequencies for adults (a) and seedling (b).

Filled bars are continuous populations and open bars fragmented populations.

Figure 4.3 (a-c). Spatial correlograms of coancestry coefficients (ρ_{ij}) for continuous populations of *Acer macrophyllum*. Dashed lines represent upper and lower 95% confidence limits for ρ_{ij} under the null hypothesis that genotypes are randomly distributed.

Figure 4.3 (d-f). Spatial correlograms of coancestry coefficients (p_{ij}) for fragmented populations of *Acer macrophyllum*. Dashed lines represent upper and lower 95% confidence limits for p_{ij} under the null hypothesis that genotypes are randomly distributed.

Chapter five

GENETIC VARIATION AND POPULATION STRUCTURE IN BIGLEAF MAPLE: A COMPARISON OF ALLOZYME MARKERS AND QUANTITATIVE TRAITS

INTRODUCTION

Knowledge of genetic variation and population structure is necessary for understanding and conserving the evolutionary potential of populations (Wright 1951). Patterns of genetic variation can be detected at both among and within-population levels (e.g. Hamrick et al. 1992; Xie and Ying 1996). Levels of genetic variation and degree of genetic control also vary among traits, ages and environments (Mullin et al. 1995; Aitken et al. 1995; Wu et al. 1995; Xie and Ying 1996).

Langlet (1971) noted that the maintenance of intra-specific variation in natural populations of plants is complex. Patterns of geographic variation result from the joint actions of underlying mechanisms that affect the associations between environmental and genetic heterogeneity, such as different selection pressures, levels of gene flow, and genetic drift (Lindhart and Grant 1996).

The use of molecular markers has several limitations in providing information that could be used to define conservation and management strategies (Lynch 1996). This is because the primary aim of conservation genetics is to quantify and maintain the evolutionary potential of a species. For this reason, studies should include an assessment of genetic variation for traits affecting fitness, many of which are polygenic (Petit et al. 2000). Most molecular genetic markers are considered selectively neutral, while the pattern of quantitative trait variation is likely to be driven by environmental factors resulting in different selection pressures in different locations (Petit et al. 2000; Lynch et al. 1999). A

comparison of molecular and quantitative measures of genetic variation allows insights into the different modes of evolution in sub-divided populations.

Studies of patterns of genetic differentiation of quantitative traits are not uncommon in forest trees. However, only a fraction of these allow for the estimation of Q_{ST} , the parameter estimating the portion of total quantitative genetic variation due to among-population differences (Spitze 1993). It is expensive to conduct sufficiently large common garden experiments with trees to include both populations and families within populations to obtain among and within-population genetic diversity estimates. As a result, the availability of such estimates in the literature is biased towards small, short-lived organisms (Ritland 2000). Studies that have reported joint estimates of quantitative (Q_{ST}) and molecular (F_{ST}) estimates of genetic variation among populations in plants include, *Pseudotsuga menziesii* (Rehfeldt 1978; Campbell 1986), *Populus balsamifera* (Riemenschneider et al. 1992), *Picea glauca* (Li et al. 1992; Jaramillo-Correa et al. 2001), *Daphnia obtusa* (Spitze 1993; Lynch et al. 1999), *Clarkia dudleyana* (Podolsky and Holtsford 1995), *Pinus contorta* ssp. *latifolia* (Yang et al. 1996), *Populus tremuloides* (Thomas et al. 1997), *Quercus petraeae* (Kremer et al. 1997), *Cerastium arvense* (Quiroga et al. 2002), and *Pinus pinaster* (González-Martínez et al. 2002). Merilä and Crnokrak (2002), in their meta-analysis comparing such studies of genetic differentiation at marker loci and quantitative traits, found that the degree of genetic differentiation coding quantitative traits (Q_{ST}) typically exceeds that of presumably neutral genetic markers (F_{ST}). These results have been attributed to the role of differential natural selection among populations in determining the population genetic structure of quantitative traits.

In forests of the Pacific Northwest of North America, bigleaf maple (*Acer macrophyllum*) is an important component of biodiversity, and a species of growing economic importance. However, breeding programs have not yet been initiated. Common garden experiments including provenance trials are being conducted to screen genetic variation in natural populations and to allow selection of the best available genotypes for reforestation or for breeding (Wright 1976). In addition,

provenance research also aims to define the genetic and environmental components of phenotypic variation between trees from different geographic regions (Morgenstern 1997). In this chapter, I use a provenance/progeny common garden experiment to estimate quantitative genetic parameters, and compare genetic differentiation among populations at allozyme loci with quantitative variation.

MATERIALS AND METHODS

In 1995 and 1996, seeds from 14 populations were collected from across the portion of the species range of distribution in British Columbia by the Ministry of Forests (Table 5.1 and Figure 5.1). Nine out of the 14 populations were located on Vancouver Island. Populations selected ranged from 48°22' to 50°21' N latitude, 121°23' to 126°35' W longitude, and 14 to 600 m elevation (Table 5.1).

QUANTITATIVE TRAITS

Seeds from 148 open-pollinated families from 14 provenances in total were sown in 614 Styroblocks[®] in mixture of peat and vermiculite in early December 1995, and maintained at 5.2°C minimum and 10°C maximum temperature in a greenhouse. Regular misting four times per day continued throughout the germination period. Germination started in mid-January, 1996. Germination of all provenances was nearly complete by the end of February. Seedlings were fertilized in February and March and moved to a cooler greenhouse for acclimatization on March 18. Overall, only about 45.3% of the seedlings germinated. Germination rates ranged from 8-86% among population and 0-100% among families. However there was no clear geographic pattern in germination rate.

The common garden test was planted from April 29 to May 1, 1997 at Surrey, British Columbia. The experiment was laid out in a split-plot design in four randomized blocks with provenances as main plots, and five-tree family rows as subplots. A total of 2925 seedlings were planted. Maintenance of the experiment to ensure high survival and good seedling growth included weeding, watering and fencing against deer.

Data collection

Height growth was measured at the end of the second year in the field (1998), third (1999) and fourth year (2000). Diameter was measured for all provenances at the end of the growing season in year three (1999). Phenological data (bud flush) was monitored and recorded two to three times a week from March 2002 to mid-May 2002. Julian bud flush data was defined by when the first unfolded leaf was observed. With the exception of bud flush, data measurements of height and diameter were made available to me from the BC Ministry of Forests.

Analysis

Analysis of variance (ANOVA) was conducted using PROC GLM (SAS Institute Inc. 1990) for height, diameter and bud flush traits using the following general linear models;

$$Y_{ijkl} = \mu + B_i + P_j + PB_{ji} + F(P)_{k(j)} + F(P)B_{k(ij)} + \varepsilon_{l(ijk)} \quad (1)$$

Where:

Y = measurement of seedling l from family k in provenance j in block i

μ = overall mean

B_i = effect of block i

P_j = effect of provenance j

PB_{ji} = interaction effect of block with provenance

$F(P)_{k(j)}$ = effect of family within provenance

$F(P)B_{k(ij)}$ = interaction effect of block with family within provenance

ε = experimental error

All effects in the model were assumed to be random. Variance components for all traits were estimated using the PROC VARCOMP (METHOD=REML) procedure (SAS Institute Inc. 1990). In addition, the GLM procedure type III sums of squares (SAS, 1990) was used to estimate the proper F-test for family and provenance effects with the null hypotheses (Ho): No family or provenance effects. I used provenance-by-block interaction (PB) as the error term to test provenance effect and family within provenance-by-block interaction (F(P)B) as the error term to test for family effect. The amount of genetic variation in growth traits and bud flush was quantified by estimating the family variances and testing their significance ($P < 0.05$). Individual tree and family heritabilities were estimated as follows:

$$\text{Individual heritability: } h^2_i = 3\sigma^2_{F(P)} / \sigma^2_{F(P)} + \sigma^2_{B \times F(P)} + \sigma^2_E \quad (2)$$

$$\text{Family heritability: } h^2_f = \sigma^2_{F(P)} / \sigma^2_{F(P)} + \sigma^2_{B \times F(P)} / b + \sigma^2_E / n \quad (3)$$

All variables are defined above with the exception of b and n which are number of blocks and number of trees in plot respectively. For estimating individual heritability, the additive genetic variance was estimated as three times the family variance (instead of four times the family variance) as suitable for half-sib progenies. It is assumed that *Acer macrophyllum* open-pollinated progenies are more closely related than half-sibs in view of the high inbreeding in this species and the relatively low number of effective pollen parents (Iddrisu and Ritland 2004). The standard errors of heritability estimates were calculated following Dickerson (1969).

To assess the associations among traits for both growth and bud phenology (bud flush), genetic correlations (r_g) between pairs of traits were calculated following Falconer (1989) as follows:

$$r_g = \text{Cov}_{F(x,y)} / (\sigma^2_{F_x} \sigma^2_{F_y})^{1/2} \quad (4)$$

where $\text{Cov}_{F(x,y)}$ is the family covariance between traits x and y , and $\sigma^2_{F_x}$ and $\sigma^2_{F_y}$ are their corresponding family variances. $\text{Cov}_{F(x,y)}$ was calculated using the following relationship:

$$\text{COV}_{F(x,y)} = (\sigma^2_{F(x+y)} - \sigma^2_{Fx} - \sigma^2_{Fy}) / 2 \quad (5)$$

Phenotypic correlations for each pair of traits, as well as correlations between traits and geographic and climatic variables were estimated as Pearson's product moment correlations using the PROC CORR procedure (SAS Institute Inc. 1990). Climatic data were obtained using a method developed by Hamann and Wang (2005).

Wright's (1951) F-statistics provide a useful measure of the level of population genetic structure at neutral marker loci by quantifying the proportion of total allelic variation found within versus among populations. Similarly, population differentiation for quantitative traits can be estimated using Q_{ST} (Spitze 1993) which is analogous to the F_{ST} estimate for marker loci. It is estimated as:

$$Q_{ST} = \sigma^2_{GB} / (2 \sigma^2_{GW} + \sigma^2_{GB}) \quad (6)$$

where σ^2_{GB} is the among population component of variance and σ^2_{GW} the within population component of variance. The neutral expectation for Q_{ST} is equivalent to F_{ST} for selectively neutral genetic markers (Lande 1992).

ISOZYME VARIATION

Vegetative buds were collected in February 2001 from two of the four blocks (1460 trees) from all 14 populations in the common garden experiment. Buds were stored at -80°C until analyzed by isozyme electrophoresis. The trees sampled from each population for isozyme analysis were the same as those used for quantitative genetic analysis. Electrophoresis buffer systems and loci assayed are those described in chapter three.

Genetic data analyses were performed using BIOSYS-2, a modified version of the BIOSYS-1 program (Swofford and Selander 1981). The following parameters were estimated: allele frequencies, mean number of alleles per locus (A), percent of loci that were polymorphic ($\%P$) (with the most common allele having a frequency of 99% or less), observed heterozygosity (H_O) and expected heterozygosity ($H_E = 1 - \sum p_i^2$, where p_i is the frequency of the i th allele). To investigate the extent of population structuring and differentiation, F_{ST} (Wright 1965) was estimated for individual loci across the 14 populations.

RESULTS

Quantitative traits

Provenances differed significantly in growth traits ($p < 0.001$). All growth traits had similar patterns of variation at all ages. Provenance means for height, diameter and bud flush are presented in Table 5.1. The highest growth rates were observed in trees from Hope, Squamish, Port Alberni and Qualicum. The difference between the most productive provenance (Hope) and the least productive (Woss) in terms of height was about 36%.

The first bud flush was recorded on Julian day 105 day. All buds in the trial completely flushed by the 129th day. In general, flushing was variable among trees within provenances. Woss, Sayward and Owl, the three northernmost provenances (Table 5.1) flushed first and Metchosin, the southernmost provenance, flushed last, this latitudinal trend was weak.

For height growth and bud flush, block, population and family within population effects were all highly significant ($p < 0.001$) (Table 5.2). The family variance for height increase slightly from 1% of the total variance for height-2 to 2.3% for height-4 (Table 5.3).

Estimates of individual and family heritability for height were relatively low, ranging from 0.15 to 0.18. Family heritability ranged from 0.37 for height-2 to 0.40 for height-4 (Table 5.3). Timing of bud flush had the highest heritability estimate both for individuals (0.21) and families (0.91) (Table 5.3). Estimates of Q_{ST} values varied from 0.12 for bud flush to 0.26 for height-2 and averaged 0.17. For height, Q_{ST} values seemed to decrease with age (Table 5.3).

There were strong genetic correlations among heights at all ages (Table 5.4). Diameter was also strongly correlated with height growth. Timing of bud flush was weakly and negatively correlated with all growth traits. Phenotypic correlations were strongly correlated among growth traits at all ages and significant at $P = 0.01$ (Table 5.4). Height growth in all years was correlated with degree days above 5°C and bud flush was mainly correlated with continentality (Table 5.5).

Molecular genetic variability

Eight of the 10 loci analysed were polymorphic in at least one population. In all populations, GDH and LAP-1 were monomorphic. The percent of loci that were polymorphic ($\%P$) varied among populations between 30% and 60%, averaging 43.5%. The mean number of alleles per locus (A) ranged from 1.3 to 1.5, averaging 1.37 (Table 5.6). The expected heterozygosity within populations ranged from 0.071 to 0.134 and averaged 0.127 across the 14 populations studied.

The proportion of inter-population genetic differentiation among populations (F_{ST}) indicated that the vast majority of total variation resided within populations, with approximately 9% of the total variation occurring among populations (Table 5.7). Locus specific estimates ranged from 0.0653 for 6PGD-1 to 0.2243 for LAP-2.

DISCUSSION

QUANTITATIVE TRAITS

The provenances of *Acer macrophyllum* sampled did not exhibit high germination rate under nursery conditions, with an overall mean of 45.3% and a range from 0% to 86% among provenances. The low germination rate, assuming seeds were healthy and well handled, may suggest that growth conditions in the nursery were not optimal and under such conditions seedlings may not fully express genetic variation at an early age (Bongarten and Hanover 1985). In addition, seedling growth can suffer following transplanting from the nursery, which could also impact expression of genetic differences in early stages (Namkoong and Conkle 1976; Camussi et al. 1995). Provenances like Hope and Chilliwack that showed higher germination rates in the nursery (greenhouse) also showed higher height and diameter growth (Table 5.1).

Genetic variation in growth traits for bigleaf maple seedlings both among and within provenances was detected at an early age. Both provenance and family variance components were significant for bud flush and height at all ages but not for diameter. This pattern is similar to that reported for young lodgepole pine (Wu et al. 1995). Although provenance, block and family within population effects were significant for height, the largest variance component was block by family within provenance interaction (Table 5.3).

The narrow-sense heritabilities for individual (h^2_i) and family (h^2_f) were moderate and remained stable with age for height (Table 5.3). The individual heritability estimate for height growth in bigleaf maple in this study is on the low side compared with those reported for forest trees by Cornelius (1994). Franklin (1979) found diminishing heritability estimates for height growth with age, as competition increased with canopy closure. Other studies, however, have reported different age trends of individual heritability for height growth. For instance, Cotterill and Dean (1988) observed an increase in individual heritability for radiata pine (*Pinus radiata*) following thinning, followed by a decrease. On the contrary, Xie

and Ying (1996) reported a decrease followed by an increase after thinning a lodgepole pine (*Pinus contorta*) early selection test. It is therefore difficult to find a consistent pattern for heritabilities for growth traits with age or silvicultural treatment. Genetic parameters for quantitative traits need to be interpreted with caution, as they are applicable only to the defined base population, reference unit of selection and specific environments where studies are performed (Zobel 1984).

Bud flush

Several studies of bud flush phenology have reported that it is under moderate to strong genetic control (reviewed in Howe et al. 2003). For example, Howe et al. (2000) and Bradshaw and Stettler (1995) reported that heritability for bud flush was moderate for F₂ hybrid poplar. Other studies indicate that bud flush is under strong genetic control in Douglas-fir (Aitken and Adams 1997), in *Populus trichocarpa* (Thomas et al. 1997) as well as in other angiosperm and coniferous tree species (Bongarten and Hanover 1985; Chuine et al. 2000) than in bigleaf maple.

In this study, bud burst phenology for bigleaf maple varied significantly among families with moderate estimates for heritability. Notwithstanding, geographically based patterns of genetic variation have been observed for bud flush (e.g., Howe et al. 2000). For some species, trees from northern locations and high elevations will tend to flush earlier than those from southern locations, especially in common garden tests, because they have been exposed to shorter frost free seasons in their native environment, leading to selection of genotypes that have either a lower chilling requirements to break bud dormancy, or a lower heat sum or threshold temperature to initiate growth therefore begin growing earlier in a common garden than populations from milder climates in the spring (Farmer 1993). For example, northern provenances (Owl, Sayward and Woss) flushed slightly earlier than the southern provenances on average. If chilling requirements are met, bud flush is mainly in response to heat accumulation in the spring (Lavender 1981). In this study, it is presumed that, chilling requirements were met

and thus bud flush timing differences among families may reflect different heat sums or threshold temperature required for bud flush (Li and Adams 1993). This result corroborates the findings of Perry and Wu (1960) from another maple (*Acer rubrum*), in which buds from northern provenances flushed earlier than southern provenances or at the same time, depending on the temperature. The test site (Surrey, BC) experienced mild winter temperatures, and according to Hunter and Lechowicz (1992), under such natural conditions, the lack of chilling temperature will be less important than the lack of forcing temperature as an agent to speed up bud flush.

Genetic correlations

There were strong genetic correlations observed among growth traits (Table 5.4). These high genetic and phenotypic correlations could be due to either pleiotropy or maternal effects (contribution of the maternal parent to the offspring phenotype via some mechanism other than the transmission of genes, e.g. seed size). The presence of maternal effects can bias estimates of seedling genetic variance, heritability and genetic correlations, especially for height (Lambeth 1980). Hence, it would be useful to study growth patterns of bigleaf maple seedlings over more growing seasons, to investigate the extent of maternal effects and age trend in genetic control of growth traits (Lambeth 1980). High age-to-age genetic correlations between growth traits detected in this study suggest that selection for fast growing trees can be done at the early ages. However the interval between ages two, three and four is too short a time to realize significant changes in family ranks with tree age for the tested families. Therefore, caution should be taken when interpreting such genetic correlations, since they might be lower over long intervals (Rweyengeza et al. 2003).

Correlations with climatic variables

Correlations between geographic and climatic variable were moderate (Table 5.5) which may reflect the capacity of bigleaf maple to adapt to varying

environmental conditions (Jaramillo-Correa et al. 2001). Height growth was significantly correlated with mean annual temperature, and degree days above 5°C (DD5) and bud flush correlated with temperature differential (TD). Since differentiation in quantitative traits (Q_{ST} ; see below) is observed for these traits, according to Jaramillo-Correa et al. (2001) these quantitative traits may be under differential selection in response to regional differences in climatic factors. For instance, the mean annual temperature (MAT) and degree days above 5°C (DD5) for Owl average 4.79°C and 891 respectively while that in Hope was 10.41°C and 2022. As one would expect, trees from the milder climate (Hope) exhibited higher growth rates than Owl (Table 5.1).

F_{ST} vs Q_{ST}

Over the past few years, joint estimates of differentiation for quantitative traits and for molecular marker loci have shown two main patterns. Some species, such as *Daphnia obtusa* (Lynch et al. 1999) and *Arabidopsis thaliana* (Kuitinen et al. 1997), have a quantitative population structure essentially identical to that for molecular markers suggesting genetic structure for both quantitative and genetic markers is determined by drift and gene flow, whereas other plant species such as *Quercus petraeae* (Kremer et al. 1997) or *Clarkia dudleyana* (Podolsky and Holtsford 1995) have highly divergent populations to quantitative traits. The latter pattern is found in coniferous species. Yang et al. (1996) found differences between allozyme ($F_{ST} = 0.019$) and quantitative genetic differentiation for specific gravity ($Q_{ST} = 0.133$), stem diameter ($Q_{ST} = 0.166$), stem height ($Q_{ST} = 0.195$) and branch length ($Q_{ST} = 0.161$) in *Pinus contorta*. In this study, estimates of Q_{ST} for five quantitative traits varied from ($Q_{ST} = 0.12$) in bud flush, diameter, and height-4 to ($Q_{ST} = 0.26$) for height-2 (Table 5.3).

By comparing estimates of differentiation from quantitative traits (Q_{ST}) and isozymes (F_{ST}) we can examine whether evolutionary processes involved in quantitative and isozyme variation in *Acer macrophyllum* are similar or not. In meta-analyses of published results that compared population structure in markers

with that of quantitative traits, Mckay and Latta (2002) and Merilä and Crnorkak (2001) found that mean Q_{ST} is typically larger than but poorly correlated with mean F_{ST} across 29 species of plants, vertebrates and invertebrates. Spitze (1993) suggested three possible outcomes from the comparison of F_{ST} and Q_{ST} : 1) If $Q_{ST} > F_{ST}$, the implication is that natural selection rather than genetic drift alone must have been involved in shaping or favouring different phenotypes in different populations; 2) if $F_{ST} = Q_{ST}$, then genetic drift alone could be responsible in the population divergence and this could be evident in smaller populations; and 3) if $Q_{ST} < F_{ST}$, then it is most likely that natural selection is convergent in that the same phenotypes are favoured in different populations. Comparison of average estimates of Q_{ST} and F_{ST} in this study ($Q_{ST} = 0.17 > F_{ST} = 0.09$) according to Merilä and Crnorkak (2001) provides evidence of involvement of differential selection in shaping phenotypic variation in different populations. Growth traits such as height have been reported to be under differential selection in *Pinus contorta* (Yang et al. 1999) and *Picea glauca* (Jaramillo-Correa et al. 2001) because individual trees must grow rapidly to escape suppression from competition from neighbouring trees yet have a sufficiently conservative growth pattern to avoid frost injury, the risk of which varies locally. *Acer macrophyllum* is an early successional species, relatively shade tolerant and growing across a wide range of sites and climatic conditions. Differential adaptation to regional and local patterns of precipitation, temperature and other climatic variables seems to be the explanation for the divergence in these traits. Notwithstanding, it is worth noting that, geographic and environmental scale of sampling will affect the magnitude of Q_{ST} .

Some studies using isozyme markers have reported greater differences between Q_{ST} and F_{ST} than the current study (e.g., Prout and Barker 1993; Spitze 1993; Long and Singh 1995; Yang et al. 1996; and Waldmann and Anderson 1998). For several other tree species, Q_{ST} values are relatively low for timing of bud flush but high for growth cessation or timing of bud set (Howe et al. 2003).

Merilä and Crnorkak (2001) and Latta and Mckay (2002) have reviewed the basic assumption underlying comparative studies of population genetic structure which included assumption of neutrality of allozymes in these comparative studies. It is worth noting that, in some instances genetic variances within populations have been overestimated because of non-genetic (maternal) effects which could lead to a downward bias of Q_{ST} (Waldmann and Anderson 1998). One way to resolve this, as proposed by Merilä and Crnorkak (2001), would be to compare the consistency of Q_{ST} estimates and direct measures of selection in different populations for different traits.

Table 5.1. Bigleaf maple populations sampled for provenance trials, and least square means for growth and bud flush traits (with standard errors in parenthesis).

Population	LAT	LONG	ELEV	HT-2	HT-3	HT-4	DIA	BF
Metchosin	48.36	123.55	40	135.5 (0.04)	184.9 (0.05)	231.4 (0.09)	32.5 (0.01)	122.5 (0.05)
Maple Bay	48.83	123.63	14	132.2 (0.03)	179.5 (0.04)	240.6 (0.06)	30.7 (0.01)	120.8 (0.120)
Chilliwack	49.15	122.00	142	135.9 (0.04)	181.6 (0.04)	236.6 (0.05)	28.6 (0.01)	121.7 (0.10)
P.Alberni	49.26	124.85	15	139.8 (0.03)	192.2 (0.05)	263.2 (0.05)	34.2 (0.01)	121.9 (0.07)
Qualicum	49.33	124.36	80	138.2 (0.03)	194.5 (0.04)	243.9 (0.05)	32.7 (0.01)	120.2 (0.06)
Hope	49.36	123.38	90	160.2 (0.03)	216.8 (0.04)	247.5 (0.04)	33.5 (0.01)	120.6 (0.20)
Courtenay	49.66	125.03	70	126.8 (0.03)	174.9 (0.05)	225.8 (0.05)	30.1 (0.01)	120.9 (0.04)
Gold River	49.75	124.73	200	112.2 (0.03)	162.1 (0.05)	201.0 (0.05)	31.4 (0.01)	121.5 (0.8)
Squamish	49.78	123.13	50	143.4 (0.03)	193.2 (0.06)	246.3 (0.05)	32.3 (0.01)	120.7 (0.05)
Lang Bay	49.78	124.36	25	136.3 (0.03)	186.6 (0.04)	236.7 (0.05)	30.2 (0.01)	120.3 (0.07)
Cowichan	49.81	124.21	200	116.0 (0.03)	166.8 (0.05)	233.8 (0.04)	31.9 (0.01)	121.0 (0.20)
Woss	50.21	126.58	160	95.8 (0.03)	147.6 (0.05)	235.5 (0.06)	25.1 (0.01)	120.2 (0.03)
Sayward	50.31	125.93	50	131.5 (0.04)	180.0 (0.04)	237.9 (0.06)	33.2 (0.01)	119.6 (0.05)
Owl	50.35	124.73	600	118.9 (0.04)	171.8 (0.04)	213.0 (0.05)	28.6 (0.01)	118.5 (0.05)

Note: LAT = latitude (°N), LONG = longitude (°W), ELEV = elevation (m), HT-2 = second year height (cm), HT-3 = third year height (cm), HT-4 = fourth year height (cm), DIA = third year diameter (cm), BF = bud flush (Julian days).

Table 5.2. ANOVA results for F approximations for the hypothesis of no family or provenance effect.

Trait	Source	DF	SS	MS	F	Pr > F
HT-2	B	3	58.24	19.41	154.85	0.0001
	P	13	62.78	4.83	38.52	0.0011
	BxP	39	53.11	1.36	10.86	0.0001
	F(P)	134	87.42	0.65	5.2	0.0004
	BxF(P)	386	160.12	0.41	3.31	0.0001
HT-3	B	3	112.85	37.62	210.32	0.0001
	P	13	71.96	5.54	30.95	0.0183
	BxP	39	90.60	2.32	12.99	0.0001
	F(P)	134	159.79	1.19	6.67	0.0004
	BxF(P)	386	291.72	0.76	4.23	0.0001
HT-4	B	3	250.22	83.41	283.01	0.0001
	P	13	77.85	5.99	20.32	0.0455
	BxP	39	120.45	3.09	10.48	0.0001
	F(P)	134	169.69	1.27	4.30	0.0383
	BxF(P)	383	466.50	1.22	4.13	0.0001
DIA	B	3	2.30	0.77	126.01	0.0001
	P	13	1.59	0.12	20.05	0.0841
	BxP	39	2.69	0.07	11.33	0.0001
	F(P)	134	4.36	0.03	5.35	0.0001
	BxF(P)	386	7.04	0.02	3.00	0.0001
BF	B	3	822.71	274.24	7.61	0.0001
	P	13	12625.82	971.22	26.96	0.0150
	BxP	39	15395.30	394.75	10.96	0.0001
	F(P)	134	17591.27	131.28	3.64	0.0285
	BxF(P)	386	46934.67	121.59	3.38	0.0001

Note: DF = degree of freedom, SS = sum of squares, MS = mean sum of squares, F = F-value approximation, Pr > F = probability of greater F-values occurring by chance.

Table 5.3. Components of variance, individual heritabilities (h^2_i), family heritabilities (h^2_f) and population differentiation (Q_{ST}) among growth and bud flush traits.

Trait	B	P	BxP	F(P)	BxF(P)	E	h^2_i	h^2_f	Q_{ST}
HT-2	0.028	0.022	0.019	0.010	0.075	0.127	0.15 (0.06)	0.37 (0.04)	0.26
HT-3	0.054	0.021	0.031	0.019	0.144	0.184	0.17 (0.02)	0.38 (0.01)	0.16
HT-4	0.093	0.019	0.036	0.023	0.165	0.190	0.18 (0.05)	0.40 (0.01)	0.12
DIA ⁺	0.001	0.004	0.001	0.005	0.004	0.001	-	-	0.12
BF	0.032	0.086	0.058	0.110	0.210	0.780	0.29 (0.01)	0.91 (0.02)	0.12

*: Not significant, all other variables are significant for all effects at the $P < 0.001$.

Table 5.4. Genetic correlations (above diagonal) and family phenotypic correlations (below diagonal) between seedling traits for bigleaf maple provenances in British Columbia.

Trait	HT-2	HT-3	HT-4	DIA	BF
HT-2		0.99	0.94	0.78	-0.19
HT-3	0.95		0.97	0.79	-0.22
HT-4	0.89	0.93		0.75	-0.15
DIA	0.69	0.70	0.64		-0.11
BF	-0.32	-0.41	-0.39	-0.20	

Table 5.5. Correlation coefficients between quantitative traits and climatic variables based on 14 provenance means.

	HT-2	HT-3	HT-4	DIA	BF
LAT ⁺⁺	-0.47	-0.42	-0.22	-0.35	0.45
ELEV ⁺⁺	-0.44	-0.38	-0.46	-0.42	-0.05
MAT	0.58*	0.51	0.32	0.45	-0.15
TD	-0.24	-0.25	-0.10	-0.27	0.55*
MAP	-0.15	-0.15	0.20	-0.23	0.25
AHM	0.23	0.21	-0.10	0.28	-0.36
DD5	0.62*	0.54*	0.33	0.42	-0.13

Note: * Significant at $P < 0.05$ after sequential Bonferroni adjustment (Rice 1989).

⁺⁺ Abbreviations as in table 5.1. MAT = mean annual temperature, TD = temperature differential, MAP = mean annual precipitation, AHM = annual heat: moisture index, DD5 = degree days above 5°C.

Table 5.6. Genetic diversity estimates for 14 juvenile populations of *Acer macrophyllum*.

Population	A	%P	H _O	H _E
Courtenay	1.43	50	0.103	0.126
Hope	1.43	50	0.101	0.134
Sayward	1.31	40	0.090	0.127
Squamish	1.37	40	0.090	0.128
Cowichan	1.37	50	0.090	0.119
Metchosin	1.37	50	0.080	0.133
Maple Mt	1.43	60	0.110	0.111
Owl	1.37	40	0.103	0.123
Woss	1.50	50	0.096	0.138
Pt. Alberni	1.31	30	0.112	0.127
Qualicum	1.37	40	0.092	0.119
Lang Bay	1.31	30	0.079	0.119
Chilliwack	1.31	30	0.109	0.148
Gold River	1.37	50	0.121	0.137
Mean	1.37	43.5	0.098	0.127

Table 5.7. Estimates of Wright's F-statistics for eight polymorphic loci in British Columbia bigleaf maple populations.

Locus	F_{IS}	F_{IT}	F_{ST}
<i>AAT-1</i>	0.721	0.747	0.091
<i>AAT-2</i>	0.050	0.124	0.106
<i>IDH</i>	0.103	0.101	0.106
<i>6PG-1</i>	0.314	0.370	0.085
<i>6PG-2</i>	0.314	0.391	0.126
<i>PGI-1</i>	0.110	0.203	0.108
<i>PGI-2</i>	-0.123	0.114	0.146
<i>LAP-2</i>	0.291	0.322	0.068
Mean	0.222	0.301	0.090

Figure 5.1. Locations of sampled populations of bigleaf maple provenance trials.

Chapter Six

CONCLUSIONS

In British Columbia there is a trend towards greater understanding and utilization of angiosperm trees, because of their important contributions to the diversity and sustainability of British Columbia's forest ecosystems as well as the value of their wood. Responsible management and utilization of this hardwood resource could provide employment opportunities in forestry and value-added sectors. In addition, these trees are a desirable ecosystem component, adding to the structural and species diversity of British Columbia's forests.

Forest fragmentation is a growing problem because of human population growth and land use conversion of forests. Therefore, what we encounter today in some areas are small patches of original habitat for species restoration and conservation of genetic diversity. In this scenario, a thorough understanding of genetic processes affecting genes, individuals and populations, and thus affecting the persistence of this species in modified landscapes, is essential for designing sound conservation practices. My study has contributed towards our understanding of some important components of genetics of bigleaf maple. By documenting genetic variation and population structure, both at the quantitative and molecular levels, investigating the mating system, and comparing genetic diversity and genetic processes in continuous versus fragmented populations of bigleaf maple, I have provided information needed to manage and conserve this species.

Major findings

In chapter three I showed that natural populations of bigleaf maple harbour moderate levels of genetic variation. However, at the northern range of the species distribution (Jericho and Fraser populations), polymorphism was high yet expected heterozygosities were low compared to more southern populations. In addition there was no evidence of deviation from random mating in northern populations, in contrast to populations from the southern portion of the range, which had substantial inbreeding in three out of the six populations. Inbreeding in bigleaf maple may result from geitonogamous pollinations by bumble bees (*Bombus spp*), from assortative mating, or from mating among relatives. In addition, as pollination is mainly by insects, the movement of pollinators among adjacent flowers within a crown or between adjacent crowns of related neighbours would also cause inbreeding or selfing. The low heterozygosity, however, may reflect overall low genetic variation at the species northern range due to genetic drift or founder effects during postglacial recolonization. Across the sampled range, populations are only weakly differentiated, suggesting extensive gene flow or recent divergence from a common ancestral population. One alternate hypothesis to explain the low differentiation among populations may be the ecological similarity between most of the sites sampled. This is supported by the non-significant correlation between geographic and genetic distances.

An analysis of mating system found that bigleaf maple populations are predominantly outcrossing with no evidence of biparental inbreeding. This result was somewhat surprising since most populations have significant levels of inbreeding ($F_{IS} > 0$). However, this estimate of outcrossing rates may be biased upwards in view of the fact I used entirely germinated or filled seeds which probably did not account for embryonic lethals due to selfing. Another interesting finding is the evidence of few pollen donors per seed parent, yet the maintenance of high outcrossing rates.

Genetic study of the effects of fragmentation on plant species so far have shown that forest fragmentation can significantly affect population genetic processes. Results from my study suggest that both seedling and adult cohorts in six populations possess similar levels of genetic variation regardless of whether population habitats were classified as fragmented or continuous. This finding suggests extensive gene flow among populations of bigleaf maple. Furthermore, the maintenance of genetic variation in fragmented populations could be attributed to the fact that there have not been a sufficient number of generations since fragmentation to generate a detectable loss of diversity due to genetic drift and inbreeding. The most important finding, however, is the evidence of spatial structuring of genotypes within all three fragmented populations as well as one continuous population. I attribute the clumping of genetically similar individuals mainly to limited seed dispersal resulting in individuals in clusters being more related than expected by chance. In this study, spatial genetic structure appeared to be affected by species density. Coincidentally, the two populations that did not show spatial genetic structure seem to have higher population density than the four populations that showed spatial genetic structure.

Genetic variation in seedling growth and bud phenology was also detected both among populations, and among families within populations. The substantial within-population variation observed in this study, coupled with the moderate heritabilities and moderate genetic correlations among growth traits and bud flush, suggest an opportunity for genetic improvement and early selection for these traits. In addition, the significant correlation between quantitative traits and climatic variables in this study seems to suggest that bigleaf maple has adapted to varying environmental conditions, with natural selection favouring different phenotypes in different environments.

Recommendations

There is no doubt that management of fragmented populations of plant species has become an important element of biological conservation, and this issue will continue to grow. Many of the plant species that are currently recognised as threatened are restricted to small habitat fragments and *in situ* conservation of large contiguous populations within a relatively pristine environment is no longer feasible. But the good news with bigleaf maple is that historic levels of genetic variation have thus far persisted and it does not appear that the species is in need of immediate conservation attention. Having said this, integrating our results with other findings, one issue that remains contentious is whether genetic processes, primarily genetic erosion and inbreeding, actually play a significant role in reducing the viability of small fragmented populations compared to the risk of habitat loss and associated demographic factors.

Future studies that examine the effects of fragmentation on plant populations should seek a standardized approach to examine habitat subdivision effects. Based on my findings of how fragmentation affects genetic variation and spatial genetic structure, mating system, and genetic variation at both the quantitative and molecular levels, the following guidelines are recommended:

1. Where economically feasible, compare original un-fragmented (continuous) populations and fragmented populations, as fragmentation is a population level process and not an individual based one. If original habitats do not exist, analyze a number of sites or fragments from smallest and more isolated to largest less isolated. Ideally, one should sample a sufficient number of sites to have the statistical power to separate out the effect of size and isolation.
2. Researchers should focus on critical aspects of the biology of the plants to be studied as they can provide evidence of how surviving individuals are

passing on their genes to the next generation or the potential for seed and pollen dispersal in among fragments.

3. Genetic diversity measures should be carefully standardized because these measures are very sensitive to sample sizes. From an analytical standpoint, new tools are needed to detect changes especially in mating patterns and dispersal rates of plant populations in human dominated landscapes that would otherwise go undetected. Hypervariable codominant markers such as microsatellites are highly recommended to shed more light on the total genetic structure and variability of bigleaf maple.
4. The study of quantitative variation in bigleaf maple needs to be extended to include more test sites as well measurements of more traits and more growing seasons in order to investigate the degree of genotype-by-environment interaction and juvenile-mature correlations. This knowledge is essential to guide the establishment of breeding and deployment zones and to develop further strategies for genetic resource management and utilization in bigleaf maple.

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Appendix I

Enzyme, buffer systems and recipes for histochemical staining solutions.

Enzyme	# of loci	Gel buffer	Stain components
Aspirate Aminotransferase (AAT)	2	Sodium Borate (Ridgeway)	50 ml 0.2 M Tris-HCL pH 8.0 1 mg Pyridoxal 5-phosphate 200 mg L-Aspartic acid 100 mg Ketoglutaric acid 200 mg Fast Blue BB salt
6- Phosphogluconate dehydrogenase (6-PGD)	2	Sodium Borate (Ridgeway)	50 ml 0.2 M Tris-HCl pH 8.0 10 mg Phosphogluconic acid 1 ml NADP 1 ml MTT 1 ml PMS
Isocitric dehydrogenase (IDH)	1	Sodium Borate (Ridgeway)	50 ml 0.2 M Tris-HCl pH 8.0 100 ml DL-Isocitric acid 1 ml NADP 1 ml MTT 1 ml PMS
Phosphoglucose isomerase (PGI)	2	Morpholine	50 ml 0.2 M Tris-HCl pH 8.0 25 mg Fructose-6-phosphate 1 ml NADP 1 ml MTT 1 ml PMS 1 ml MgCl ₂
Leucine Aminopeptidase (LAP)	2	Morpholine	50 ml Aminopeptidase buffer pH 6.0 0.4% L-Leucine 30 mg β -naphtylamide 20 mg Black K salt
Glutamete Dehydrogenase (GDH)	1	Morpholine	50 ml 0.1 M Tris-HCl pH 8.0 400 mg Glutamic acid 3 ml NADP 3 ml MTT 3 ml PMS

Appendix II.

Allele frequency distribution of ten loci of bigleaf maple provenance trials.

Locus		POP1	POP2	POP3	POP4	POP5	POP6	POP7
AAT-1								
(N)		89	63	48	79	85	82	67
	1	0.719	0.849	0.882	0.715	0.235	0.634	0.743
	2	0.281	0.151	0.118	0.285	0.765	0.366	0.257
AAT-2								
(N)		88	62	47	80	85	86	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH								
(N)		96	72	51	70	79	84	64
	1	0.708	0.861	0.686	0.700	0.184	0.673	0.706
	2	0.292	0.139	0.314	0.300	0.816	0.327	0.294
6PG-1								
(N)		90	73	46	78	88	80	59
	1	0.822	0.747	0.685	0.763	0.42	0.587	0.795
	2	0.178	0.253	0.315	0.237	0.58	0.412	0.205
6PG-2								
(N)		92	70	45	78	91	87	60
	1	0.891	0.786	1.000	0.904	1.000	1.000	1.000
	2	0.109	0.214	0.000	0.096	0.000	0.000	0.000
PGI-1								
(N)		88	72	50	80	91	88	60
	1	1.000	1.000	1.000	1.000	0.835	0.966	0.975
	2	0.000	0.000	0.000	0.000	0.165	0.034	0.025
PGI-2								
(N)		96	72	51	80	91	89	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	0.878
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.122
GDH								
(N)		82	73	52	80	92	89	63
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	1.000	1.000	0.000	0.000	0.000	0.000	1.000
LAP-1								
(N)		87	72	50	79	89	80	63
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	1.000	1.000	0.000	0.000	0.000	0.000	1.000
LAP2								
(N)		84	71	47	80	89	84	66
	1	0.917	0.782	0.543	0.781	0.966	0.821	1.000
	2	0.083	0.218	0.457	0.219	0.034	0.179	0.000