A Microsatellite Analysis of the Western Canadian Mountain Pine Beetle (*Dendroctonus ponderosae*) Epidemic: Phylogeography and Long Distance Dispersal Patterns

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Abstract

The mountain pine beetle (MPB) is an eruptive insect that is currently causing an outbreak of record size in Western Canada. A lack of long distance MPB dispersal data has limited our understanding of and ability to manage MPB epidemics. My goal was to determine the MPBs Western Canadian population structure, upon which dispersal patterns may be superimposed. I analyzed MPBs from 35 infested lodgepole pine stands at six microsatellite loci. The MPB exhibited strong and significant Western Canadian population structure. This population structure was incongruent with the structure of its primary symbiont, *O. clavigerum*, but congruent with the structure of its primary host, *P. contorta*. Novel fungal selection pressures have probably caused the discrepancy in beetle/fungus phylogeography. A result of Western Canadian MPB population structure alternately contrasts and supports population structures previously reported for Scolytids, including MPBs. The partitioning of MPB population structure into a Northern and Southern group is most likely the result of postglacial recolonization and differences in MPB population dynamics. Primarily using my genetic data, I inferred the historical movement patterns of the MPB in Western Canada. I found no evidence that the epidemic spread from an epicenter in Tweedsmuir Provincial Park. My data support multiple sources for the current epidemic; I suggest that regional population expansions have caused the rapid escalation in the severity of the current epidemic. MPB movement patterns and atmospheric wind data were concordant; winds in Western Canada are predominantly westerly or southwesterly, which was the predominant direction of inferred movements among MPB populations. In contrast, current MPB population structure best fits a 30-year climatic suitability distribution for a historical (1921-1950) as

opposed to the most current (1971-2000) period. The population genetics of long distance MPB dispersal, an evolutionary theory for MPB population dynamics, and MPB "range expansion" are extensively discussed. Potential biases and research limitations are noted. Based on my results and inferences, future areas of investigation are noted. An executive summary, with management recommendations, is provided as a conclusion.

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Introduction

1.1. Background

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins, is the most destructive pest of pine forests in western North America (Safranyik and Carroll, 2006). This bark beetle attacks at least 11 tree species in the Pinaceae (Kelley and Farrell, 1998). Many of these species, such as lodgepole pine (*Pinus contorta* var. *latifolia* and *murrayana*), western white pine (*P. monticola*), and ponderosa pine (*P. ponderosa*), have wide distributions. The mountain pine beetle (MPB) has killed 100's of millions of trees in the United States over the past century (Stock and Guenther, 1979; McGregor, 1985). However, a current MPB epidemic in British Columbia has caused new tree mortality over a record 10.1 million hectares based on BC Ministry of Forests and Range surveys at the end of 2007 (Westfall and Ebata, 2007). This epidemic has extended into Alberta and is the most severe insect outbreak in Canadian history.

Tree mortality events of this magnitude can have considerable environmental effects (Uunila *et al.*, 2006). As a positive force, MPB outbreaks are a crucial feedback mechanism for the regeneration of senescent pine forests (Samman and Logan, 2000). The seral consequences of outbreaks, as well as the conditions during outbreaks, are beneficial for many species ranging from understory vegetation to large mammals (Schmid and Mata, 1996; Martin *et al.*, 2006).

Despite positive aspects, the MPB epidemic is overwhelmingly negative, especially from a human perspective (Samman and Logan, 2000). Losses of mature forests will negatively impact climax wildlife species and entire aquatic ecosystems due to habitat loss and changes in water parameters, respectively (Uunila *et al.*, 2006).

Threatened and endangered species, both terrestrial and aquatic, will face higher extinction risks. The contiguous expanses of dead trees left by the MPB epidemic will present an unprecedented risk for large-scale forest fires and mitigation costs will be high. These expanses of dead trees are also making large contributions to global carbon dioxide emissions (Kurz *et al.*, 2008). Tourism and property values will also be impacted, particularly in resort towns. Economic damage from loss of commodity value is expected to have extremely adverse effects on the British Columbia and Alberta forest industries (Wagner *et al.*, 2006).

The mountain pine beetle has been extensively researched because of the severity of its impacts on forest ecosystems and consequent effects on industry (Safranyik and Carroll, 2006). Particularly well understood is the life history of the MPB. Most MPBs complete their life cycle within one year (univoltine). Adult MPBs emerge during late summer by boring to the outside from the subcortical tissues of the host tree. These MPBs disperse, the majority of beetles flying below the stand canopy until they locate a suitable host tree. Female beetles initiate construction of galleries in the subcortical tissues of the host, simultaneously inoculating attacked trees with symbiotic fungi. These fungi are important for stopping host defenses and killing the tree, as well as for MPB food sources (Six and Paine, 1998). During gallery construction, volatile chemicals produced by both the defending host and attacking MPB strongly attract conspecifics, causing a mass attack of the tree (Borden, 1982).

After mating, females lay eggs along their galleries. Eggs hatch within two weeks into larvae, which pass through four growth stages, or instars. Larvae excavate horizontal tunnels in the water- and nutrient-conducting subcortical tissues of the tree.

Larval girdling and fungal infection combine to kill the host tree (Safranyik and Carroll, 2006). Depending on the timing of oviposition, as well as site climatic factors, various instars over-winter under the bark. In spring, larvae resume feeding and as fourth instars, construct pupal chambers and become pupae. After two to four weeks, pupae develop into callows, or soft-bodied and pale coloured adults, which in turn develop into adults in about ten days, completing the MPB life cycle. Because the MPB life cycle is dictated by ambient temperatures, in cool locations such as mountain sites, life cycles may take two years to complete.

The epidemiology of the MPB is also well-studied (Safranyik and Carroll, 2006). In the endemic stage, MPB population densities are so low that only unhealthy (suppressed) trees are attacked. Three to six trees are infested in a mature lodgepole pine stand per year, at a density of two to three attacks per tree. Thus a maximum population size for the MPB in the endemic phase is roughly 36 beetles/ha. Through processes that suppress the health of trees or that increase beetle populations, MPBs reach the incipient-epidemic stage and can successfully mass attack (kill) a large diameter tree in a stand. Jackson *et al.* (2008) estimated that ~577 beetles are required to successfully attack a mature lodgepole pine tree. A few years of two- to eight-fold population increases allow MPBs to reach the epidemic stage, characterized by widespread infestations over large spatial and temporal scales. Through extreme cold-weather events or resource depletion, epidemic populations eventually crash and revert to endemic populations.

A lack of long distance MPB dispersal data has limited our understanding of the development of the current epidemic (Furniss and Furniss, 1972; Safranyik *et al.*, 1992; Safranyik and Carroll, 2006). The effectiveness of MPB outbreak management has also

been affected (Safranyik *et al.*, 1989; Robertson *et al.*, 2007). For example, many complex models assess stand susceptibility to MPB attack (Negron *et al.*, 1999; Shore *et al.*, 2000; Nelson *et al.*, 2006; Shore *et al.*, 2006). Such models provide only a mechanistic understanding of MPB population dynamics after stands are attacked.

In the assessment of long-range MPB dispersal, the use of mark-recapture techniques is not economically feasible (Linton *et al.*, 1987; Salom and McLean, 1990). The use of genetic techniques, such as allozyme and RAPD (Random Amplification of Polymorphic DNA) analysis, has also failed to yield significant results (Stock *et al.*, 1984; Calpas *et al.*, 2002). A relatively new molecular technique, called microsatellite analysis, uses genomic markers within individual beetles and has the greatest potential to provide critical data on "long distance" (beyond stand) MPB dispersal.

Microsatellites are among the most powerful markers available for genetic analyses of population structure (Balloux and Lugon-Moulin, 2002). The population structures derived from microsatellite data allow for high-resolution estimates of dispersal. As codominant markers, microsatellites produce allele frequency data that allows for hetero- and homozygotes to be distinguished at a given locus (Goldstein and Schlötterer, 1999). Microsatellites are also ideal for population genetic study because they are neutral and thus free of the confounding effects of selection.

Microsatellites are grouped repeats of short nucleotide sequences, from 2 to 6 nucleotides long, with unique flanking DNA sequences (Beebee and Rowe, 2004). Primers are developed that anneal to these unique DNA sequences, amplifying (mass-copying) the grouped repeats. Different lengths of these repeats at a locus correspond to different alleles. Microsatellites evolve through genetic drift and mutation, which

remove and add repeats, respectively. Because drift and mutation occur randomly, given sufficient time, isolated populations will show divergence in the number of grouped repeats at a random locus, allowing for quantification of population differences. Long distance dispersal patterns can then be elucidated through statistical comparisons of population allele frequencies.

An undergraduate thesis (Bartell, 2007) on a subset of this Master's dissertation was among the first to use microsatellite markers to determine the population genetic structure of a Scolytid beetle (Sallé *et al.*, 2007). In this thesis, the five populations most likely to be differentiated, primarily because of geographic isolation (distance and mountain ranges), were selected for analysis. These populations were Houston, Mount Robson, Banff, Lac la Hache, and Manning Park (see Table 1). Using only two microsatellite loci, I demonstrated the efficacy of microsatellite techniques by finding that Houston was significantly different from the other four populations and that Banff was significantly different from three other populations (AMOVA at $\alpha = 0.05$). I reported that the use of more loci would considerably increase resolution, *i.e.* the ability to differentiate populations (Sallé *et al.*, 2007). I concluded that geographic isolation was a dominating force in MPB population genetics and found no evidence that the current epidemic originated from dispersal from an epicenter. Instead, my results suggested that the current MPB epidemic arose from multiple sources. I emphasized that this Master's dissertation, by analyzing all 35 sampled MPB populations at a greater genetic resolution (more microsatellite loci), would be able to identify the relative importance of genetic relationships among populations and the extent of dispersal within the outbreak area.

The success of this undergraduate thesis was crucial for determining the feasibility of this Master's project.

There are numerous plausible explanations for the spread of the current MPB epidemic. Public perception is that the epidemic originated from an epicenter in Tweedsmuir Provincial Park. Also, there is a governmental and public perception that outbreaks in Alberta have originated via dispersal from numerous, apparently isolated BC infestations. Indeed, MPB populations are endemic to many regions of BC (Wood and Unger, 1996; Nelson *et al.*, 2007c).

Thus, there are two competing theories for the spread of MPB epidemics over landscapes. Epidemics may arise from numerous endemic (native) populations simultaneously expanding in response to favourable ecological conditions such as reduced winter mortality and mature, susceptible forests. On the other hand, epidemics may arise from one or a few epicenters from which massive dispersal events take place. A complex combination of the two theories is also a likely explanation (Namkoong *et al.*, 1979). Indeed, in an analysis of the current epidemic, Aukema *et al.* (2006) found evidence for both a true epicenter in North Tweedsmuir Provincial Park and simultaneous geographically-isolated outbreaks in southern BC. The main goal of my research is to use microsatellite analysis to determine the phylogeography and dispersal patterns of the MPB over the current epidemic area, and in the process, to determine the most likely mechanisms for the spread of this epidemic.

1.2. Research Objectives:

The main goal of my research was to use microsatellite markers to determine the phylogeography of the MPB over the current epidemic area, upon which dispersal patterns may be superimposed. In the process, I determined the most likely mechanisms for the spread of this epidemic. Specific objectives were:

1) To analyze MPB phylogeography and dispersal patterns at regional (fine) and provincial (coarse) scales to determine the relative influence of historical isolation versus recent immigration on the current genetic structure of each MPB population.
2) To compare dispersal patterns derived from MPB population genetic data with patterns predicted by spatiotemporal and climatic (prevailing atmospheric winds) analyses of the epidemic's spread, to gain insight into both the spread of MPB epidemics and the ecology of long distance MPB dispersal.
3) To compare the population genetic structure found for the MPB to the population structures reported for the MPBs primary symbiotic fungus, *G. clavigera,* and for the MPBs primary host tree, *P. contorta.*

1.3. The Mechanisms Behind Long Distance MPB Dispersal

Long distance bark beetle dispersal is generally viewed as a passive process in which emerging beetles are caught in updrafts, are moved above the stand canopy, and are transported many kilometers by atmospheric winds. In *Ips typographus*, the eightspined spruce bark beetle, Forsse and Solbreck (1985) estimated from vertical trapping and subsequent modeling that ~10% of beetles may be above the stand canopy and disperse many kilometers by wind during dispersal flights. A similar long distance dispersal percentage might be expected for MPBs (Safranyik *et al.*, 1992), which have similar dispersal ecology and are only slightly larger than these spruce beetles.

Recent research on long distance MPB dispersal (Jackson *et al.*, 2008) has found beetles up to 850 m above the forest canopy. However, most beetles dispersing in the atmosphere are found within a few hundred meters of the canopy. Jackson *et al.* (2008) suggested that MPBs likely actively regulate their height in the atmosphere in response to temperature, as hypothesized by Geerts and Miao (2005). Moreover, Jackson *et al.* (2008) conservatively estimated that MPBs may move 110 km in a single day. This estimate assumed an average flight duration of four hours (standard deviation of two hours) and an average wind speed of 4.8 m/s (standard deviation of 1.3 m/s). If MPBs undergo passive transport in the atmosphere, then dispersal distances could reach several hundreds of kilometers, as reported for a 2006 dispersal event into the Peace River region of BC (Westfall and Ebata, 2007) and as reported for the outbreak which crossed the Alberta prairies in the 1980's (Cerezke, 1989).

These remarkable dispersal distances are undoubtedly because of the upward convection currents associated with the warm, fair-weather days needed for Scolytid

flight (Chapman, 1962; Safranyik *et al.*, 1989). Furniss and Furniss (1972) hypothesized that these currents could sweep up Scolytids in flight and move them above the stand canopy; data support this conjecture (Schmid *et al.*, 1992). Though the majority of dispersing MPBs fly just above the undergrowth, about 2.4% of dispersing MPBs may be above the canopy (Safranyik *et al.*, 1992). Though Botterweg (1982) argued that bark beetles need to disperse longer distances under endemic rather than epidemic conditions, Safranyik and Carroll (2006) suggested an increase in the percentage of above-canopy dispersers during epidemics, when host tree shortages occur. Regardless of how beetles reach an above-canopy position, once there they may travel 100's of kilometers before deposition into new stands (Jackson *et al.*, 2008).

Regarding atmospheric transport, it is unknown whether such transport requires beetles to be actively flying throughout, whether they are "gliding," or are neutrally-buoyant with their wings folded (Jackson *et al.*, 2008). Thus, at this time, the maximum potential dispersal distance of bark beetles is a difficult parameter to estimate. Atmospheric analyses and aerial transects are useful for determining general directions of long distance bark beetle dispersal but cannot provide data on dispersal between specific populations or locations (Jackson *et al.*, 2008). My study will help determine if and how long-distance dispersal is occurring in epidemic MPB populations. More specifically, I will determine if there is a correlation between the epidemic phylogeography of the MPB and the prevailing atmospheric wind patterns of BC and AB. Any correlation will be examined at both coarse (provincial-level) and fine (regional) scales.

1.4. Limitations of Mark-Recapture Studies of Long Distance Bark Beetle Dispersal

The most common method for researching dispersal, particularly in bark beetles (Scolytinae), is the use of mark-recapture techniques. The scale of these bark beetle studies has been predominantly within-stand. This scale is directly related to the limitations of mark-recapture: labour intensive; high cost; prone to considerable error; prone to study failure. Salom and McLean (1990; 1991) provide an example of the labour intensive nature of this technique while studying dispersal of *Trypodendron lineatum* (Scolytinae) in a small, 3 km^2 valley. They used up to 100 Lindgren (1983) multiple-funnel traps that had to be pheromone-baited, monitored, and maintained regularly, while capturing, marking, and releasing beetles over the field season.

Error can be difficult to minimize in mark-recapture. Linton *et al.* (1987) used florescent powders to mark MPBs and in a test they recaptured significantly fewer marked compared to unmarked individuals after dispersal flights. Rainfall and high moisture can remove external markings, as can abrasion and preening (Cook and Hain, 1992), potentially compromising a study. Internal markers, such as rubidium (Rb), are ingested by beetles but are expensive and are removed by feeding (Thoeny *et al.,* 1992), placing constraints on the spatial and temporal extent of study. Moreover, the use of immunomarkers continues to be spatially and economically constrained (Jones *et al.*, 2006). Safranyik *et al.* (1992) have used mark-recapture to create a solid understanding of the within-stand mechanisms and extent of MPB dispersal. But because of the currently insurmountable limitations of mark-recapture, long-distance dispersal is poorly understood across the Scolytinae (Nilssen, 1984).

1.5. Using Population Genetics to Infer MPB Dispersal? Past Genetic Studies of Long Distance Bark Beetle Dispersal

The primary advantage of a genetic approach to studying dispersal is that markers are contained within the genome of every sampled individual. With a genetic approach "resolution" is equivalent to the recapture rate in mark-recapture studies. In the case of bark beetles, high resolutions are primarily achieved by sampling many stands over a region (eg. a grid pattern with a 100km separation between stands), genotyping ~50 beetles/stand, and using a large number of polymorphic neutral markers.

The population genetic approach to determining long distance dispersal, and thus my research approach, is stepwise. The fundamental requirement for genetically determining dispersal is significant prior population structure, in other words the existence of genetically-differentiated populations. Dispersal patterns are superimposed upon this structure and are detected by instances of genetic similarity between stands.

There are several other advantages associated with a population genetic approach to dispersal research. Population genetic data allow for the determination of: the source of invading populations, the presence of selective differences between genotypes, and the mechanism/s that preserve genetic diversity within and between populations (Civetta *et al.*, 1990; Beebee and Rowe, 2004). Determining patterns of gene flow can provide critical information for both preventative and reactive forest management (Stauffer *et al.*, 1992; Kerdelhué *et al.*, 2003; Mock *et al.*, 2007). In the long term, population genetic data may also help us understand how the MPB will evolve under a changing climate.

Most population genetic studies of Scolytids support the idea that significant geographic barriers, such as mountain ranges and large distances, can prevent inter-population gene flow, causing among-population genetic differentiation and generating population structure. Pioneering population genetic studies that used conservative allozyme techniques clearly support this idea. In the Douglas-fir beetle (*Dendroctonus pseudotsugae*), geographically isolated coastal and inland populations in the Pacific Northwest may be diverging into separate species (Stock *et al.*, 1979). Moreover, in the southern pine beetle (*D. frontalis*), peripatric populations from Arizona and Mexico were genetically divergent (Anderson *et al.*, 1979; Namkoong *et al.*, 1979). In a follow-up study, Roberds *et al.* (1987) found significant genetic differences among *D. frontalis* populations from Texas, Arkansas, North Carolina, and Mississippi. Six *et al.* (1999) assessed the range-wide population genetic structure of the Jeffrey pine beetle (*D. jeffreyi*) and discovered a northern and southern group, consistent with that predicted by geographic isolation.

Population genetic studies utilizing more modern methods, such as mitochondrial and nuclear sequencing, consistently support the role of geographic barriers and isolation in Scolytid population dynamics. The majority of recent Scolytid population genetics research has occurred in Europe on *Ips typographus*, *Tomicus piniperda*, and *Tomicus destruens*. In the spruce beetle *I. typographus*, Stauffer *et al.* (1999) found mitochondrial evidence for the existence of Central European, Scandinavian, and Central Russian groups. In the pine shoot beetle *T. piniperda,* Duan *et al.* (2004) confirmed the existence of a new *Tomicus* species in southern China that was divergent from *T. piniperda* in Europe and northern China. Ritzerow *et al.* (2004) supported this "splitting" of *T.*

piniperda by providing evidence for barriers to gene flow between the two species. In Spain, isolation has caused populations of *T. piniperda* and its primary host, *P. sylvestris*, to evolve in parallel for ~0.3 million years (Soranzo *et al.*, 2000; Ritzerow *et al.*, 2004).

A similar pattern of isolation and parallel evolution has been found between the pine shoot beetle *T. destruens* and its host *Pinus pinaster* in Portugal (Salvador *et al.*, 2000; Vasconcelos *et al.*, 2006). *T. destruens* has well-supported longitudinal population genetic structures that are embodied by separate groups in Spain, France and Italy (Faccoli *et al.*, 2005). Faccoli *et al.* (2005) also found strong genetic evidence for separate populations of *T. destruens* within Italy. Horn *et al.* (2006) completed a range-wide study of *T. destruens* in the Mediterranean basin and found genetic evidence for groups in Portugal, Spain/France, Italy, Greece, and the Middle East; populations in North Africa were hybrids of groups on the Iberian Peninsula (Portugal and Spain/France).

Considerably fewer population genetic studies of Scolytids have occurred in North America but these studies have confirmed the importance of geographic barriers in Scolytid population dynamics. In the western pine beetle (*D. brevicomis*) there is high mitochondrial differentiation between western and eastern populations isolated by the Great Basin (Kelley *et al.*, 1999). Cognato *et al.* (2003) found three geographically-defined mitochondrial haplotype lineages in the pinyon pine beetle (*Ips confusus*). In a preliminary analysis, Bartell (2007) found significant differences among five isolated MPB populations in BC and AB at microsatellite loci. Using mitochondrial and microsatellite techniques, Maroja *et al.* (2007) delineated three haplotype lineages

associated with different geographical regions across the spruce beetle's (*D. rufipennis*) North American range.

However, two recent papers have argued that geographical barriers are not a dominant force in Scolytid population dynamics. Both studies used microsatellite markers. Sallé *et al.* (2007) found non-significant European population structure in *Ips typographus*, the eightspined spruce beetle. They explained this lack of structure by arguing that *I. typographus* has a consistently high dispersal rate. Kerdelhué *et al.* (2006) found a lack of population structure in *Tomicus piniperda*, the pine shoot beetle, in France and also argued for a high dispersal rate. However, the potential for sampling and (fungal) contamination bias was high in these studies; Stauffer *et al.* (1992; 1999) and Kerdelhué *et al.* (2002) provided opposing results for Sallé *et al.* (2007) and Kerdelhué *et al.* (2006), respectively.

Two potentially biased studies on the MPB have also disputed the role of geographical barriers in Scolytid population dynamics. These studies of the MPB failed to detect population structure and both doubted that MPB dispersal could be determined genetically. Calpas *et al.* (2002) could not distinguish MPB populations isolated by large distances. Stock *et al.* (1984) found high genetic similarity between populations from seven western states in the United States. While Stock *et al.* (1984) used allozyme analysis, a conservative technique, Calpas *et al.* (2002) used random amplified polymorphic DNA (RAPD) analysis, which is highly sensitive to between-population differences. However, the sample sizes of both studies: 15 per population for Calpas *et al.* (2002) and not reported for Stock *et al.* (1984), may have caused their negative results. Calpas *et al.* (2002) also used a technique with low reproducibility (Beebee and

Rowe, 2004) and did not control for fungal contamination of MPB DNA. Thus Calpas *et al.* (2002) may have amplified and analyzed results derived from fungal DNA.

Most studies of the MPB have found some degree of population structure, relegating the studies of Stock *et al.* (1984) and Calpas *et al.* (2002) as anomalies. In the Pacific Northwest, a study of isozyme variation in MPBs from six sites indicated that the sites are genetically diverging (Stock and Guenther, 1979). In support of this conclusion, one population isolated from all others by a large geographic barrier, a desert, was genetically unique. In Utah and British Columbia, Bentz and Stock (1986) found high levels of allozyme differentiation between *D. ponderosae* populations. In BC and AB, high levels of genetic divergence have been found among MPB populations using allozymes (Langor and Spence, 1991). In California, significant differences were found between northern and central MPB populations using mitochondrial markers (Kelley *et al.*, 2000). Over its North American range, the MPB exhibited significant population structure at mitochondrial and AFLP (Amplified Fragment Length Polymorphism) loci (Mock *et al.*, 2007), with gene flow around rather than across the Great Basin and Mohave deserts. These studies support the idea that geographic barriers, such as mountain ranges and large distances, can prevent gene flow and cause genetic differentiation between populations.

1.6. Major Influences on the Genetics of MPB Populations – Implications for Study

In this study I carefully designed a sampling regime that maximized power and minimized bias. Given that determining MPB population structure was my primary objective, a major area of concern was controlling for the effects of host species and epidemic-induced population genetic homogenization on genetic diversity in and differentiation among MPB populations.

There are genetic ramifications for sampling MPBs from different host tree species in a study and treating the samples as identical. Stock and Amman (1980; 1985) found that genetic differences in MPBs were highly correlated with host tree species (ponderosa versus lodgepole pine). Indeed, host species influences the survival and genetics of attacking MPBs (Stock and Amman, 1985). Langor and Spence (1991) also found significant genetic differences among beetles from different host species (limber versus lodgepole pine). Notably, all three of the above studies used allozyme analysis. Allozymes, as protein markers, have low apparent mutation rates (Beebee and Rowe, 2004). Also, allozymes are often not selectively neutral and may have important roles in cell function and/or maintenance (Bert *et al.*, 2002). In sum, allozymes are very conservative markers relative to polymorphic markers such as microsatellites (eg. Ross *et al.*, 1999).

Considering the conservative nature of allozyme analysis, the results of Stock and Amman (1980; 1985) and Langor and Spence (1991), that the MPB exhibits genetic differences associated with host preference, are more significant than they appear. The existence of host-associated genetic differences in MPBs was also supported by the allozyme study of Sturgeon and Mitton (1986). However, recent research using a

modern, neutral genetic method (AFLP) found no significant differences among MPBs from different host species (Mock *et al.*, 2007). Kelley *et al.* (2000), moreover, found host associated genetic differences among MPBs using allozymes (loci potentially under selection) but not using neutral mitochondrial markers.

These two recent studies indicate that Stock and Amman (1980; 1985), Sturgeon and Mitton (1986), and Langor and Spence (1991) detected selective differences among MPBs from different species of host tree. The results of Kelley *et al.* (2000) and Mock *et al.* (2007) also indicate that the genetic differences they found among MPBs from different host species were due to host-mediated selection (host effects) and not MPB host preference. Importantly, modern results suggest that there are no significant differences among MPBs from different host species at neutral loci. The existence of sympatric MPB population structure, however, is still an active area of research. I sampled exclusively from MPB-infested lodgepole pine to prevent any possible genetic bias in my data.

The fundamental requirement for genetically determining MPB dispersal is significant prior population structure, in other words the existence of genetically-differentiated populations, upon which recent dispersal patterns are superimposed. However, during epidemics, massive immigration and gene flow may erase the genetic identities of endemic (native) populations, making the identification of source populations impossible.

However, based on southern pine beetle epidemics, Scolytid epidemics are not continuous random-mating populations; some populations that are separated by sufficient

distance and/or geographic barriers maintain their genetic identity (Namkoong *et al.*, 1979). In addition, Roberds *et al.* (1987) found that allele frequencies were similar in populations that declined from epidemic to endemic status. Among MPB populations in British Columbia and Alberta, Langor and Spence (1991) found high within-site and higher between-site genetic differences, which suggests that selection and drift are extremely important in the dynamics and divergence of populations. If epidemics are relatively infrequent, then the following model of MPB population genetics may be accurate.

Native MPB populations are randomly distributed in habitats. In the endemic phase, these populations genetically diverge by drift but also by selection at a local scale (Namkoong *et al.*, 1979). During epidemics, populations are connected by levels of gene flow correlated with distance, such that nearby populations become genetically similar while distant populations remain different. Once the epidemic declines, native populations in the endemic phase are once again isolated and influenced by drift and local selection. Bark beetle population genetic structures should be maintained by barriers, despite the homogenizing effects of infrequent epidemics, allowing for dispersal inferences.

The population structure derived from neutral genetic markers is a product of the opposition of two forces. These forces are genetic drift and gene flow. In tandem, these two forces allow for a population genetic approach to dispersal research. In isolation, populations will diverge over time due to genetic drift, the random loss of alleles each generation. Because genetic drift is a random process, isolated populations with an

initially identical gene pool will randomly fix or lose alleles at different rates, becoming differentiated over time. In contrast, gene flow genetically homogenizes populations. Matings with migrants introduce new alleles and create recombinant offspring. Low levels of gene flow, such as between isolated populations, simply slow rates of population divergence but high gene flow can create panmixia (high genetic similarity) among populations.

A population genetic approach also allows for the determination of effective dispersal. Researching effective dispersal entails sampling the surviving brood of successfully dispersing and reproducing adult MPBs; most stands were sampled in June-July (one exception – May). This approach determines the source/s of immigrating MPBs that are most important for outbreak propagation. Dispersers may cause tree mortality at a site but if their brood do not survive, then these dispersers did not contribute to outbreak propagation. Genotyping effective dispersers provides data crucial to halting the rapid advance of the current epidemic. Indeed, MPB mortality during dispersal, especially long-distance dispersal, is extremely high as MPBs must land in areas with suitable hosts, climate, and in sufficient numbers (Schmid, 1969). Moreover, after long-distance dispersal, MPB fat reserves may be exhausted and host colonization, mating, gallery construction, as well as maternal investment in eggs (Elkin and Reid, 2005), could be compromised. Identifying source populations producing effective dispersers capable of overcoming the energetic demands of a long flight and of surviving and reproducing in a foreign environment, possibly hundreds of kilometers from their origin, is critical for halting the advance of epidemics.

1.7. Current Methods and Ideas for MPB Management

A number of different management options are available to control present MPB outbreaks and prevent future MPB outbreaks; however, these techniques are highly limited in scope and effectiveness. Short-term management involves a number of techniques, such as prescribed burns to infested stands, sanitation harvesting in brood-containing stands, tree baiting and removal, pesticides, and fall-and-burn treatments, all of which reduce MPB populations with varying levels of success (Samman and Logan, 2000; Carroll *et al.*, 2006). Prescribed burns in uninfested stands have also been used to create discontinuous host tree distributions and inhibit dispersal, as in some BC Provincial Parks.

Alternatively the defenses of host trees may be improved. There is a long history of research supporting the role of stand thinning in reducing tree losses to MPB outbreaks (Amman and Logan, 1998). As an example, Waring and Pitman (1985) modified lodgepole pine stands in central Oregon by lowering canopy densities or treating with nitrogen fertilizer, significantly increasing MPB-attack resistance. However, modern research has disputed the supposed benefits of stand treatments (Hindmarch and Reid, 2001; Safranyik *et al.*, 2004). In the Western US, stand thinning to reduce forest fuel loads increased the incidence of Scolytid attacks on pines by 15 times but caused no significant increase in tree mortality (Fettig *et al.*, 2006). Safranyik *et al.* (1999), in the East Kootenays of BC, found that stand thinning had no effect on Scolytid attacks on lodgepole pine. Nevertheless, many researchers and government ministries, such as Natural Resources Canada, assert that stand density management is important for optimizing stand microclimate, tree vigor, and inter-tree spacing, to

collectively "beetle proof" stands (Safranyik *et al.*, 1998; Safranyik *et al.*, 2004; Whitehead *et al.*, 2004; Whitehead and Russo, 2005; Whitehead *et al.*, 2006).

Regulation of stand composition has also been suggested for minimizing future MPB outbreaks (Samman and Logan, 2000). Past management practices have promoted the widespread dominance of climax species, such as lodgepole pine, due in part to fire suppression and reduced overall disturbance (Taylor and Carroll, 2004), as well as due to silvicultural practices. Undoubtedly, the abundance of decadent, mature or over-mature stands of lodgepole pine across BC has been a major factor in the massive scale of the current MPB epidemic (Taylor *et al.*, 2006). Samman and Logan (2000) recommend removing these old-growth stands, which are more susceptible to insect infestations, and promoting more landscape-level seral diversity. But effective prevention of future large-scale MPB outbreaks may not be as simple as creating more heterogeneous landscapes.

The ideal British Columbia forest landscape of the future must be heterogeneous in terms of age and genus (Burton, 2006). Since MPBs are generalist mortality agents on *Pinus,* which have a very wide distribution in BC, forest managers planning for landscape heterogeneity cannot only consider the MPBs primary host, lodgepole pine. Future "landscape heterogeneity" must avoid large regions of contiguous *Pinus* species, regardless of age diversity. This distribution of *Pinus* may not be possible to achieve given that *Pinus* species are dominant in many BC ecosystems.

1.8. The Symbiotic Fungus Grosmannia clavigera

One of my research goals was to compare the population genetic structure found for the MPB to the population structures reported for the MPBs primary symbiotic fungus, *G. clavigera* (previously *Ophiostoma clavigerum*), and for the MPBs primary host tree, *P. contorta*. A major goal of modern research is to determine the comparative phylogeography, and thus the comparative evolutionary ecology, of closely associated species (Stauffer *et al.*, 1999; Ritzerow *et al.*, 2004; Horn *et al.*, 2006; Vasconcelos *et al.*, 2006; Maroja *et al.*, 2007). I begin with the MPBs most critical symbiont. Given my comparative analyses (see Discussion), it is pertinent to thoroughly introduce *G. clavigera* and review both its mutualism with the MPB and the factors affecting its evolution.

Grosmannia clavigera is the main symbiotic fungus of the MPB (Solheim, 1995; Yamaoka *et al.*, 1995; Solheim and Krokene, 1998). Both species benefit from their mutualistic relationship. *G. clavigera* benefits by being dispersed between suitable host trees (Harrington, 1993; Paine *et al.*, 1997), which are scarce during the temporally-dominant endemic MPB population phase (Preisler and Mitchell, 1993; Safranyik and Carroll, 2006). This fungus may be entirely dependent on the MPB for dispersal and thus long-term persistence (Six and Paine, 1999). Indeed, *G. clavigera* individuals produce a wide morphological range of asexual forms, or anamorphs, many of which are highly adapted for insect dispersal (Harrington, 1993). The fungus may benefit the MPB through: 1) protecting against antagonistic blue-stain fungi (Klepzig and Wilkens, 1997; Paine *et al.*, 1997); 2) helping overcome host defenses (Berryman, 1972; Owen *et al.*,

1987); 3) favourably altering the chemical and/or moisture composition of the phloem (Reid, 1961; Wagner *et al.*, 1979); 4) providing nutrients required for MPB reproduction and/or development (Whitney *et al.*, 1987; Goldhammer *et al.*, 1990; Six and Paine, 1998).

The mutualistic relationship between fungi and bark beetles has been widely supported but some researchers dispute such a relationship (Harrington, 1993). The key traits of mutualistic bark beetle fungi are pathogenicity and a degree of bark beetle specificity but most bark beetle fungi do not exhibit these traits (Harrington, 1993). However, *G. clavigera* is highly pathogenic in *Pinaceae* (Yamaoka *et al.*, 1995; Solheim and Krokene, 1998) and is the main symbiont carried by MPBs (Six, 2003). Also, *G. clavigera* is only vectored by two species, the MPB and its sister species the Jeffrey pine beetle (Lee *et al.*, 2007). The *G. clavigera* – MPB relationship is likely mutualistic (Six and Paine, 1999; Six, 2003). *O. montium* is also commonly associated with the MPB but is less pathogenic and species-specific compared to *G. clavigera* (Harrington, 1993). A new symbiotic fungus, *Leptographium longiclavatum*, was recently isolated from the MPB (Kim *et al.*, 2005; Lee *et al.*, 2006a) and has pathogenicity similar to *G. clavigera* (Lee *et al.*, 2006b), but given the wealth of research on *G. clavigera*, it is most likely the MPBs primary symbiont.

G. clavigera, like many fungi associated with bark beetles, is an ascomycete (Harrington, 1993). Its mycelia are predominantly haploid anamorphs that reproduce asexually, but during sexual reproduction, short-lived diploid teleomorphs are formed (Six and Paine, 1999). Asexual spores, or conidia, are produced in slimy masses that line

MPB galleries. These conidia are acquired by MPBs by adhering to the exocuticle or by ingestion into the mycangia of newly eclosed adult MPBs (Six and Paine, 1996).

The population genetic structure of *G. clavigera*, the MPBs main fungal symbiont, may yield insight into MPB population genetics. Lee *et al.* (2007) found low genetic diversity in *G. clavigera* isolated from MPBs or MPB-infested trees at seven sites in Canada (Houston, Fort St. James, William's Lake, Manning Park, Banff) and the United States (Hellroaring, Idaho; Hidden Valley, Montana). Lee *et al.* (2007) also found moderate but significant differences among the populations, partially attributable to distinct Rocky Mountain and BC Interior population genetic structures. They also found two very strongly supported genetically-distinct groups. Most individuals belonged to Group 1 (with representatives from all populations) while Group 2 contained nine individuals (representing the Rocky Mountain populations). Since both Group 1 and 2 were found in the Rocky Mountains but only Group 1 was found in the BC Interior, and since the MPB epidemic is spreading from the BC Interior, Lee *et al.* (2007) suggested that Group 2 may represent *G. clavigera* populations endemic to the Rocky Mountains while Group 1 recently came into secondary contact via MPB immigration. The two groups are so strongly supported (genetically unique) that they may represent cryptic species (Lee *et al.*, 2007).

The combination of results from Bartell (2007) and from Lee *et al.* (2007) show significant population structures in the MPB and its main fungal symbiont. These results support the hypothesis that geographic barriers are a dominant force in MPB population dynamics. Finally, Lee *et al.*'s (2007) results also suggest that highly isolated MPB populations exist in the Rocky Mountains that are only connected to BC Interior

populations by immigration during extremely severe MPB epidemics. There is an inconsistency, however, in Lee *et al.*'s (2007) results.

Low levels of genetic diversity in populations of MPB-associated *G. clavigera* (Lee *et al.*, 2007) are not consistent with several studies of haploid fungi, which found levels of genetic variation comparable to outbreeding diploid organisms (Spieth, 1975; Perkins and Turner, 1988). However, in a close relative to the MPB, *Dendroctonus jeffreyi* (Higby and Stock, 1982), Six and Paine (1999) also discovered low genetic variation in *G. clavigera*. The low genetic diversity of *G. clavigera* from both *D. ponderosae* and *D. jeffreyi* may be explained by immediate selection against deleterious alleles (Perkins and Turner, 1988), dominance of genetic drift over mutation in small populations (Christiansen and Feldman, 1986; Hartl and Clark, 1997; Beebee and Rowe, 2004), low levels of sexual reproduction (Harrington *et al.*, 1996), poor dissemination of sexual spores (Six and Paine, 1999), evolution of clonality to prevent the breaking up of high fitness genotypes essential for mutualism (Wulff, 1985), or by founder effects (Six and Paine, 1999).

Given its wide host preference within the genus *Pinus* (Wood, 1982), the MPB likely hasn't suffered species-wide founder effects. Moreover, *G. clavigera* associated with MPBs commonly reproduces sexually (Robinson-Jeffrey and Davidson, 1968). However, the ascospores derived from sexually-produced parents may not be produced fast enough for MPBs to acquire them before dispersal (Six and Paine, 1996); this may be especially true in most areas, where MPB life cycles are completed within one year. Notably, MPB contact with *G. clavigera* ascospores may be possible in mountain environments such as the Rocky Mountains, where MPB life cycles can last two years or

more (Safranyik and Carroll, 2006). A greatly prolonged MPB life cycle may ensure that a higher proportion of (sexual) ascospores relative to (asexual) conidia are ultimately acquired by dispersing MPBs. However, in sum, poor dissemination of sexual spores, the diversity reducing effects of clonality, and the rapid elimination of deleterious alleles may be the cause of low genetic diversity in MPB-derived *G. clavigera*. In mountain environments such as Banff, widespread sexual reproduction, combined with the predominant dissemination of ascospores, may maintain the higher levels of *G. clavigera* genetic diversity from Rocky Mountain populations found by Lee *et al.* (2007). Indeed, the higher levels of genetic variation in *G. clavigera* from the Rocky Mountains may be needed for the fungus to maximally respond to selective pressures and evolve (Six and Paine, 1999).

1.9. Primary Selective Pressures on the MPB and G. clavigera

It is important to understand the major host-mediated selective pressures on the MPB and its primary symbiont. Such pressures may explain regional differences in population structure. There are four major additive ways MPBs overcome host tree defenses and kill trees after attack in late summer/early fall (Franceschi *et al.*, 2005; Safranyik and Carroll, 2006). First, the beetles use efficient aggregation pheromones to attack healthy trees with the timing and numbers needed to overcome their defenses. Second, the beetles need a tolerance to host defense chemicals, most likely a high tolerance given ever-present constitutive defenses. Third, the beetles physically damage the bark and inner tissues of the host through adult and larval tunneling. Lastly and perhaps most critically, upon tree penetration MPBs introduce symbiotic fungi, present in their mycangia and/or on their exoskeleton, which infect the tree and prevent water conduction. This is critical because water and resin conduction must be stopped, or impeded to very low levels, very rapidly upon MPB attack (Franceschi *et al.*, 2005; Safranyik and Carroll, 2006). If not, constitutive defenses will pitch out and kill MPBs. The development of induced defense responses will follow, causing invader-specific damage.

The first three ways MPBs overcome host defenses most likely show low variation in MPB populations given their fundamental role in MPB ecology. The low variation of these three MPB-intrinsic traits is likely persistent across population phases. During the endemic phase, mass attacks do not occur and both tolerance to host chemicals and physical damage to the inner bark tissues are probably not important in

stressed trees. However, these three traits must be retained to overcome the strong defenses of mature trees during epidemics.

Of the above four factors, the most important is most likely the presence of symbiotic virulent fungi. MPB attacks are unsuccessful without these fungi (Yamaoka *et al.*, 1995; Six and Paine, 1998); there should be heavy selective pressure for fungal strains that are increasingly virulent and stop sap flow in trees expeditiously. It has been shown (Solheim, 1995; Solheim and Krokene, 1998) that *G. clavigera* is the most virulent of MPB symbiotic fungi. Among MPB symbiotic fungi, only *G. clavigera* is aggressive enough to kill lodgepole pine when introduced alone (Yamaoka *et al.*, 1995). Within the heavy selective pressure on symbiotic fungi, the Canadian Rocky Mountain environment, compared to the Interior, may differentially constrain or enhance selection on individual beetles for novel strains of symbiotic fungi. Since harboring more virulent strains of *G. clavigera*, which have regional adaptations, would represent a considerable fitness advantage for MPBs, this may explain the genetically distinct group of *G. clavigera* from Rocky Mountain MPB populations (Lee *et al.*, 2007).

My research may clarify the relationship between the Rocky Mountain and BC Interior populations of the MPB and its primary mutualist (*G. clavigera*). There are some large genetic differences between Rocky Mountain and BC Interior populations of *G. clavigera*. Lee *et al.* (2007) found that the number of unique AFLP markers (38 out of 469), heterozygosity, and polymorphism was higher in Rocky Mountain compared to Interior BC populations of *G. clavigera*. As previously mentioned, Lee *et al.* (2007) also found that most individuals in their study belonged to Group 1 (with representatives from all populations) while Group 2 contained nine individuals (representing the Rocky

Mountain populations). They hypothesized that Group 2 was an original endemic population while Group 1 was a widespread population that spread from the BC Interior during the current epidemic. Lee *et al.* (2007) suggested that Group 1 may be more prevalent because it is more pathogenic or is more beneficial for MPBs while Group 2 may be better adapted to the colder climate of the Rocky Mountains. This latter suggestion is concordant with my above prediction of differential selection for novel strains of *G. clavigera* in the isolated Rocky Mountains compared to the Interior.

In sum, my results will shed light on the association between the MPB, its primary symbiont, and its primary host, by providing key data on MPB population genetic structure. If Rocky Mountain MPB populations are very different from Interior MPB populations but show recent immigration from the Interior, matching the structure of *G. clavigera* (Lee *et al.*, 2007), there is evidence that these populations have evolved allopatrically. But if Rocky Mountain MPB populations are similar to Interior MPB populations, conflicting with the structure of *G. clavigera*, there is evidence for differential fungal selection in the Rocky Mountains.

Materials and Methods

2.1. Site Selection and MPB Collection

In the summers of 2005, 2006, and 2007, MPBs were collected, prior to adult dispersal, from nine regions throughout BC and Alberta for a total of 35 geographically distinct sampling sites (Table 1). In 2007 only Grande Prairie was sampled. Regions were selected based on: historical data on past MPB outbreaks; the perceived locations of native (endemic) populations; and geographic barriers to dispersal. Sampling was balanced between the edges of the current infestation, to determine the origin of recent MPB dispersal flights, and a distribution of sites across the epidemic area of BC and Alberta. Collection permits for Jasper, Banff, and Yoho National Parks, and for several BC Provincial Parks, were obtained, and landowner permissions were obtained as needed.

At each sampling site, 13 to 20 infested trees separated by 10 meters or more were selected. For each tree, bark was removed from each of four sides with a hatchet. A GPS location was taken at each sampled tree. At least four individual offspring were sampled from each of four separate breeding galleries per tree. Different breeding galleries were sampled to ensure genetically independent samples. Only lodgepole pine (*Pinus contorta* Douglas var. *latifolia*) trees were sampled to avoid bias due to potential host-associated MPB preferences.

Table 1. Sampled stands (35), by region, for the mountain pine beetle with GPS locations, year sampled, number of beetles fully-genotyped (n), mean observed heterozygosity, and mean number of alleles. Given locations are indicative of regions; sampling did not always occur within noted towns.

Location by Region	Abbr.	Latitude (N)	Longitude (W)	Year Sampled	n	Mean H_{et} Observed	Mean Number of Alleles
Rocky Mountains							
Chetwynd / Pine Pass	C/PP	55.6352	122.2522	2006	55	0.51	5.33
Willmore Wilderness	PC	53 5707	119.7928	2006	48	0.51	4.83
Mount Robson Provincial Park	YH	52.8949	118.7348	2005	46	0.54	5.17
Banff (Banff National Park)	TM	51 1770	115.5593	2006	45	0.61	5.67
Lake Louise (Banff National Park)	LL	51.4172	116.1793	2006	48	0.58	6.33
Kootenay National Park	KP	50 6436	115.9784	2005/2006	48	0.65	6.00
Golden	G	51 2385	116.6530	2005	47	0.66	5.83
Northeast of Rocky Mountains							
Tumbler Ridge	TR	54 8914	121 2252	2005/2006	48	0.53	4.50
Grande Prairie	GP	54 7270	118.9736	2007	30	0.47	4.17
Nechako Plateau							
Fort St. James	JP	54.6463	124 4196	2005	46	0.48	4.33
Francois Lake	FL	54.0317	124.9391	2006	48	0.51	4.17
Houston	HO	53.9938	126.6522	2006	47	0.48	4.83
Telkwa	TE	54 6677	127.0890	2006	48	0.47	4.33
West of Rocky Mountains							
Mackenzie	KL	54.6963	122.8203	2005	47	0.42	4.33
Prince George	PG	53.9068	122.8066	2005	47	0.54	5.17
McBride	Rf	53 3120	120.1266	2005	24	0.49	4.17
Valemount	V	52.6590	118.9965	2005	48	0.56	5.67
Cariboo-Chilcotin							
Quesnel	QU	53.0423	122.2519	2006	46	0.55	5.33
Bowron Lake Provincial Park	BL	53.2488	121.4162	2006	47	0.54	5.50
Farwell Canyon	FC	51.6590	122.9177	2006	48	0.48	5.17
Tatla Lake	TA	51.9715	124.4130	2006	44	0.52	4.67
Lac La Hache	LA	51.7342	121.6071	2006	46	0.57	5.50
Wells Gray Provincial Park	SP	51.7410	120.0121	2006	47	0.60	6.33
Coast Mountains							
Whistler	GL	50.1683	122.9260	2006	48	0.56	5.50
Cascade Mountains							
Manning Park	MP	49.2163	121.0698	2006	46	0.58	5.67
Thompson-Okanagan							
Lillooet	LC	50.4568	121.6346	2006	44	0.56	6.33
Merritt	IR	50.0343	120.6575	2006	48	0.58	6.83
Kamloops	KA	50.4859	120.5321	2006	43	0.64	6.33
Falkland	RR	50.5199	119.6018	2006	48	0.58	6.17
Kelowna	KE	49.9978	119.6657	2006	46	0.59	6.17
Kootenays							
Nancy Greene Provincial Park	NG	49.2592	117.9277	2006	48	0.64	6.00
Valhalla Provincial Park	VA	49.7500	117.4949	2006	48	0.55	6.00
West Arm Provincial Park	WA	49.5250	117.2321	2006	46	0.55	6.17
Argenta	AR	50.1566	116.9164	2006	48	0.64	5.83
Kimberley	ANG	49.6416	116.2124	2005	49	0.62	6.50

The 35 populations were sampled over two years, from mid-June to mid-July, to maximize collection of the adult MPB life stage (two exceptions: May – Willmore Wilderness; from bolts – Grande Prairie). Sampling occurred during this "window" because most adult MPB offspring disperse in mid-July, requiring an earlier collection date. Different rates of development and different macro- and microclimatic conditions between sampling sites, province-wide, resulted in collection of larval, pupal, and adult life stages. In some high elevation stands, for example, only overwintering adults and tiny, unsuitable larvae were found because of reduced temperatures, shorter summers, and reduced phloem thickness as compared to valley bottom sites (Safranyik and Carroll, 2006). Conversely, at sites with a high southern exposure at low elevations, some beetles had emerged by mid-June. Analyses show no difference in the quality of DNA extracted from different MPB life stages.

Sampled beetles were placed in labeled vials containing 95% ethanol and stored at -80°C for DNA preservation. From each gallery, one beetle was randomly selected for genetic analysis (at least 48 beetles/sampling site).

2.2. DNA Extraction and Evaluation

Using a standard proteinase K and phenol/chloroform procedure (Sambrook and Russell, 2001), DNA was extracted from one beetle per gallery for each site. Adult offspring were selected when possible. Sterile plastic micropestles were used to homogenize each beetle sample, followed by two applications of proteinase K and incubation, culminating in overnight digestion in an incubator at 37°C. DNA was extracted as above, precipitated in ethanol/sodium acetate solution, and resuspended in

Tris-EDTA buffer (pH 8.0) for storage at –80°C. The amount and quality of extracted DNA was evaluated through electrophoresis on 1% agarose gels using ethidium bromide staining. To normalize DNA to 100 ng/μL in Tris-EDTA buffer (pH 8.0), extraction concentrations were determined using a NanoDrop® ND-1000 UV-Vis Spectrophotometer.

2.3. PCR of Microsatellites

Through an initial collaboration with a research team led by Dr. Karen Mock (Utah State University), I had access to the microsatellite markers they developed. Only two marker systems, CAL 1-1 and MPB 017, were confirmed at UNBC to amplify beetle DNA (Steinke, 2006). Even in highly-controlled, sterile laboratory environments with enrichment protocols, building bark beetle microsatellite libraries is extremely difficult (Sallé *et al.*, 2007). My second collaboration, with Dr. Janice Cooke of the University of Alberta, is a prime example of such difficulty. Approximately 80% of all primers developed for the mountain pine beetle (*Dendroctonus ponderosae*), despite considerable controls, were developed for fungal mutualists co-occurring in beetle DNA extractions (C. Davis, unpublished data). Four marker systems were confirmed at UNBC to amplify beetle DNA (MPB25, MPB30, MPB35, and MPB40) while a fifth marker system (MPB50) was only sporadically successful and was discontinued. Thus six microsatellite systems were used in this project.

PCRs were run individually, using one microsatellite per beetle, for each selected sampling site. The reagent concentrations for PCR were (for CAL 1-1 and MPB 017): 2.4mM $MgCl_2$, 200μM (each) dNTP's, 1X PCR buffer, 200nM of dye-labelled forward

primer, 200nM of reverse primer, 1 unit of Taq DNA polymerase, and 20ng of DNA template, to a final volume of 25μL. The reagent concentrations for PCR were (for MPB25, MPB30, MPB35, and MPB40): 2.4mM $MgCl_2$, 200μM (each) dNTP's, 1X PCR buffer, 80nM of M_{13}-tagged left primer, 320nM of right primer, 640nM of dye-labelled M_{13} primer, 1 unit of Taq DNA polymerase, and 20ng of DNA template, to a final volume of 15μL.

Negative controls substituted 1μL of PCR water for template beetle DNA. For CAL 1-1 and MPB017, thermal cycling involved temperatures of 94°C for 4 minutes, followed by 36 consecutive cycles of: one minute of denaturation at 92°C, annealing (49°C for Cal 1-1 and 56°C for MPB017) for 1 minute, and extension for 1 minute at 72°C with extension in the final cycle lasting 11 minutes. For MPB25, MPB35, and MPB40, thermal cycling was 94°C for 4 minutes, followed by 16 consecutive cycles of (touchdown cycles): one minute at 94°C, 56°C (decreasing by 0.5°C each cycle) for 1 minute, and 72°C for 1 minute, then 17 consecutive cycles of (main cycles): one minute at 94°C, one minute at 53°C, and one minute at 72°C with extension in the final cycle lasting 11 minutes. Thermal cycling was similar between MPB 25, 35, 40 and MPB 30 except that MPB 30 had a 59°C initial touchdown annealing temperature and a 50°C main cycle annealing temperature. Completed reactions were stored at –20°C.

2.4. Analysis of Microsatellites

PCR reactions underwent fragment analysis on Beckman-Coulter CEQ8000 automated DNA sequencers. All allele scoring was done manually to prevent both the erroneous scoring of artefacts and the occurrence of null (unrecognized but real) alleles

(Jarne and Lagoda, 1996; Dakin and Avise, 2004). Different alleles were determined for each microsatellite locus and genotypes were generated for each beetle (~50 beetles per sampling site).

2.5. Statistical Analyses

2.5.1 Data Set Validation

I analyzed my data set for concordance with Hardy-Weinberg Equilibrium (HWE) expectations of random mating as well as for linkage disequilibrium, the non-random association of alleles between loci. Genetic studies across taxa have confirmed that the majority of populations conform to HWE expectations of random mating. Under HWE, population genotype frequencies remain relatively constant over time. Although HWE is theoretically violated by: assortive mating, inter-population gene flow, natural selection on markers, mutations, and genetic drift, many populations that are considerably influenced by one or more of the above factors remain in HWE (Beebee and Rowe, 2004). Deviation from HWE in a population may imply Wahlund effects (sampling across populations) or null alleles (alleles that fail to PCR amplify) (Beebee and Rowe, 2004). I used Arlequin 3.11 (Excoffier *et al.*, 2005) to test stands for HWE using an expansion of Fisher's exact test (Guo and Thompson, 1992). Six thousand dememorization steps, to ensure the independence of results from the starting configuration, and 150,000 MCMC steps were used. Bonferroni corrections for multiple statistical comparisons (Rice, 1989) were applied at the stand level (.008333 = .05 / 6 loci) and for all pairwise comparisons (.000238 = .05 / 210 tests).

I tested for linkage disequilibrium to ensure that loci were independent. I used some statistical programs that assume linkage equilibrium within populations. Significant linkage disequilibrium can arise because of inbreeding or hybridization, but also because loci are physically proximate on chromosomes. I used Arlequin to test stands for linkage disequilibrium using an extension of Fisher's exact test (Raymond and Rousset, 1995) with 6,000 dememorizations and 150,000 MCMC steps. Bonferroni corrections for multiple statistical comparisons were applied at the stand level (.003333 = .05 / 15 tests per stand) and for all pairwise comparisons (.000095 = .05 / 525 tests).

2.5.2 AMOVA

I used Arlequin to assess population structure using AMOVA (Analysis of Molecular Variance). AMOVA tests *a priori* population structure in which stands were defined based on location of collection (35 stands or populations). Creating an x-dimensional matrix (x = number of loci), AMOVA calculates the genetic distance between individuals. Total genetic variation is hierarchically partitioned into: among groups; among populations within groups; among individuals within populations, and significance is tested by permutation (Excoffier *et al.*, 1992). Each AMOVA was run with 10,000 permutations at a .05 significance level.

Pairwise F_{ST} values were also generated, as F_{ST} is a good correlate of short-term genetic distance between populations (Reynolds *et al.*, 1983; Slatkin, 1995). The calculation of F-statistics has been refined since the pioneering work of Wright (1951) but the theoretical basis remains. For F_{ST}, deviations from Hardy-Weinberg Equilibrium are quantified as:

$$F_{ST} = (H_t - H_s) / H_t$$

Where H_t is the heterozygosity across populations and H_s is the average heterozygosity of a given population. Arlequin was used to calculate F_{ST} values with Weir and Cockerham's (1984) correction for unequal sample size. Using AMOVA, individual genotypes are permuted between populations to assess significance. Pairwise F_{ST} values were calculated with 10,000 permutations (a=.05). However, tests other than AMOVA, such as Nei's (1987) multilocus G-test of population differentiation, are also widely used to determine the significance of pairwise F_{ST} values. I thus validated the Arlequin AMOVA pairwise F_{ST} analyses by conducting the multilocus G-test included in the program FSTAT 2.9.3.2 (Goudet, 1995) with 11,900 permutations (a=.05).

2.5.3 Structure

To strengthen the population structure inferred from the Arlequin AMOVA results, I used an innovative population genetics program. Structure 2.2 (Pritchard *et al.*, 2000; Falush *et al.*, 2003; Falush *et al.*, 2007) does not test for population structure using populations that are defined *a priori*, often by collection site. This intellectual advance allows for the detection of cryptic population structures (eg. Rosenberg *et al.*, 2002; Harter *et al.*, 2004). Structure is also well-suited to data from a range of genetic markers, including microsatellites. Using a Bayesian approach, Structure groups individuals with similar genotypes into clusters based on likelihoods of cluster membership.

I used a model with admixture and correlated allele frequencies. This was the most biologically-realistic model for the MPB; each individual MPB has likely inherited a fraction of its ancestry from each of the K clusters, due to population admixture, while

population allele frequencies are likely to be correlated due to inter-population breeding and/or shared ancestry. I did not use the linkage model as the incidence of linkage disequilibria in my data was rare and apparently random. Population structure was determined by examining Structure's output, in terms of stand membership, for a user-defined range of cluster values (K). Typically, population structure will be most apparent at a lower K. Population structure will decline with increasing K as the likelihood of individual membership in one cluster is diluted by membership in other clusters. Runs were conducted for K-values ranging from 2 to 35. For each run, 100,000 iterations of burn-in and data collection were performed. Multiple runs confirmed the consistency of results. The program Distruct (Rosenberg, 2004) was used to convert Structure results to a visual format.

2.5.4 SAMOVA

SAMOVA (Spatial Analysis of Molecular Variance; Dupanloup *et al.*, 2002) was used to confirm the population structure supported by the AMOVA and Structure results. This program uses inputs of both GPS data, with one point for each collected stand, and genotype data. Similarly to Structure (Falush *et al.*, 2007), SAMOVA determines population structure using only genotype data. However, while Structure assigns individuals to groups, SAMOVA assigns stands to groups. SAMOVA additionally considers but does not require stand membership in a group based on geographic proximity. SAMOVA uses a simulated annealing procedure in which stands are randomly assigned to groups until maximum differentiation between groups is achieved; in AMOVA terms, maximum-differentiation entails optimizing the amount of genetic

variance due to differences between groups and is represented by the statistic F_{CT}. Similar methods, such as the Monmonier algorithm (Simoni *et al.*, 1999), have been shown to be less powerful than SAMOVA (Dupanloup *et al.*, 2002). The boundaries between groups should correspond to genetic barriers. Runs were conducted for K values from 2 through 15. To minimize the influence of initial configurations on the results, the simulated annealing process was repeated 100 times for each run.

Molecular distances were calculated as pairwise differences, which are more typical for DNA sequence data, as well as calculated as sums of squared size differences, typical of microsatellite data. Both distance calculations were used for all runs to compare outputs. However, sums of squared size differences were less appropriate for my data set because 1/2 and 1/3 repeat allele size differences were present. Microsatellite-based measures calculate distance based on number of mutation events but my data set made assumptions regarding mutational events unclear. Moreover, microsatellite-based measures are based on the assumption that allele size differences and divergence are correlated (Balloux and Lugon-Moulin, 2002). This assumption is unrealistic for many species (Shriver *et al.*, 1993) and is often violated (Paetkau *et al.*, 1997; Zhang and Hewitt, 2003). Pairwise measures, in which degree of difference is not considered, were a more conservative and realistic analysis choice. Population structure was evaluated by the magnitude of F_{CT} and the significance of the structure (Arlequin – AMOVA). Multiple runs confirmed the consistency of results.

2.5.5 Isolation by Distance

Isolation by distance (IBD) is a correlate for population structure under a stepping stone model. In IBD, the genetic distance and geographic distance between pairs of populations is positively correlated. Thus nearby populations are expected to be highly related and relatedness is expected to decline linearly with increasing distance between populations. Genetic distances between stands (Arlequin – F_{ST}) were plotted against the straight-line geographic distances between stands. While some population genetics studies have used more-derived IBD models (eg. Sallé *et al.*, 2007), these models are optimized for the estimation of demographic parameters from population genetic data (Rousset, 1997). In my IBD analyses, I was looking for trends in my data; I thus did not seek to indirectly estimate parameters such as the number of migrants between populations per generation, or population density, as examples.

In my IBD analyses, I did not consider the distribution of suitable host tree species in the calculation of geographical distances between stands. Unlike Mock *et al.* (2007), whose research area included the Great Basin and Mohave Deserts, I studied a portion of the MPBs range where pine stands are largely contiguous.

Because each IBD data point was not independent, standard regression analyses were not used. Instead, I used the Mantel test (Mantel, 1967; Smouse *et al.*, 1986) within Arlequin to test the significance of the relationship between genetic and geographic distance between stands. The Mantel procedure tests the significance of the correlation between two matrices, using a permutation procedure to account for auto-correlation. This test was performed with 10,000 random iterations (a=.05).

2.5.6 Other Analyses

I conducted a number of other analyses at either coarse provincial or fine stand-level scales. MPB population genetic structure was overlain with maps of historic distributions of climatic suitability classes for the MPB (Carroll *et al.*, 2004). Two related but different measures of the genetic diversity of stands, mean observed heterozygosity and mean number of alleles, were plotted against the north-south distance of stands from 49° latitude, the BC/US border, to investigate possible north-south genetic gradients. I hypothesized that north-south genetic gradients may exist based on two ideas: the MPBs genome should reflect Ice Age patterns of refugial isolation and subsequent recolonization of BC; moreover, the strongest abiotic influence on the MPB is climate and climate follows a general north-south gradient in BC.

At a stand scale, the incidence of private alleles, alleles which are found in only one stand, is a simple estimate of the genetic distinctiveness of stands (Kalinowski, 2004). Private alleles were tabulated by locus and stand. The mean pairwise F_{ST} values of unique stands, those that were significantly different from all others in AMOVA analyses (Arlequin; a=.05), were compared with mean F_{ST} values across all 35 stands for evidence of population bottlenecks and/or founder effects.

I did not conduct statistical tests within Bottleneck (Piry *et al.*, 1999) because at least ten loci are required to obtain reasonable power. I did conduct per-stand tests for mode-shift distortions of allele frequencies. Stable populations tend to have an L-shaped allele frequency distribution as most alleles are rare and by definition exist at low frequencies (< 0.10). Bottlenecks cause most rare alleles to be lost such that the

dominant peak in the allele frequency distribution shifts to an intermediate frequency (eg. 0.2 to 0.3; Luikart *et al.*, 1998).

Mean number of alleles and number of private alleles were not adjusted for variation in sample size as statistics (mean n = 46; standard deviation = 5.17) did not warrant adjustment. Outlier stands, McBride with 24 samples and Grande Prairie with 30 samples, were highlighted instead; these two stands were responsible for 60% of the above standard deviation.

Results

3.1. Hardy-Weinberg Equilibrium and Linkage Disequilibrium

Tests for HWE at each locus for each stand revealed thirteen significant deviations out of 210 tests using a stand-level Bonferroni correction and only three deviations using a correction for all tests (data not shown). Of the former, ten deviations were due to a deficiency of heterozygotes while three deviations were due to an excess of heterozygotes. Using two levels of Bonferroni correction confirmed that deviations were distributed across stands and loci. Given the stochastic and rare occurrence of deviations, I assumed populations were in HWE for subsequent analyses.

Tests for linkage disequilibria between pairs of loci for each stand revealed twenty significant deviations out of 525 tests using a stand-level Bonferroni correction and only eight deviations using a correction for all tests (data not shown). Of the former, most stands had one instance of disequilibria and only five (Banff, Argenta, Tatla Lake, Kimberley, Golden) had two disequilibria. Using two levels of Bonferroni correction confirmed that deviations were distributed across stands and loci; deviations remained distributed after the more stringent correction was applied. The incidence of linkage disequilibrium was highest between MPB35 and MPB40 (five deviations using the former correction). Given the stochastic and rare occurrence of deviations, for subsequent analyses I assumed populations were in linkage equilibrium at all loci.

3.2. AMOVA

AMOVA analyses revealed shallow but highly significant MPB population structure in Western Canada (global F_{ST} = .03828; $P < .00001$). F_{ST} is the variation among stands relative to the total variance. Nearly 81% of 595 pairwise between-stand AMOVA tests rejected the null hypothesis and thus found stands were significantly different. I created a map of genetic similarities where paired stands that were not significantly different (F_{ST} calculation with AMOVA; a=.05) were joined by lines (Figure 1). Based on my population genetic data, Figure 1 suggested the existence of a Northern and Southern group with genetic similarities existing through the Fraser Valley (defined in this Thesis as the portion of the Fraser River from Prince George to Vancouver). Besides geographical separation, the groups were also characterized by the incidence of genetic similarities. Similarities were sparse in the Northern group as compared to the complicated "web" in the Southern group (Figure 1). Notably, five stands: Whistler, Mackenzie, Tumbler Ridge, McBride, and Willmore Wilderness, were significantly different from all other sampled stands. A multilocus G-test conducted using FSTAT was found to be less conservative than the Arlequin AMOVA method for assessing the significance of pairwise F_{ST} values (data not shown). The multilocus G-test found more significant differences between populations. Using the results from FSTAT (a=.05), I created another map of genetic similarities (figure not shown). This map supported the AMOVA map, confirming the existence of: a Northern and Southern group with similarities through the Fraser Valley; few similarities in the north and many similarities in the south; and the same five unique populations.

Figure 1. Instances of genetic similarity among 35 MPB stands in BC and western AB analyzed at six microsatellite loci. Connecting lines denote that stands are not significantly different and thus are genetically similar (Arlequin – AMOVA F_{ST}; a = .05; 9999 permutations). Sampling sites are represented as circles. Similarities imply some level of past and/or recent gene flow. This population structure (H_a = all stands are a different population) was shallow but highly significant (AMOVA – global F_{ST} = .03828; $P < .00001$). The inset map denotes the names of sampling locations.

3.3. Structure

Structure analyses gave the strongest support to the existence of two population clusters (K=2). Population structure increasingly dissolved at runs with higher K-values. Figure 2 shows the likelihood of membership in all clusters for each individual beetle, grouped by stand. Runs with K=2 captured the most structure in my data and subsequent runs at higher K-values, such as at K=3, quickly dissolved apparent population structure (Figure 2). Clines of likelihood of cluster membership (K=2) were plotted onto a map of stand locations (Figure 3). This Structure-derived map clearly supported the existence of a Northern and Southern group. In Figure 3, the Northern and Southern group are demarcated by a .50/.50 isocline of likelihood of membership in either cluster. In both the Northern and Southern groups, stands farthest from the .50/.50 isocline had the highest likelihood of membership in their respective group. Multiple runs yielded identical likelihood-of-cluster-membership values for each stand. An AMOVA with Arlequin determined that the Northern and Southern groups defined by Structure were highly significant (F_{ST} = .05543; F_{CT} = .03665; both $P < .00001$). F_{CT} is the variance among groups relative to the total variance. The possible existence of groups defined by the isoclines in Figure 3 was tested with an AMOVA in Arlequin but this five-group population structure (F_{ST} = .04420; F_{CT} = .02947; both $P < .00001$), while significant, explained less total genetic variation compared to the two-group Northern and Southern population structure.

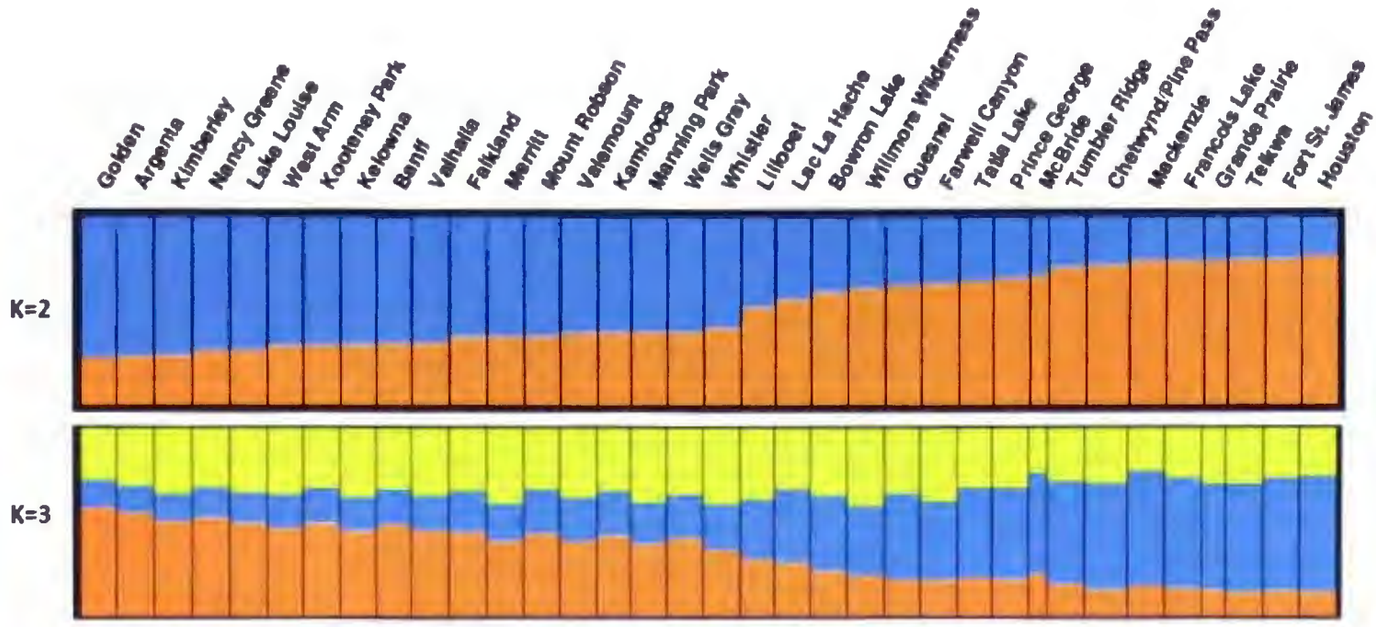

Figure 2. Population structure estimated using the program Structure. Each individual is represented by a thin vertical line that is partitioned into K coloured segments that represent the individual s likelihood of membership in each of the K clusters. Individual likelihoods were summed into a mean population likelihood of membership. Black vertical lines separate populations. Explanation: at K=2 there are two groups (blue and orange); at far left Golden has the highest likelihood of membership in the blue group; membership in the blue group declines moving right· at far right Houston has the highest likelihood of membership in the orange group. At K=2 Structure partitioned the 35 stands into a Southern (blue) and Northern (orange) group. Notably, at higher K values, as represented on this figure by K=3, at far left Golden clearly belongs to the orange group and at far right Houston clearly belongs to the blue group. However, the membership of the remaining populations in the middle of the figure is approximately equally shared among the three groups (yellow, blue, and orange)· thus, population structure dissolved in Structure analyses using K-values higher than two.

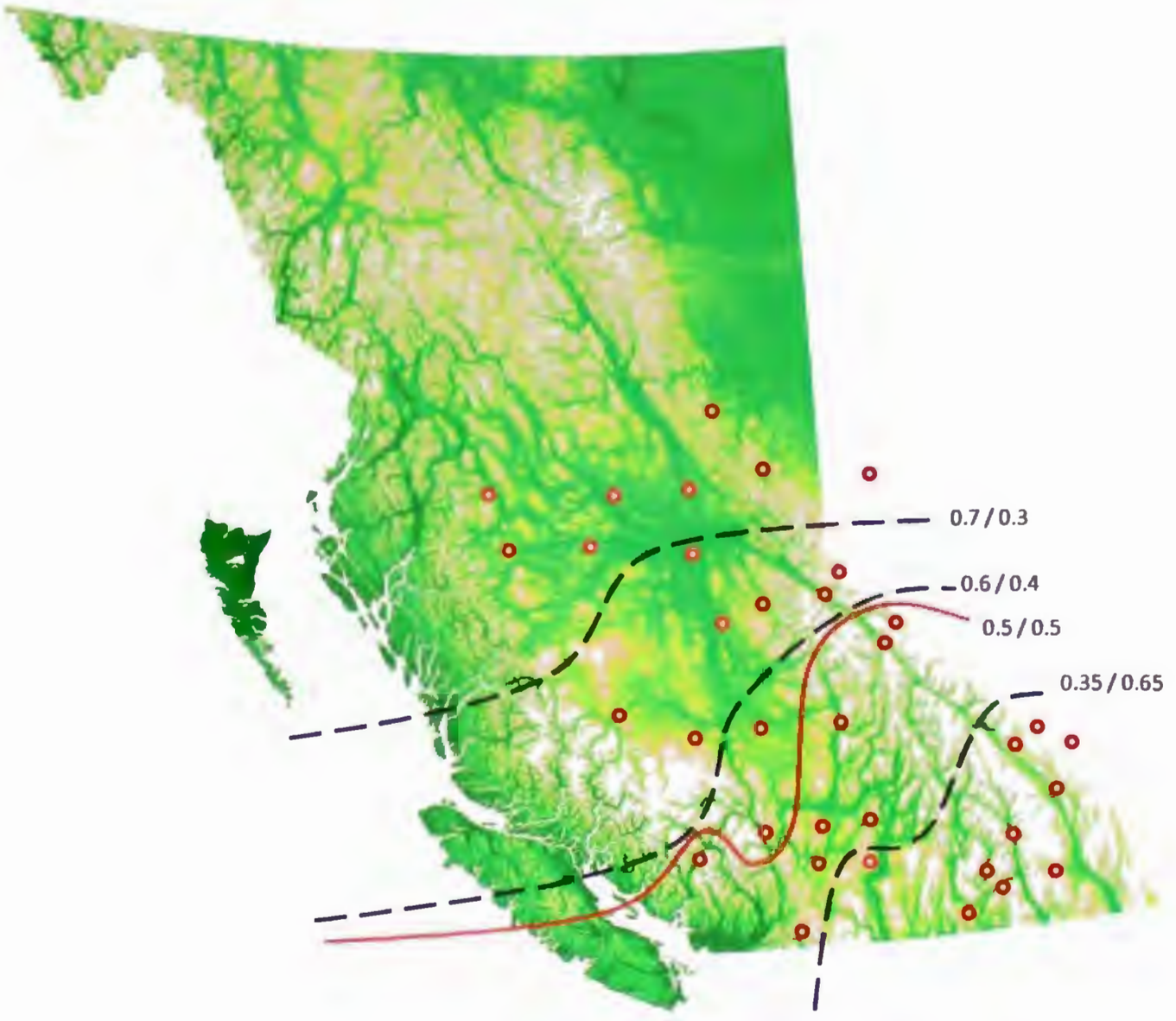

Figure 3. Clines of likelihood of cluster membership (K=2) derived from a Structure analysis of population structure among 35 sampled MPB stands in BC and western AB. Structure most strongly supported the existence of two groups, Northern and Southern, which are separated in this figure by a .50/.50 membership isocline; for each cline, likelihood of stand membership values are for the Northern group on the left and for the Southern group on the right. Sampling sites are represented as circles. The Northern and Southern group population structure (all stands above the .50/.50 line in the Northern group and vice versa) was highly significant (AMOVA – F_{ST} = .05543; F_{CT} = .03665; both $P < .00001$).

3.4. SAMOVA

SAMOVA analyses both confirmed and refined my analyses using Structure. Similarly to Structure, SAMOVA gave the strongest support to the existence of a Northern and Southern group (K=2). However, SAMOVA refined the Northern group / Southern group boundary (Figure 4), which Structure defined very loosely. The Northern and Southern group population structure defined by SAMOVA explained the most total genetic variation of all the population structure models (F_{ST} = .05794; F_{CT} = .03449; both $P < .00001$). Hierarchical partitioning placed 94.2% of total genetic variance within stands and most of the remainder (3.5%) was among the Northern and Southern groups; 2.3% of variation was among stands within each group. SAMOVA analyses at higher K-values did not rearrange stands among groups or split groups. Instead, a single population was excluded with each successive run at a higher K-value. Thus, the SAMOVA population structure at K=2 was most strongly supported by both patterns of stand assignment as well as the maximal amount of total variance explained. All further references to a Northern and Southern MPB group are specific to the SAMOVA analyses.

Using pairwise and sums of squared size differences for genetic distance calculations had no effect on SAMOVA population structures. I thus used results derived from pairwise differences, which are more conservative. Multiple runs at each K-value confirmed the consistency of results.

Figure 4. Population structure derived from a SAMOVA analysis of 35 stands of MPBs sampled in BC and western AB. SAMOVA most strongly supported the existence of two groups (Northern and Southern); the boundary between these groups is the solid black line. Sampling sites are represented as circles. This Western Canadian MPB population structure explained the most genetic variation of all of my analyses (AMOVA – F_{ST} = .05794; F_{CT} = .03449; $P < .00001$).

3.5. Isolation by Distance

I found a highly significant and strong effect of isolation by distance ($r = .55$; $P < .000001$; Figure 5). I then tested whether this IBD pattern was driven by the population structure indicated by previous analyses. Specifically, I tested whether this strong IBD pattern was due to the Northern group, the Southern group, or interactions between the groups. IBD within groups only included within-group stand comparisons while IBD between-groups only included comparisons between Northern and Southern stands. Strong and significant IBD was found for the Southern group ($r = .60$; $P < .000001$; Figure 6) and for the Northern group versus the Southern group ($r = .31$; $P < .000001$; Figure 7). There was no IBD effect for the Northern group ($r = .11$; $P = .6887$; Figure 8).

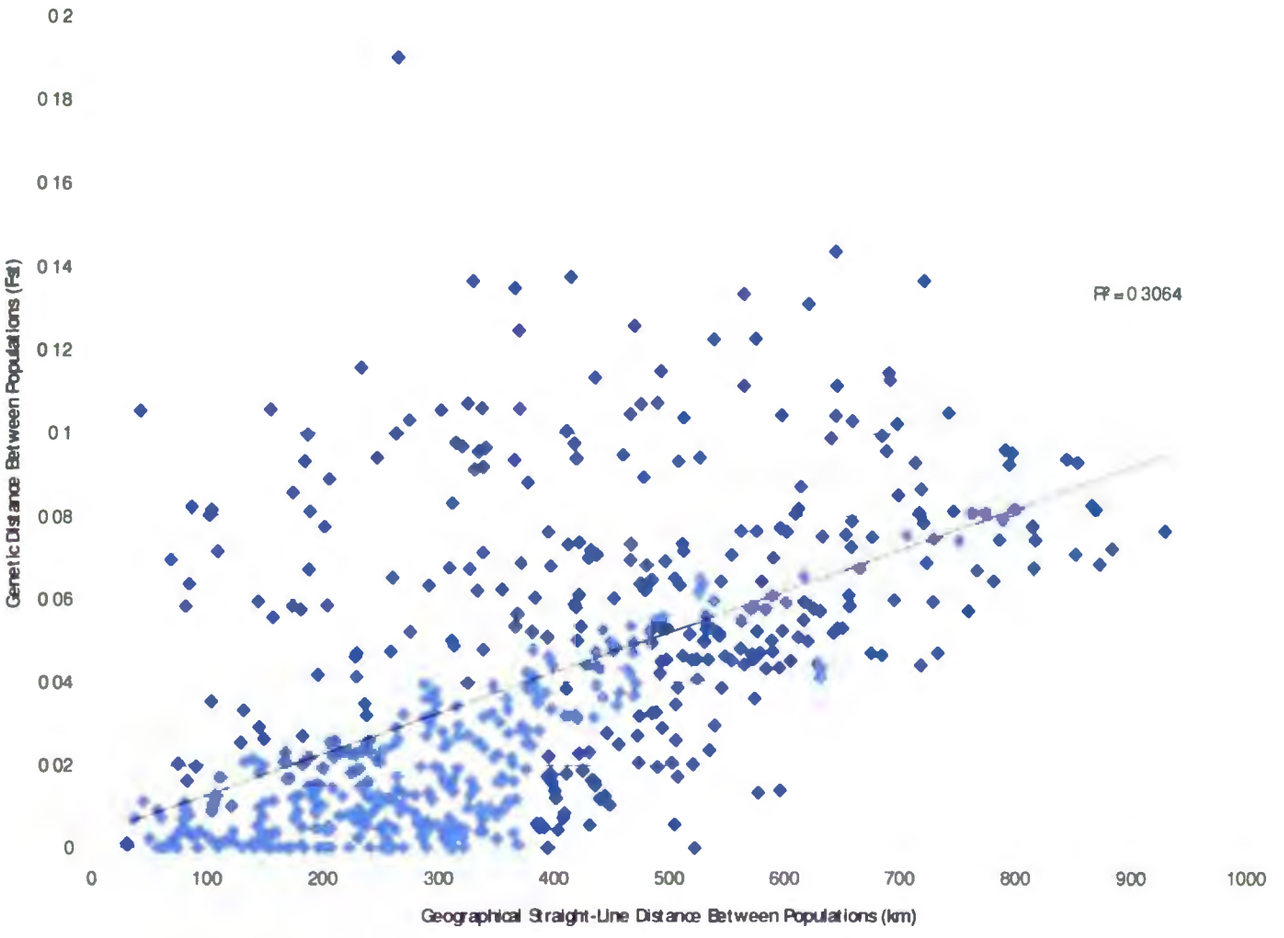

Figure 5. Isolation by distance (IBD) pattern resulting from comparison of F_{ST}-values and straight-line geographic distances between all pairs of stands. Thirty-five MPB stands were sampled in BC and western AB and analyzed at six microsatellite loci. This relationship was highly significant (Arlequin – Mantel test with 10,000 permutations; $P < 0.000001$).

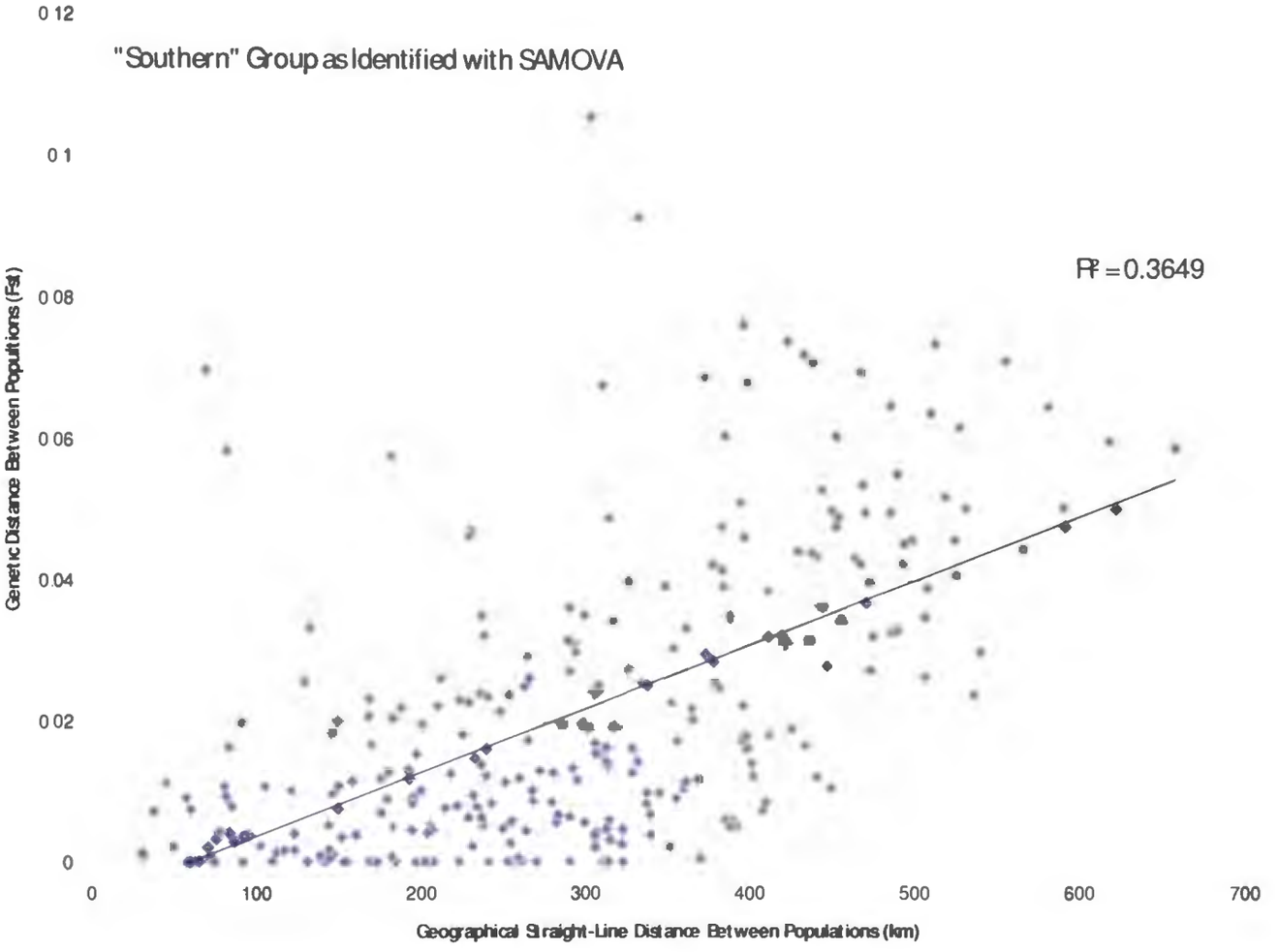

Figure 6. Isolation by distance (IBD) pattern resulting from comparison of F_{ST}-values and straight-line geographic distances between all pairs of stands within only the SAMOVA-defined Southern MPB group. This relationship was highly significant (Arlequin – Mantel test with 10,000 permutations; $P < 0.000001$).

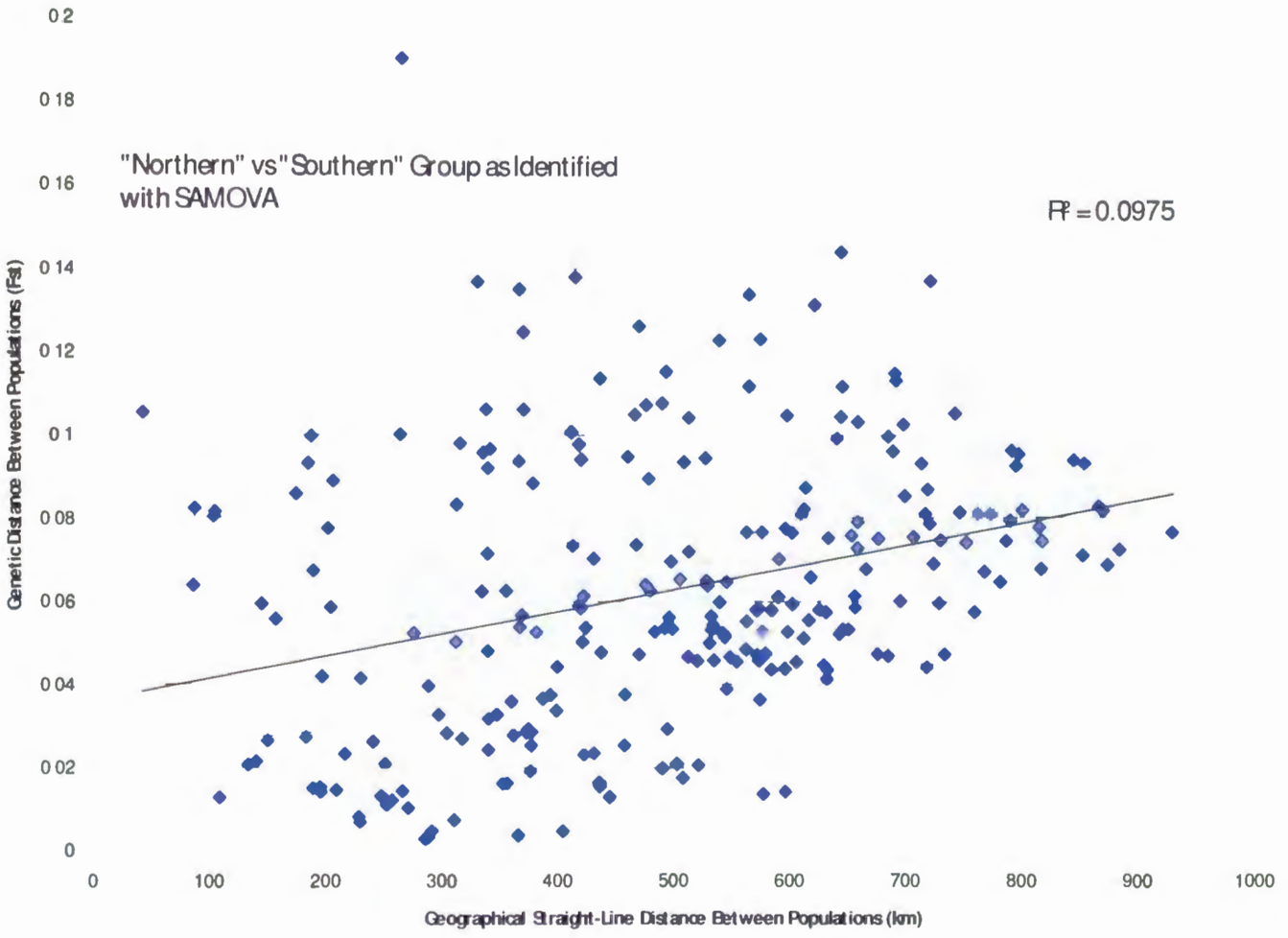

Figure 7. Isolation by distance (IBD) pattern resulting from comparison of F_{ST}-values and straight-line geographic distances between all pairs of stands between only the SAMOVA-defined Northern and Southern MPB groups. This relationship was highly significant (Arlequin – Mantel test with 10,000 permutations; $P < 0.000001$).

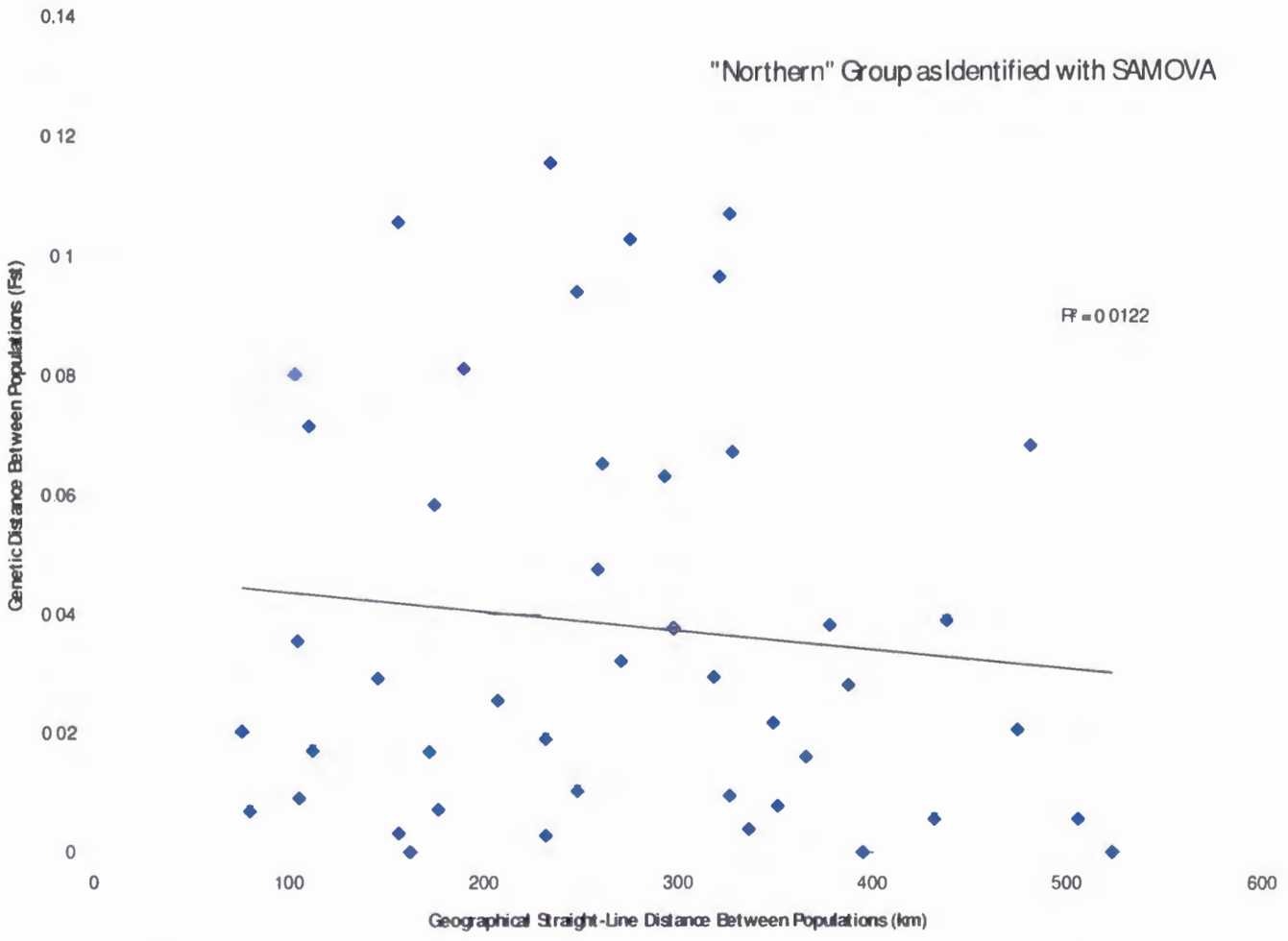

Figure 8. Isolation by distance (IBD) pattern resulting from comparison of F_{ST}-values and straight-line geographic distances between all pairs of stands within only the SAMOVA-defined Northern MPB group. This relationship was not significant and a regression line is provided for illustrative purposes (Arlequin – Mantel test with 10,000 permutations; $P = .6887$).

3.6. Other Analyses

Overlaying maps of historical distributions of MPB climatic suitability classes (CSC; Carroll *et al.*, 2004) with my SAMOVA-derived population structure indicated that climate is a likely driver of MPB population structure (Figure 9). The habitat of the Southern group is typified by high or extreme CSCs, indicating that the Southern group generally occupies a climatically-optimal environment. In contrast, the habitat of the Northern group is typified by roughly equal proportions of extreme CSCs, from very low/low to high/extreme. This pattern, and thus the concordance between historical distributions of MPB CSCs and current MPB population structure, is weakest for the most recent 30-year period (1971-2000; Figure 9). Current MPB population structure has the highest visual concordance with the climatic suitability class distribution over the period of 1921-1950 (Figure 9). As the 20th century progressed, there was a major expansion of climatic suitability into the regions west of and including Prince George; however, MPB population structure did not reflect this change.

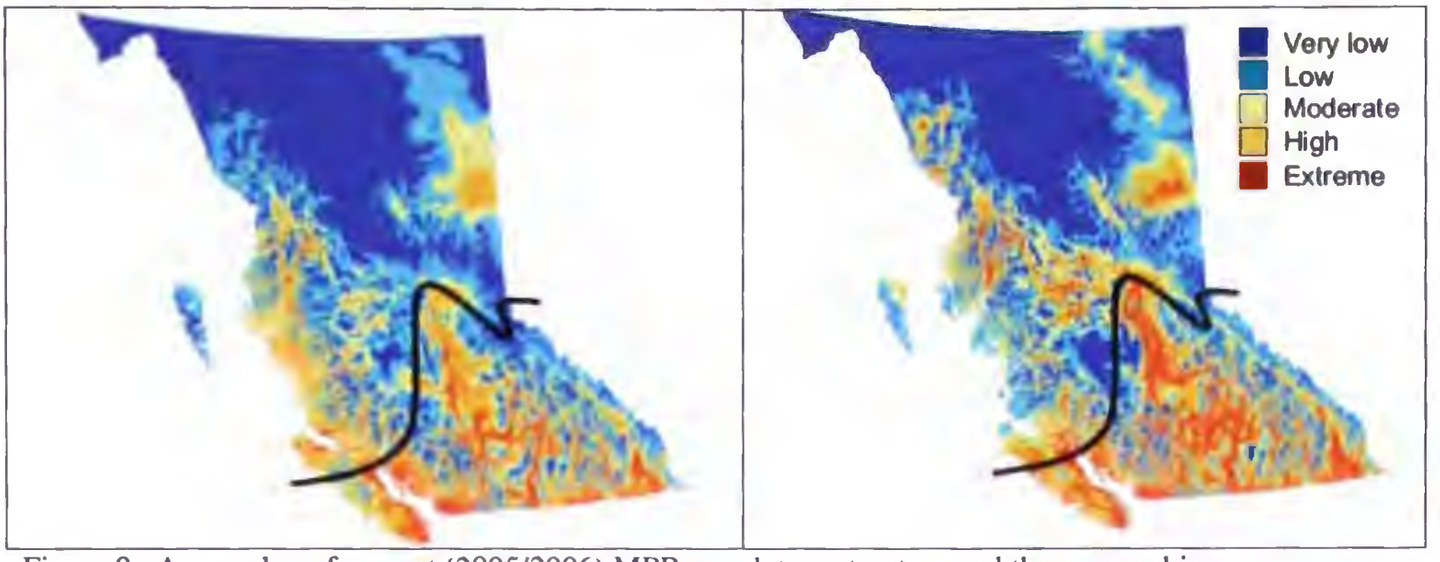

Figure 9. An overlay of current (2005/2006) MPB population structure and the geographic distribution of regions that are climatically favourable for the MPB (Left: 1921-1950 climatic data; Right: 1971-2000 climatic data; Carroll *et al.*, 2004). The SAMOVA-derived MPB population structure is shown, in which the northern and southern groups are demarcated by a solid black line. "Very low" CSCs indicate regions which are climatically unsuitable for the MPB while "Extreme" CSCs indicate regions climatically optimal for MPBs. CSC distribution maps modified and reproduced with permission of A. Carroll.

I found evidence for two north-south genetic gradients among stands. Mean number of alleles per stand had a highly significant inverse relationship with the straight-line north-south distance of each stand from the BC/US border (49° latitude; Figure 10; r = .81; $P < .00000001$). Mean observed stand heterozygosity also had a highly significant inverse relationship with straight-line north-south distance of each stand from the BC/US border (49° latitude; Figure 11; $r = .73$; $P < .000001$). In these relationships, southern stands had higher values for each response variable, intermediate populations had intermediate values, and northern stands had the lowest values. Sample size biases towards lower genetic diversity values were possible for McBride (n=24) and Grande Prairie (n=30). Sample size was reduced in McBride because MPB 30 amplified poorly, possibly because of one or more stand-specific mutations in the primer annealing site. However, since McBride was fully genotyped at five of six loci for 48 samples, at n=48 at five loci I calculated genetic diversity and found it was identical to diversity at n=24 for six loci. This suggests that a sample size of 24 genetically-independent beetles can be representative of a MPB population and that the potential for sample size bias in Grande Prairie was low.

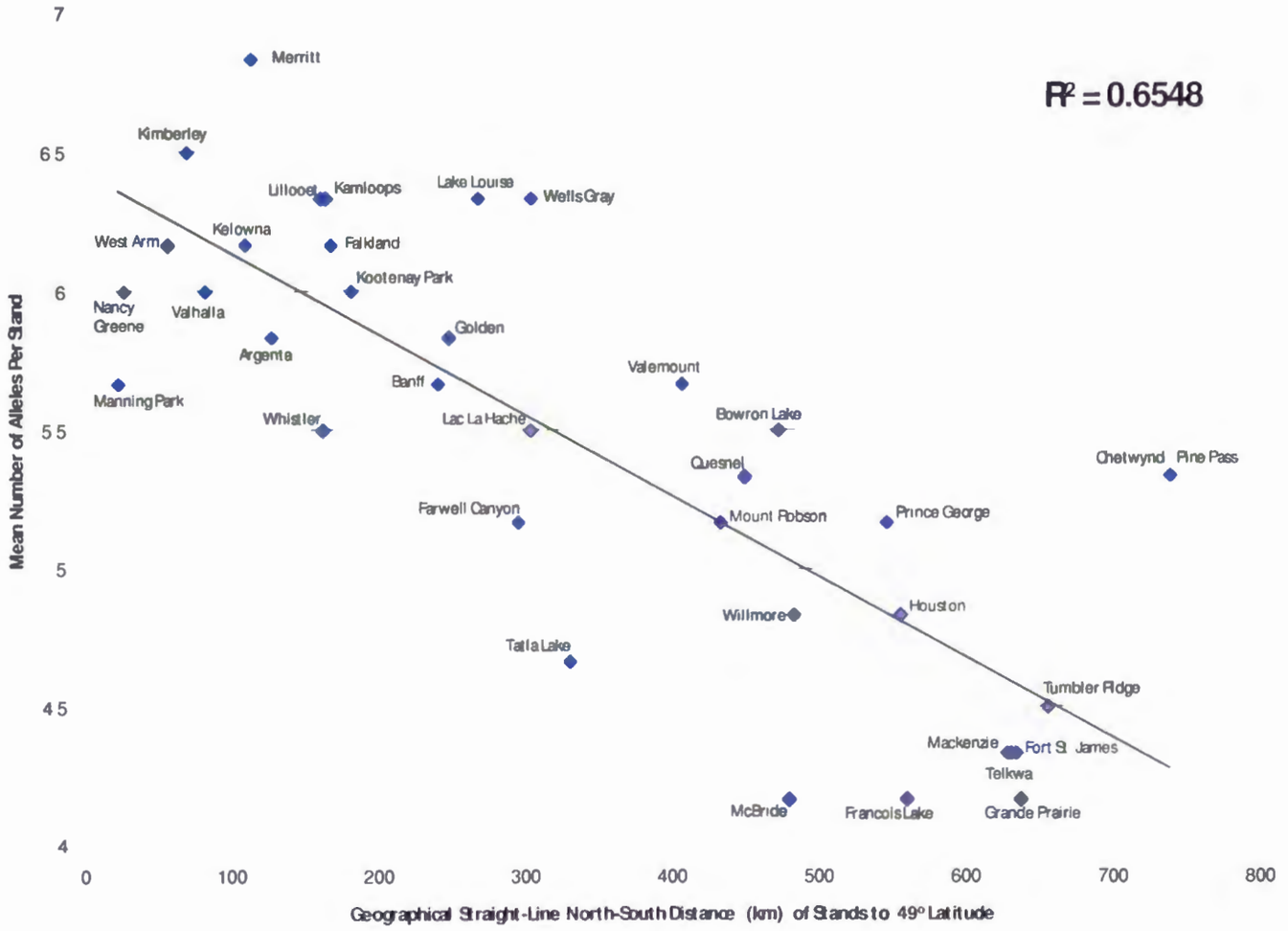

Figure 10. Inverse relationship between mean number of alleles and straight-line north-south distance of stands to the BC/US border (49° latitude). This relationship was highly significant ($P < .00000001$).

Figure 11. Inverse relationship between mean observed heterozygosity and straight-line north-south distance of stands to the BC/US border (49° latitude). This relationship was highly significant ($P < .000001$).

Tabulation of private alleles by locus and stand revealed the existence of 18 private alleles distributed among 13 stands (Table 2). Lake Louise had three private alleles while three stands, Kimberley, Willmore Wilderness, and Merritt, had two private alleles. Private alleles occurred singly in the remaining populations. In terms of bottlenecks, I found no evidence for any distortion of allele frequencies within stands (data not shown).

Table 2. Private alleles, by locus and population, for 35 populations of mountain pine beetles that were analyzed at six microsatellite loci.

Locus	Population	Allele Size	Number of Individuals With Allele	Hetero- (Het) or Homozygote (Hom)
MPB 30	Lake Louise	142	1	Het
MPB 40	Merritt	179	1	Het
	Manning Park	187	1	Het
	Quesnel	203	1	Het
MPB 25	Chetwynd/Pine Pass	235	1	Het
	Lake Louise	236	1	Het
	Lillooet	256	1	Het
	Valemount	259	1	Het
	Merritt	261	1	Het
	Lake Louise	265	2	Het
MPB 35	Banff	279	1	Hom
	Kelowna	288	1	Hom
	Willmore Wilderness	305	1	Het
CAL 1-1	Kamloops	206	2	Het
MPB 017	Kimberley	247	1	Het
	Willmore Wilderness	252	1	Het
	Fort St. James	256	1	Het
	Kimberley	263	1	Hom

A comparison of mean pairwise F_{ST}-values between unique stands and the mean across all 35 stands revealed some between-stand differences. Compared to a mean F_{ST} of 0.0398 across stands, Mackenzie (mean F_{ST}=.1051), McBride (mean F_{ST}=.0899), and Willmore Wilderness (mean F_{ST}=.0625) had much higher mean values. Tumbler Ridge (mean F_{ST}=.0479) and Whistler (mean F_{ST}=.0440) had values close to the across-stand mean.

Analyzing the incidence of among-stand genetic similarities on a per stand basis revealed some potentially important patterns. Genetic similarities between populations (Figure 1) imply recurrent inter-population gene flow. In terms of range expansion within the current epidemic, both Grande Prairie and Chetwynd/Pine Pass were consistent with coming from sources in North-Central BC. Grande Prairie was not significantly different from Chetwynd/Pine Pass, Telkwa, Houston, Francois Lake, Fort St. James, Quesnel, and Bowron Lake. Chetwynd/Pine Pass was not significantly different from Houston, Fort St. James, Tatla Lake, and Quesnel. In the Northern group, Telkwa and Houston had many instances of genetic similarities; thus gene flow involving these populations may have been strong. In contrast, Fort St. James and Francois Lake had fewer genetic similarities with stands and seem to have been involved in less inter-population gene flow.

The Prince George/Quesnel region seems to be the hub through which most similarities, whether ancestral or due to more recent dispersal, occurred between the Northern and Southern groups. Most gene flow was between stands from the same group. Thus stands in the Northern group were most likely to be similar to other Northern stands and vice versa for the Southern group. However, there was an apparent

hub, defined by similarities between the two groups, at the boundary between them. Prince George (PG) was genetically similar to Quesnel (QU) and Bowron Lake (BL), as well as to three other Southern stands (Farwell Canyon, Lillooet, Lac La Hache) but only one Northern stand (Telkwa). QU was genetically similar to BL and PG, as well as to three other Southern stands (Farwell Canyon, Lillooet, Lac La Hache) and five other Northern stands. BL was genetically similar to QU and PG, as well as to three other Southern stands (Farwell Canyon, Lillooet, Lac La Hache) and two other Northern stands. Notably, PG, QU, and BL were all genetically similar to only three other Southern stands (Farwell Canyon, Lillooet, Lac La Hache). Farwell Canyon was an intermediate stand by way of its similarity to the PG/QU/BL group in the north and the Lillooet/Lac La Hache group in the south. By contrast, Tatla Lake was another boundary stand but was only genetically similar to the Chetwynd/Pine Pass outbreak. The remaining boundary stands in McBride and the Willmore Wilderness were significantly different from all other stands.

"Connection" hub stands were common within the current epidemic area. By labeling stands as hubs, I implied that these stands were focal routes for gene flow involving recent and/or more ancient dispersal. Only stands that were characterized by about ten or more instances of genetic similarity were labeled hubs. Numbers of "connections" are given in parentheses. Lac La Hache (8) and Lillooet (11), Wells Gray (12), Lake Louise (10), Kootenay Park (11) and Kimberley (10), Valhalla (11) and West Arm (15) and Nancy Greene (12), and Kamloops (10) and Merritt (12) and Falkland (12) were all hubs within the current epidemic.

Several stands that were not significantly different from all other stands nonetheless appeared to be comparatively isolated based on the incidence of genetic similarities, with numbers of "connections" given in parentheses. These ten stands were Fort St. James (4), Francois Lake (2), Tatla Lake (1), Farwell Canyon (5), Mount Robson (5), Manning Park (4), Golden (4), Banff (2), Argenta (4), and Kelowna (3). Fort St. James was connected by gene flow to Houston and Quesnel and may have contributed to the new infestations in Chetwynd/Pine Pass and Grande Prairie. Francois Lake was connected by gene flow to Telkwa and also may have contributed to infestations in Grande Prairie. Tatla Lake was only genetically similar to Chetwynd/Pine Pass; however, by a ranking of significant F_{ST}-values, Tatla Lake was next most related to Lillooet, Farwell Canyon, Bowron Lake, and Quesnel. Farwell Canyon, as mentioned above, was an intermediary stand connected to boundary hubs in Prince George/Quesnel/Bowron Lake in the north and Lillooet and Lac La Hache in the south. Mount Robson had only five connections compared to the neighboring Valemount stand, which had 14 connections. Mount Robson was genetically similar to Valemount, Wells Gray, Falkland, West Arm, and Kootenay Park. Given its mountainous location at the edge of the MPBs Western Canadian range, Mount Robson was likely a sink for these five regions. Manning Park was genetically similar to nearby stands in Lillooet, Lac La Hache, Wells Gray, and Merritt. Golden was genetically similar to three stands in the Kootenays (Nancy Greene, Valhalla, West Arm) as well as to Lake Louise. Banff was genetically similar to only two stands, Nancy Greene and West Arm, both in the Kootenays. Argenta was genetically similar to stands from only the Kootenays: Nancy

Greene, Valhalla, West Arm, and Kimberley. Kelowna was genetically similar to Merritt, Valemount, and West Arm.

Relative comparisons of mean pairwise F_{ST}-values (in parentheses) provided mixed support for the comparative isolation of the ten stands noted above. Fort St. James and Francois Lake appeared isolated based on their incidences of genetic similarity to other stands, but the mean F_{ST}-values (~.050) for these stands were consistent with other northern stands. Farwell Canyon (.034) and Tatla Lake (.036) also appeared isolated based on "connections" and this isolation was supported by mean F_{ST}-values higher than nearby stands (Lac La Hache - .020, Lillooet - .018, Quesnel - .029, Bowron Lake - .022). Mount Robson had a mean F_{ST}-value (.030) slightly higher than neighboring Valemount (.023) and Wells Gray (.023). Manning Park had a mean F_{ST}-value (.030) slightly higher than nearby Okanagan stands (Merritt - .025; Kamloops - .026). Kelowna had a mean F_{ST}-value (.035) 40% higher than neighboring stands in the Okanagan (average for Merritt, Kamloops, and Falkland - .025). Similarly, Argenta had a mean F_{ST}-value (.044) ~30% higher than neighboring stands in the Kootenays (average for Nancy Greene, Valhalla, West Arm, Kimberley, Kootenay Park - .034). Both Golden (.051) and Banff (.047) had mean F_{ST}-values considerably higher than nearby stands: Lake Louise (.036); Kootenay Park (.033); Kimberley (.036).

Each of the five unique stands that were significantly different from all other stands exhibited distinctive inter-stand relationships (F_{ST} data not shown). Since all genetic distance (F_{ST}) values involving these stands were significant, these non-zero F_{ST} values could be ranked to determine levels of relatedness. Mackenzie was most closely related to Tumbler Ridge (F_{ST}=.035). Tumbler Ridge was most closely related to

Chetwynd/Pine Pass (F_{ST}=.009), then to Lac La Hache (F_{ST}=.016) and Houston (F_{ST}=.016). McBride was most closely related to Fort St. James (F_{ST}=.030), then Houston (F_{ST}=.039) and Grande Prairie (F_{ST}=.058). In contrast, McBride's geographical neighbor in the Willmore Wilderness was most closely related to Quesnel (F_{ST}=.022) and Bowron Lake (F_{ST}=.026). Whistler was most closely related to Manning Park (F_{ST}=.013), then Kamloops (F_{ST}=.017) and Lillooet (F_{ST}=.020).

Discussion

4.1. MPB Population Structure in Western Canada

I found shallow but highly significant MPB population structure in Western Canada (SAMOVA: F_{ST} = .05794; P < .00001). This population structure was probably superficially-shallow because I employed microsatellites, which are highly polymorphic markers. The high mutation rates of microsatellites deflate F_{ST} statistics (Balloux and Lugon-Moulin, 2002). As an example, two subpopulations each having ten equifrequent private alleles are clearly differentiated as much as possible and should have an F_{ST}-value of 1.0. However, high levels of polymorphism (due to mutations) result in an F_{ST}-value of only 0.053 (Balloux *et al.*, 2000), which is indicative of weak population structure. In sum, seemingly small F_{ST}-values do not imply negligible population structure (Wright, 1978).

Based on these inferences, the MPB exhibits strong and significant Western Canadian population structure. This population structure was supported and progressively refined by AMOVA, Structure, and SAMOVA analyses. This study was the fourth to use microsatellite markers to infer the population structure of a Scolytid beetle. However, of these studies, this was the first microsatellite analysis of Scolytid population structure at a high resolution (many sites over a relatively small region). Kerdelhué *et al.* (2006) used five microsatellite markers to study populations of the pine shoot beetle (*Tomicus piniperda*) sampled from seven sites in France. Also using five microsatellites, Sallé *et al.* (2007) examined the phylogeography of the European spruce beetle (*Ips typographus*) at 28 sampling sites across Europe. Using nine microsatellites, Maroja *et al.* (2007) studied the phylogeography of the spruce beetle (*D. rufipennis*

Kirby) at 14 sites across its North American range. My study, in consideration of not only the three studies above but also the past studies of Scolytid population structure, is the highest-resolution study of Scolytid population structure to date.

My relatively high-resolution population genetic analysis of the MPB partitioned stands into a Northern group and a Southern group. I had hypothesized that a larger number of groups might exist in BC and western AB given that geographic barriers, in the form of mountain ranges and/or distance, are pervasive and significant in Western Canada. Geographical barriers are expected to isolate MPB populations, minimizing the homogenizing effects of inter-population gene flow and allowing for the largely drift-mediated divergence of populations into distinct groups. Based on detailed MPB outbreak maps for BC and western AB covering the past century (Wood and Unger, 1996), I expected distinct MPB groups in the Rocky Mountains (Banff and Lake Louise), the Kootenays, the Okanagan, the Chilcotin, the Robson Valley, and the NW region of BC centered on New Hazelton. I also expected several groups in other regions of Northern BC. However, my results provide no evidence for any of these expected regionally-based MPB population structures.

4.1.1. Congruency Among the Phylogeography of the MPB, its Primary Symbiont, and its Primary Host

I had hypothesized that the population genetic structure of the MPB may reflect that of the species with which it has ostensibly associated over countless generations: its primary symbiont, the pathogenic blue-stain fungus *G. clavigera*, and its primary host, the lodgepole pine (*Pinus contorta*). Comparing the phylogeography of these species revealed some interesting insights into their respective evolutionary ecology.

Recent research on *G. clavigera* found weak phylogenetic support for separate northern and southern groups (Lee *et al.*, 2007). Though Lee *et al.* (2007) only sampled seven sites, their latitudinally-defined groups match with MPB population structure. In both species, sites in Houston and Fort St. James were in the northern group and sites in Manning Park, near Williams Lake, and in Banff were in the southern group. However, Lee *et al.*'s (2007) most-supported finding was the significant subdivision of *G. clavigera* population structure into distinct Interior BC and Rocky Mountain groups. Based on the strongest available evidence, MPB population structure is apparently not concordant with that of its main symbiont. Instead of the longitudinal two-group population structure found for *G. clavigera* (Lee *et al.*, 2007), I found a latitudinal two-group population structure for the MPB.

Differences in the population structure found by Lee *et al.* (2007) for *G. clavigera* and the structure I found for the MPB are not readily explained by a comparison of methods. Lee *et al.* (2007) employed six AFLP (Amplified Fragment Length Polymorphism) primer sets, yielding a very large number of markers for analyses (469 in total). DNA was isolated from 170 different cultures. These cultures were taken from

seven sampling sites, five in Canada (Houston, Fort St. James, Williams Lake, Manning Park, Banff) and two in the United States. Given that their statistical analyses were also robust, Lee *et al.*'s (2007) research was as rigorous as my research. Both studies also used selectively-neutral markers; neither study's results were confounded by natural selection.

I hypothesize that the differences in province-wide population structure between the MPB and *G. clavigera* are likely because of the different evolutionary pressures acting on these species. These differential evolutionary pressures are evident when comparing the genetic diversity of Rocky Mountain and Interior samples of the MPB and *G. clavigera*. The genetic diversity reported for *G. clavigera* across the Interior and Rocky Mountain groups was low (H_s = .053). However, *G. clavigera* from Rocky Mountain stands had higher numbers/levels of unique AFLP markers (38 out of 469; Interior = 6 out of 469), mean genetic diversity over all loci (Rocky Mountains = .066; Interior = .0435), and mean polymorphism (Rocky Mountains = 19.3%; Interior = 12.65%). In contrast, I found that MPBs from the Rocky Mountains (Banff and Lake Louise) showed levels of genetic diversity comparable to other stands within the southern group. The incidence of private alleles suggested higher MPB genetic diversity in Rocky Mountain stands compared to Interior BC stands; Lake Louise and Banff had four of the 18 private alleles found across all 35 stands. If private alleles were randomly distributed among stands, then an average of ~1 per pair of stands would be expected. Unfortunately, the incidence of private alleles is significantly affected by sampling bias; though my sample sizes had a mean of 46 and were consistent, I did not explicitly evaluate this bias and thus my private allele results must be interpreted with caution.

In sum, *G. clavigera* forms a Rocky Mountain group that is strongly supported by genetic data and has higher levels of genetic diversity than Interior samples. In contrast, my MPB results support neither a Rocky Mountain group nor higher genetic diversity in the Rockies. Overall, these results suggest that: 1) in the Rocky Mountains, a more ancient lineage of *G. clavigera,* compared to the Interior, has been maintained and has retained considerable genetic diversity while some level of gene flow has occurred between MPBs from Rockies and Interior stands for some time; and/or 2) as suggested by Lee *et al.* (2007), *G. clavigera* is a species complex and the Rocky Mountain and Interior BC groups are different species; MPBs may thus carry different species of symbionts characterized by regional adaptations; and/or 3) the mountain environment of the Rockies places selective constraints on MPB symbiotic fungi, primarily on *G. clavigera*, that are distinct from the constraints of typical Interior environments.

The last hypothesis above is particularly compelling in that it suggests strong selection for novel strains of *G. clavigera* in mountain environments. Moreover, all three of the above hypotheses imply that the Rocky Mountain and Interior BC strains of *G. clavigera* are sexually incompatible and/or produce less-fit hybrids. This is important because the effects of natural selection predicted by hypothesis three could not normally be detected by the neutral genetic markers of Lee *et al.* (2007) unless selection had considerable effects on *G. clavigera* population structure.

Any selection for novel strains would be strong in that it would influence mortality and reproductive success at multiple levels in the MPB/fungus symbiosis. For the fungus alone, there should be an evolutionary arms race for multiple traits which may include: speed of colonization (inter-fungus competition for space and resources) and

synchrony of sporulation (for dissemination to beetle vectors). From the vantage of the fungus-vectoring MPB, there should be a fungal pathogenicity arms race for traits that stop resin flow expeditiously, which would increase beetle survival and fecundity. Pathogenicity-related selection would act on both beetles and fungi, as their success is all-or-none; unsuccessful beetle attacks ("pitchouts") usually cause unsuccessful fungal attacks and vice versa (N. Bartell, pers. obs.). There should also be a fungal arms race in terms of traits that enhance the provision of nutrients to beetle brood (dispersing beetles with larger fat reserves should have higher survival and fecundity).

In a Rocky Mountain environment, however, there may be metabolic or life history constraints on selection for the above traits; specifically, *G. clavigera* from the Rocky Mountains may be unique because it is cold-adapted (Lee *et al.*, 2007). Interior *G. clavigera* may not face the same level of climatic constraints and thus may be undergoing selection as above; indeed, the prevalence of *G. clavigera* strains from the Interior BC group may be because these strains are more pathogenic and/or beneficial for MPBs (Lee *et al.*, 2007). Thus *G. clavigera* populations endemic to different regions, the Rocky Mountains versus the Interior, likely evolve very differently over the long-term. Similarly to adaptations along altitudinal gradients, Rice *et al.* (2008) hypothesized that *G. clavigera* may exhibit growth adaptations that are a response to latitudinal temperature gradients across the MPBs North American range.

Regional evolutionary pressures may also have immediate, not just long-term, effects on introduced *G. clavigera* individuals. Novel selective pressures in the Rocky Mountains compared to the Interior are strongly supported by Lee *et al.*'s (2007) result that although most Rockies *G. clavigera* samples were genetically tied to Interior

sources, Rockies individuals still exhibited higher genetic diversity; this result indicates sink and not source effects. I suggest sink effects in which incoming *G. clavigera* from the Interior underwent selection for higher genetic diversity in the Rocky Mountains. Higher genetic diversity likely entails higher phenotypic plasticity; thus the Rocky Mountains environment may favour *G. clavigera* strains with a greater ability to respond to conditions atypical of the Interior region.

To complete my argument, I suggest that selective pressures on *G. clavigera* far outweigh those on the MPB and that the pressures MPBs face are unlikely to differ between the Rocky Mountains and Interior. Indeed, without virulent symbiotic fungi MPB attacks are unsuccessful (Yamaoka *et al.*, 1995; Six and Paine, 1998). These fungi play arguably the most important role in MPB survival and reproduction in the host by quickly stopping water conduction and thus resin flow. By halting water conduction, these fungi stop constitutive defenses that may kill the MPB (and fungus) and also prevent the development of induced host defenses. As mentioned previously, there should be heavy selective pressure for increasingly virulent fungal strains that stop water conduction more quickly. Such selective pressure likely acts on *G. clavigera*, the MPBs most virulent symbiont (Solheim, 1995; Solheim and Krokene, 1998); *G. clavigera* is the only MPB symbiont that is aggressive enough to kill lodgepole pine when introduced alone (Yamaoka *et al.*, 1995).

In sum, symbiotic fungi, primarily *G. clavigera*, are an important factor allowing MPBs to successfully colonize their hosts. Selection pressures are likely much greater for such fungi compared to the MPB because the MPB likely shows little variation in its three main adaptations for successful host colonization. There are likely regional

selection pressures on *G. clavigera* where in the Rocky Mountains, selection is novel or constrained compared to the Interior; such selection on *G. clavigera* acts immediately and in the long-term. MPBs are probably more likely to undergo direct selection for traits that produce more brood, such as locating more suitable hosts or for higher fecundity (eg. optimization of egg number versus size; Elkin and Reid, 2005), rather than for fundamental traits involved in overcoming host defenses. If differential selection does occur for the MPB in Interior versus Rocky Mountain stands, then effects on overall population structure (at neutral loci) have been minimal. I believe that the Northern and Southern groups I have found for the MPB have arisen not by selection, but by 2 other factors/events (see Section 4.1.5.).

The phylogeography of the MPBs primary host, lodgepole pine (*Pinus contorta*), appears to reflect both deep and shallow history. Indeed, Marshall *et al.* (2002) found post-Ice Age genetic patterns of a rapid recolonization (a "big-bang") starting 12kyr BP that was overlain by the recent growth and differentiation of local populations ("galaxy formation") in the past 1000-3000 years. This conclusion was supported by the results of Fazekas and Yeh (2006) who notably found two population groups: northern BC/Yukon versus southern BC/Alberta/Idaho/Montana for *P. contorta* var. *latifolia*. The population genetic structures of the MPB and its primary host, *P. contorta* var. *latifolia*, are concordant in British Columbia. This is consistent with population genetic studies of the beetles *T. piniperda* and *T. destruens* and their primary host trees, which have found concordant beetle/host population structures in regions of Europe (Salvador *et al.*, 2000; Soranzo *et al.*, 2000; Korol *et al.*, 2002; Ritzerow *et al.*, 2004; Horn *et al.*, 2006;

Vasconcelos *et al.*, 2006). In sum, these results suggest that the MPB and its primary host were intimately associated during recolonization. Since the MPB and *P. contorta* var. *latifolia* have roughly congruent population structures, these species may be evolving in response to similar factors, such as climate.

4.1.2. Significant MPB Population Structure - Context

The idea that bark beetles exhibit significant population genetic structure is opposed by a portion of research on Scolytids, including the MPB. These studies argued or implied that Scolytids are characterized by consistent long distance dispersal, despite the presense of significant geographic barriers. In some instances significant methodological problems were likely. Stock *et al.* (1984) and Calpas *et al.* (2002) failed to genetically differentiate isolated MPB populations using allozyme and RAPD analyses, respectively. Small sample sizes likely plagued both studies, while Stock *et al.* (1984) used a very conservative genetic technique and Calpas *et al.* (2002) may have had significant fungal contamination of isolated DNA. As an example, both my study and Calpas *et al.*'s (2002) sampled from four of the same general areas but I was able to genetically identify stands in Mount Robson, Falkland, Kootenay National Park, and Banff.

The remaining studies that support an absence of Scolytid population structure were likely biased. In the first study to use highly polymorphic genetic markers (microsatellites) to resolve the population structure of a Scolytid, high levels of genetic differentiation were expected between stands of the European spruce beetle *Ips typographus*. Instead, Sallé *et al.* (2007) found a complete lack of Europe-wide

population structure. As is often typical when non-significant population structures are found, Sallé *et al.* (2007) argued that *I. typographus* has a consistently high dispersal rate but extended this argument to Scolytids in general. However, the potential for sampling and contamination (fungal DNA) bias was high in this study; indeed, Stauffer *et al.* (1992; 1999) used allozymes and mitochondrial markers, respectively, and found significant population structure for the same species over the same region.

Kerdelhué *et al.* (2006) also employed microsatellite markers but in an analysis of the population structure of the pine shoot beetle, *Tomicus piniperda*. Though it could be argued that *T. piniperda* actually had shallow but significant population structure subdivided into groups in northern France, central France, and the Pyrenees, Kerdelhué *et al.* (2006) argued that *T. piniperda* exhibited a near-complete lack of population structure, similarly to Sallé *et al.* (2007). Prototypically, Kerdelhué *et al.* (2006) argued that *T. piniperda* is characterized by frequent long distance migrations that genetically homogenize populations. Like Sallé *et al.* (2007), sampling and contamination bias were potentially manifest in this study.

In sum, both Sallé *et al.* (2007) and Kerdelhué *et al.* (2006) found weak population structure in two beetle species using microsatellites. Past studies using lower-resolution mitochondrial markers have found more significant European population structure in these species (Stauffer *et al.*, 1999; Kerdelhué *et al.*, 2002). Indeed, many population genetic studies find significant structure using mitochondrial but not microsatellite markers. This trend is commonly caused by male-biased dispersal; mitochondrial DNA is maternally-inherited and the development of significant population structure at these loci is aided by a relative lack of female dispersal.

However, there is no evidence for sex-biased dispersal in Scolytids (Safranyik and Carroll, 2006). The results of Kerdelhué *et al.* (2006) and Sallé *et al.* (2007) are suggestive of fungal contamination. Mitochondrial sequencing allows for post-amplification contamination avoidance through the exclusion of sequences that are similar to those reported for fungi. However, as suggested by data on the MPB (C. Davis, unpub. data), microsatellite development for bark beetles most often results in fungal-specific markers despite considerable efforts to avoid contamination of starting DNA.

I suggest that the above two potentially contamination-affected studies amplified fungal and beetle DNA. The microsatellite-derived population structures that Kerdelhué *et al.* (2006) and Sallé *et al.* (2007) obtained probably reflect the population structures of not only the beetle, but also the structures of various fungi. Indeed, neither study conducted primer screening on fungal isolates to test for beetle specificity. Both of these studies' data was in HWE but this would be expected with contamination; indeed, each individual beetle would have randomly sampled a number of fungal strains of each species before dispersal. The sum effect of beetle-fungus amplifications would be the obscuring of any one species' population structure, as seen in both of these studies. Indeed, as previously mentioned, the MPB and *G. clavigera* have incongruent population structures; the population structure of either species would be obscured by DNA contamination by the other. I suggest that significant population structures, concordant with mitochondrial results, may be found for the two above species if microsatellites are screened against fungal DNA isolates to confirm beetle specificity.

Patterns of population genetic structure between the MPB and its primary symbiont, *G. clavigera*, are very supportive of significant population structuring. Lee *et al.* (2007) found that all seven of their MPB-cultured *G. clavigera* stands were significantly different from each other; this pattern was similar to my analyses of the MPB, in which ~80% of pairwise tests found significant differences between stands. Clearly, neither the MPB nor its primary symbiont is panmictic over its range or even within its genetically-defined groups. As confirmed by patterns of population structure in an intimately associated fungus, Western Canadian MPB populations exhibit significant genetic structure.

Most population genetic studies of the MPB have found significant population structure. Stock and Guenther (1979), Bentz and Stock (1986), and Langor and Spence (1991) all used allozymes and found large differences among MPB populations across the Pacific Northwest, western United States, and Western Canada, respectively. Kelley *et al.* (2000) used allozyme and mitochondrial markers and found large differences among MPB populations in California. The most recent phylogeographic study of the MPB used AFLP and mitochondrial markers and found significant North American population structure (Mock *et al.*, 2007), with gene flow around rather than across the Great Basin and Mohave deserts. On a per sampling site basis, I cannot compare my results to past studies of MPB population genetics, which have not sampled BC and AB to any large extent. However, on a theoretical level, these studies support the idea that significant geographic barriers, such as mountain ranges and large distances, can prevent gene flow and cause genetic differentiation between populations.

Even early population genetic studies that used conservative allozyme techniques support the role of geographic barriers and isolation in Scolytid population dynamics. Speciation may be occurring among specific, geographically isolated populations of the Douglas-fir beetle (Stock *et al.*, 1979) as well as in the southern pine beetle, *D. frontalis* (Anderson *et al.*, 1979; Namkoong *et al.*, 1979). Roberds *et al.* (1987) and Six *et al.* (1999) have found concordance between geographic isolation and significant population structure in *D. frontalis* and *D. jeffreyi*, respectively.

Population genetic studies utilizing more modern methods, such as mtDNA sequencing, support the role of geographic barriers and isolation in Scolytid population dynamics. In *I. typographus*, Stauffer *et al.* (1999) found significant Eurasian population structure divided into three groups. Duan *et al.* (2004) confirmed the existence of a new *Tomicus* species in southern China that was divergent from *T. piniperda* in Europe and northern China, due to geographic barriers (Ritzerow *et al.*, 2004).

T. destruens has well-supported population genetic structure with groups throughout the Mediterranean basin, which is divided by such barriers as the Pyrenees, the Alps, and the Mediterranean Sea (Faccoli *et al.*, 2005; Horn *et al.*, 2006). There is also genetic structure within these groups, as in Italy (Faccoli *et al.*, 2005).

Considerably fewer population genetic studies of Scolytids have occurred in North America but these studies have confirmed the importance of geographic barriers and isolation in Scolytid population dynamics. Most of these studies also employed mitochondrial markers, while Maroja *et al.* (2007) used both mtDNA and microsatellites. These studies found significant geographically-influenced population structure in: the western pine beetle, *D. brevicomis*, (Kelley *et al.*, 1999), the pinyon pine beetle, *Ips*

confusus, (Cognato *et al.*, 2003), and the spruce beetle, *D. rufipennis*, (Maroja *et al.*, 2007).

Many Scolytid population genetic studies that have been mentioned above as supporting the role of geographic barriers in population divergence have supported such roles over spatial scales considerably larger than the essentially one-province scale of my study. These above studies (eg. Stauffer *et al.*, 1999; Ritzerow *et al.*, 2004; Maroja *et al.*, 2007; Mock *et al.*, 2007) have often not found significant differences among Scolytid populations separated by similar barriers (distance and/or mountains) to those separating MPB stands that I found differences for. I suggest that these studies' inability to resolve Scolytid population structure at a finer scale is a direct function of their sampling intensity and the number and polymorphism of genetic markers they used. Increased regional sampling and the use of a higher number of polymorphic markers may have found fine scale Scolytid population structure. The lack of fine scale population genetic studies of Scolytids is currently a major gap left to be filled in this field of research.

4.1.3. MPB Genetic Diversity - Context

Placing the genetic diversity I found among MPB stands into context was made difficult by the possible fungal contamination of past microsatellite studies of Scolytids. Observed genetic diversity had a range of 0.42 to 0.66 in the MPB. Kerdelhué *et al.* (2006) found a range of 0.63 to 0.70 among seven infestations of *T. piniperda* in France; however, one marker was suspiciously polymorphic. Sallé *et al.* (2007) reported a range of 0.42 to 0.62 among 28 infestations of *I. typographus* in Europe. Unfortunately, as previously mentioned, Kerdelhué *et al.* (2006) and Sallé *et al.* (2007) may have cross-amplified beetle and fungal DNA. The probable contamination of these studies by multiple strains of multiple fungal species may have kept overall "beetle" genetic diversity within a normal range. In the North American spruce beetle, Maroja *et al.* (2007) found a range of 0.30 to 0.50 among 16 infestations.

4.1.4. Comparison of Beetle/Symbiont Genetic Diversity – D. ponderosae, D. jeffreyi, and G. clavigera

My genetic diversity data on the MPB allow for an evolutionary, not a phylogeographic (as previously detailed), comparison of bark beetles and their primary mutualists. As previously mentioned, *G. clavigera* is the primary symbiont of *D. ponderosae* and *D. jeffreyi* and is vectored only by these beetles. The genetic diversity values reported in this section are Nei's (1973; 1987) gene diversity, which is equal to observed heterozygosity for randomly mating sexual diploid organisms; these values are appropriate for comparison between diploid/haploid and sexual/asexual organisms (Six and Paine, 1999). Thus the genetic diversity values reported in this section can be compared among beetles and fungi. MPB-associated *G. clavigera* exhibits low genetic diversity (H_s = .053) over the same general range as my study; however, *G. clavigera* from the Rocky Mountains has higher genetic diversity (H_s = .066). I found high levels of genetic diversity in the MPB (0.552) but Rocky Mountain stands were not auspicious. This contrasts with the patterns of genetic diversity found for the *G. clavigera* mutualism in the MPB sister species *D. jeffreyi* (Six and Paine, 1999; Six *et al.*, 1999). Low genetic diversity was found for both *G. clavigera* (0.014) and *D. jeffreyi* (0.040). The results for the *D. jeffreyi* mutualism may have been an artifact of the use of genetically conservative allozyme markers. However, using polymorphic mitochondrial loci, Kelley *et al.* (2000) confirmed that *D. jeffreyi* has ~10 times less genetic diversity compared to *D. ponderosae*. Beyond marker properties, there are more logical explanations for the discrepancies in genetic diversity between these two symbioses.

In both *D. ponderosae* and *D. jeffreyi*, the low genetic diversity of *G. clavigera* may be because it is a haploid fungus. Although low genetic diversity in *G. clavigera* may be explained by factors that influence both haploid fungi and diploid beetles, such as dominance of genetic drift over mutation in small populations (Christiansen and Feldman, 1986; Hartl and Clark, 1997; Beebee and Rowe, 2004) or founder effects (Six and Paine, 1999), many evolutionary processes, relative to diploid bark beetles, are unique to haploid fungi. These unique processes include: immediate selection against deleterious alleles (Perkins and Turner, 1988), low levels of sexual reproduction (Harrington *et al.*, 1996), poor dissemination of sexual spores (Six and Paine, 1999), and/or evolution of clonality to prevent the breaking up of high fitness genotypes essential for mutualism (Wulff, 1985).

Given the MPBs wide host preference (Wood, 1982) and the high levels of MPB genetic diversity I have found, the MPB likely did not suffer species-wide bottlenecks during and/or after speciation. Though *G. clavigera* associated with MPBs commonly sexually reproduces (Robinson-Jeffrey and Davidson, 1968), such reproduction is delayed relative to asexual reproduction (Six and Paine, 1996). Thus, MPB dissemination of sexual *G. clavigera* spores (ascospores) may only be possible in semivoltine mountain environments, such as the Rocky Mountains (Safranyik and Carroll, 2006). In sum, poor dissemination of sexual spores, clonality, and the rapid elimination of deleterious alleles may be the cause of low genetic diversity in MPB-derived *G. clavigera*. In mountain environments such as Banff, the predominant dissemination of ascospores may maintain higher levels of *G. clavigera* genetic diversity; such diversity may maximize responses to selection (Six and Paine, 1999).

For the *D. jeffreyi* / *G. clavigera* mutualism, when *D. jeffreyi* diverged from its common ancestor and became a monophagous beetle with a single-species host range, the most parsimonious explanation is that both the beetle and its fungus suffered severe founder effects that have not been rectified (Higby and Stock, 1982; Six and Paine, 1999; Kelley *et al.*, 2000). Thus, in the *D. jeffreyi* / *G. clavigera* mutualism it is important to distinguish between speciation-caused founder effects and founder-like population dynamics (effects of population fluctuations) since speciation. Genetic diversity in *D. jeffreyi*-associated *G. clavigera* has likely been restricted since founding by a rarity of sexual reproduction (Six and Paine, 1999), as well as factors common to MPB-associated *G. clavigera*: clonality and the rapid elimination of deleterious alleles.

Thus the origin of low genetic diversity in *D. jeffreyi* is most likely a severe bottleneck during speciation. Explaining the maintenance of low genetic diversity in *D. jeffreyi* relative to the MPB, as they are sister species with similar population dynamics, is more difficult. It is possible that bark beetle population dynamics, which are characterized by temporally-dominant small populations and punctuated by widespread population explosions, may be neutral with respect to the maintenance of population genetic diversity. In other words, mutation/migration and genetic drift averaged over the phases of the *D. ponderosae* / *D. jeffreyi* population cycle may be in equilibrium. The diversity-reducing effects of genetic drift during the endemic phase may be offset by the diversity-increasing effects of massive rates of mutation and migration (Petit *et al.*, 2005) during epidemics.

In terms of mutations, a microsatellite mutation rate of 6×10^{-4} per locus per gamete per generation is standard (Hewitt, 2000). For every locus, there is a probability

of ~1/14 that a mutation will occur in one of the 60 offspring of a MPB mating pair. Based on Safranyik and Carroll's (2006) review, I assume that each male and female effectively contributes 60 gametes (120 gametes/pair) and that there is one generation per year (univoltine). Potentially enormous numbers of mutations are generated because of MPB epidemics. For example, 1,000,000 mating pairs yields 72,000 mutations for every locus in the MPB genome, distributed among their 60,000,000 offspring. This is a rough estimate of mutation generation. Microsatellite mutation rates vary significantly among and within both species and loci (Zhang and Hewitt, 2003). Within loci, there are upper limits for allele size (Paetkau *et al.*, 1997); moreover, allele size affects both the rate and direction of mutation at a given locus (Kimmel *et al.*, 1998; Ellegren, 2000; Xu *et al.*, 2000).

When epidemics decline, I hypothesize that most new genetic variation is maintained in myriad small MPB populations that are distributed over the landscape. Drift is hypothesized to be heavy in these populations with small effective sizes, as individuals and populations are extirpated at high rates, while some extirpated regions are recolonized by migrants. If the population dynamics of bark beetles approximate (mutation and migration)/drift equilibrium across population phases, then the genetic diversity of Scolytid species reflects their diversity upon founding. I thus hypothesize that the MPB, as a generalist mortality agent of *Pinus*, did not suffer species-wide founder effects upon speciation and reflects ancient (high) levels of genetic diversity. I concordantly hypothesize that *D. jeffreyi*, as a host specialist, suffered severe species-wide bottlenecks during and/or since speciation (Six and Paine, 1999; Kelley *et al.*, 2000) and reflects consequent levels of genetic diversity.

4.1.5. Western Canadian MPB Population Structure – A Northern and Southern Group

I propose two explanations for the existence of a genetically-defined Northern and Southern group for the MPB in Western Canada. These explanations involve: 1) post-Ice Age range expansion; or 2) MPB population dynamics. Indeed, population structure arises from the contribution of past history and current processes (Hewitt, 1999; Vucetich and Waite, 2003).

There are two extremes of recolonization from refugia: "pioneer" and "phalanx" (Hewitt, 1996). Both involve stepping stone dispersal with founder effects upon expansion. These extremes differ in the speed of recolonization (Ibrahim *et al.*, 1996). Stepping stone dispersal involves spreading from the leading edge, where bottlenecks reduce diversity (and increase homozygosity) upon the successive founding of pioneer populations (Nei *et al.*, 1975). "Pioneer" recolonization is very rapid, causing severe founder events and thus high levels of homozygosity over the colonized range. In contrast, "phalanx" recolonization is slower and allows more genetic diversity to survive over the colonized range.

Because I argued that the MPB does not suffer founder effects (see Section 4.2.3), I alter the above dispersal model in that for the MPB, bottlenecks likely occurred after founding. This is because during recolonization, newly-founded MPB populations were located at northern extremes where climate-induced bottlenecks were likely prevalent. Short and severe climatic oscillations throughout the interglacial warming would have caused additional bottlenecks (Hewitt, 1999). Thus, the MPB probably fits a non-

traditional model of stepping stone recolonization that is likely moderate with respect to the "pioneer" and "phalanx" extremes.

Under a "moderate" model of post-Ice Age range expansion, I would expect MPB populations to be the oldest in southern BC as these areas were recolonized first. Population age (and genetic diversity) should thus decline with increasing north-south distance to the BC/United States border. A hypothesis of post-Ice Age recolonization further predicts that older populations in southern BC should be closer to mutation/drift equilibrium compared to younger populations in northern BC, which should be heavily influenced by genetic drift, causing reduced diversity.

This predicted genetic pattern is concordant with my results. A similar pattern of southern richness and northern purity has been found in many taxa (Hewitt, 1999; 2004), such as grasshoppers (Cooper *et al.*, 1995), newts (Wallis and Arntzen, 1989), whitefish (Bernatchez and Dodson, 1991), and woodrats (Hayes and Harrison, 1992). Such patterns are also found among taxa endemic to North America's Pacific Northwest (eg. Green *et al.*, 1996; Conroy and Cook, 2000).

The results of Mock *et al.* (2007) confirm that the population genetic structure of the MPB reflects post-Ice Age recolonization patterns to some degree. Indeed, Mock *et al.* (2007) used other genetic markers (AFLP and mitochondrial sequencing), and thus studied a different portion of the MPB genome, yet also found that genetic diversity decreased with increasing distance north from an apparent core MPB range in southern Idaho and Utah. Combined with my results, this indicates that a molecular signal of post-Ice Age recolonization exists in the MPB. Indeed, the persistence of such signals

has been found for many European taxa (Taberlet and Bouvet, 1994; Lunt *et al.*, 1998; Santucci *et al.*, 1998).

I suggest that the persistence of this signal may have been enhanced by the probable lag in the MPBs recolonization of BC (Hewitt, 1999). The MPB did not follow the retreating glacial edge but instead followed the advancing distribution of its host trees, which likely exhibited time lags in recolonization. For example, the retreat of the ice sheet did not necessarily create immediately available and suitable habitat for host trees as there was also a treeless permafrost region in front of the continental ice sheet. Once the ice sheets and permafrost had retreated from a region, the glacial substrate left behind would have to drain, stabilize, and be initially colonized by pioneer species such as lichens and grasses to promote soil "development" at the site; this process may have taken hundreds of years at a site. Even then, host trees may not have been able to colonize a recently glacially-abandoned site due to other factors.

MPB population dynamics may also explain the presence of a Northern and a Southern group of MPBs in Western Canada. Climate is the most important source of MPB mortality (Safranyik, 1978; Cole, 1981). Climate is typically more important to the (overwintering) mortality of MPB brood in terms of early- or late-season cold snaps or prolonged periods of -40°C temperatures in mid-winter (Yuill, 1941; Safranyik *et al.*, 1974). However, during the dispersal period, poor weather can cause significant MPB mortality by causing asynchronous flights (Safranyik and Carroll, 2006).

MPB populations in southern BC are generally located in the most climatically suitable region of BC (Carroll *et al.*, 2004). Thus southern BC populations are likely

larger and more stable in time and space compared to populations from northern BC. This inference is supported by both general landscape patterns of demography and the incidence of MPB outbreaks in BC during the past century. Populations nearer the core of a species' range (southern BC) have higher abundance and lower temporal variability in abundance compared to peripheral (northern BC) populations (Vucetich and Waite, 2003). Given lower effective population sizes, Vucetich and Waite (2003) estimated that rates of genetic drift may be two to thirty times higher in peripheral (northern BC) populations.

Perhaps over 90% of MPB outbreaks over the past century have occurred in southern BC (Wood and Unger, 1996). Larger and more stable southern BC populations will retain and generate considerable genetic diversity as these populations undergo fewer and less severe bottlenecks. Also, as historical outbreak maps suggest, in southern BC populations are more numerous and outbreaks are more common compared to northern BC. The high degree of inter-population breeding among populations in the south would maintain and increase levels of genetic diversity. Thus, a hypothesis of population dynamics predicts that: southern BC has larger, more numerous MPB populations in which outbreaks are more frequent, causing higher genetic diversity compared to the smaller, fewer MPB populations in northern BC, where outbreaks are more rare. The predictions of the population dynamics hypothesis are also concordant with my results.

4.1.6. Western Canadian MPB Population Structure – Inferred Patterns of Post-Ice Age Recolonization and Population Dynamics

I suggest that current MPB population structure is the result of a complex synergy between hypotheses of post-Ice Age recolonization and MPB population dynamics. It is difficult to explore these questions without further advances in genetic marker development and in statistics. However, since my results reflect patterns from both hypotheses, patterns may be explained by either or both of these hypotheses.

Notably, north-south gradients in the genetic diversity or allele frequencies of MPB populations have been reported by past studies. To this author's knowledge, such gradients have not been seriously considered until now. Though they did not report this result, plotting the genetic diversity values found by Mock *et al.* (2007) on a map of their sampling sites revealed that genetic diversity decreased with distance north or south from an apparent core range in Idaho and Utah. Stock *et al.* (1984) found a north/south gradient in allozyme frequencies among MPB populations in Idaho. At two polymorphic allozyme loci, Kelley *et al.* (2000) found that the genetic diversity of MPB populations decreased with increasing distance south from northern California. The reduced genetic diversity of populations at both the northern and southern extremes of the MPBs range is likely due to post-glacial recolonization and population dynamics. The effects of these two factors have been detailed for the northern extreme in Western Canada. In the southern extreme, recolonization effects would be similar but climate would have adverse impacts on population dynamics in terms of multiple, poorly-synchronized flights per year (Safranyik and Carroll, 2006).

Thus the MPB likely survived in a single refugium located in the "core range" of Idaho and Utah that I inferred from Mock *et al.* (2007). In this core range, MPBs may have existed continuously over tens or hundreds of thousands of years, despite repeated glacial advances and retreats. Post-Ice Age recolonization thus occurred from this core range and moved north into BC. The decreasing genetic diversity from Oregon into California and from Utah to Arizona may have been caused by recolonization from the core range; however, it is possible that MPBs also persisted near the southerly portions of its current range during the last glacial and have suffered bottlenecks due to the warming and the increasingly fragmented host distribution of the current interglacial (Hewitt, 2000).

In terms of the last Ice Age, the MPBs primary refugium was either dominantly or solely populated by *Pinus contorta* var. *latifolia* (Fazekas and Yeh, 2006), the lodgepole pine subspecies from which I exclusively sampled MPBs. My results suggest that the MPB repopulated Western Canada from a single refugium. Following the last retreat of continental glaciers, the region occupied by the southern MPB group was recolonized first. Recolonization of Northern BC appears to have occurred through the Fraser Valley. I found no evidence for recolonization of Northern BC via the Rocky Mountain Trench (through Valemount and McBride) or via the Chilcotin region of western BC; such a pattern may have been maintained by the discontinuous *Pinus* distributions in these regions.

I found no genetic evidence that the MPB persisted in a refugium north of the ice sheet as there was no apparent hybrid zone, where colonists from different refugia meet, within the region occupied by the Northern group. I also found no evidence for other

significant MPB refugia south of the ice sheet; no hybrid zone was apparent within the region occupied by the Southern group. The existence of a single major MPB refugium during the last glaciation is confirmed by modern population genetic research on the MPBs primary host, *P. contorta* var. *latifolia* (Fazekas and Yeh, 2006).

Modern research appears to confirm a single refugium south of the ice sheet for *P. contorta* var. *latifolia*. This is the most widely distributed subspecies of lodgepole pine. Fossil pollen data (MacDonald and Cwynar, 1985) indicated that recolonization by *P. contorta* var. *latifolia* progressed northwest from southeast BC to the Yukon. There is no genetic (Marshall *et al.*, 2002; Fazekas and Yeh, 2006) or fossil pollen (MacDonald and Cwynar, 1985; Cwynar and MacDonald, 1987) evidence that any of the four lodgepole pine subspecies persisted in Beringia. However, Pleistocene persistence in refugia along the West Coast of BC, especially in the Queen Charlotte Islands region, has been supported for *P. contorta* var. *contorta*, the shore pine (Heusser, 1960; Peteet, 1991; Fazekas and Yeh, 2006). Since the MPB currently infests all *P. contorta* subspecies, I could safely assume that the MPB persisted in West Coast refugia. However, an evidence-based assumption is possible.

Importantly, Fazekas and Yeh (2006) found no genetic evidence for the subspecies designations of *P. contorta*. They suggested that before the current interglacial and its subsequent recolonization, *P. contorta* was a panmictic species. Historical panmixia is suggested by the apparent gene flow between var. *latifolia* and var. *contorta* in northern BC and the Yukon (Wheeler and Guries, 1982; Fazekas and Yeh, 2006). Thus, *P. contorta* has probably existed as a single species (not a species complex) since the last glacial.

Since all refugia populated by *P. contorta* were MPB-suitable, I suggest that the MPB persisted in var. *latifolia* in the major refugium south of the ice sheet and that the MPB persisted in var. *contorta* in several minor refugia on the West Coast and Queen Charlotte Islands of BC. The coastal refugia would have been minor for the MPB on several fronts: shore pine stands have low stem density and uneven age distributions (long-term effective MPB population sizes would be small); the geographical extent of these refugia was probably highly constrained; the extirpation of MPB populations was more probable because West Coast refugial populations were isolated from the main refugium (the ice sheet covered continental BC and Vancouver Island) and likely from one another (fiords, mountains, islands, straits). Though the vast majority of MPB recolonization of BC appears to have originated from the southern refugium, coastal and inland refugia may have contributed to the MPBs recolonization of northwest BC, such as via the Skeena or Nass River valleys (Fazekas and Yeh, 2006). My MPB populations in this region, near Telkwa and Houston, indicate that these have arisen from the major southern refugium. However, over the past 100 years MPB outbreaks have occurred in the New Hazelton region, where outbreaks have been absent during the current epidemic. Efforts should be made to sample MPBs endemic to northwest BC, or to obtain museum specimens, to make a phylogeographic comparison with beetles that have recolonized from the southern refugium. MPBs endemic to Vancouver Island should also be sampled for similar phylogeographic comparison.

In terms of population dynamics, the "mess" of instances of genetic similarities between stands in the Southern group (Figure 1) is likely representative of postglacial

recolonization and thousands of years of inter-population breeding since recolonization. I found a strong and very highly significant pattern of isolation by distance in the south. Such a pattern is strongly indicative of stepwise (short distance) movements between populations over thousands of generations. Southern BC is dominated by north-south geographic barriers in the form of mountain ranges, such as the Monashees, Selkirks, and Purcells. Given the pattern of outbreaks in the south over the past century (Wood and Unger, 1996), it is likely that in any one year or period, one population will erupt and may breed with an adjacent population/s. Such a pattern of outbreaks is the most probable contributor to my overall results in the Southern group. However, some of the genetic similarities in the south may have arisen or been enhanced by widespread outbreaks, such as the severe Cariboo-Chilcotin outbreak in the 1980's. No other severe outbreaks occurred in southern BC during the past century (Wood and Unger, 1996). Unfortunately, MPB outbreak incidence data predating the twentieth century are not available.

The sparse number of connections in the Northern group seems to imply that long distance dispersal events, which are known to have occurred within the current epidemic, are important in this region. I found that isolation by distance was absent in the north; a dominance of long distance dispersal would disrupt the correlation between gene flow and geographic distance upon which IBD is predicated. The lack of IBD in the north also suggests strong genetic drift. As previously detailed, populations in the north are probably more prone to bottlenecks and probably have fewer opportunities for inter-population breeding. In sum, in the north, long distance dispersal events are more

prevalent than in the south and are followed by periods of strong genetic drift that is characteristic of all northern populations.

4.2. The Spread of the Western Canadian MPB Epidemic

MPB outbreaks may arise in two relatively distinct ways. Outbreaks can arise locally from the expansion of native, endemic-phase MPB populations in response to favourable ecological conditions. Outbreaks can also arise via migrants, where expanding local populations supply long distance dispersers, as often occurs during MPB epidemics. In a spatiotemporal analysis of the current epidemic, Aukema *et al.* (2006) found evidence for both. Indeed, their findings supported the occurrence of long distance dispersal events in Northern BC and also indicated that many parts of the epidemic arose from the expansion of native MPB populations, especially in Southern BC.

My results do not support the widespread perception that the current MPB epidemic has spread via mass migrations from an epicenter in Tweedsmuir Provincial Park. The Houston stand (sampled in 2006) was likely the most representative of the Tweedsmuir populations. This stand was geographically closest to Tweedsmuir. Moreover, based on aerial survey maps, Houston was overrun in summer 2004 by a mass of beetles coming from the south (the Tweedsmuir region) and/or southeast. Contrary to the Tweedsmuir epicenter hypothesis, most stands were significantly different from Houston. Indeed, I found no evidence for the homogenization of population allele frequencies that typically occurs when epidemics spread from epicenters (Namkoong *et al.*, 1979). Each sampled stand was significantly different from the majority of the other stands; despite the incidence of genetic similarities among stands, I was able to use these "connections" to comparatively characterize and distinguish stands.

A time series of outbreak maps of the MPB epidemic indicates that, especially in southern BC, outbreaks have erupted simultaneously in isolated regions (Aukema *et al.*,

2006). This observation is strongly suggestive that outbreaks in these regions were initiated by the climate-induced expansion of native MPB populations. I thus suggest that MPB populations are endemic to the Whistler Valley, Manning Park, various regions of the Okanagan Plateau, the west Kootenays near stands at Nancy Greene and West Arm Parks, the east Kootenays near Kimberley, Kootenay National Park, Golden/Yoho National Park, Valemount, Mount Robson Park, and McBride. In some of these regions (eg. the Okanagan and the Kootenays), outbreaks seem to have undergone massive spatial expansions that may be due to significant MPB migrations, contributing to the population genetic patterns that I found. Inferences regarding the endemism of MPB populations in Northern BC are made difficult by the apparent dominance of long distance dispersal events.

In sum, I suggest that most outbreaks in the current epidemic were initiated by the expansion of MPB populations endemic to various regions of BC and AB. Massive dispersal events in the years following these initial expansions, especially in North-Central BC, have likely caused the large severity and scale of current outbreaks. The general dispersal patterns I have inferred represent the main factor (massive dispersal events) in the rapid expansion of MPB infestations. I argue that southern BC is dominated by significant geographic barriers and that dispersal events are typically stepwise (not long distance) through weaknesses in these barriers, such as mountain passes. The long distance dispersal patterns I have inferred should be fully-applicable to future MPB epidemics. However, I strongly argue that in the absence of long distance MPB dispersal, the isolated expansion of native MPB populations, especially in southern BC, would have eventually caused the severity and scale of the current epidemic. The

prolonged expansion of isolated native populations may simply make them more susceptible to climatic events.

Given the above general inferences, I have also inferred main dispersal patterns throughout the current epidemic (Figure 12). As previously mentioned, genetic similarities ("connections") between stands most likely reflect: 1) ongoing gene flow, or 2) recent gene flow (dispersal) with insufficient time for divergence.

DISCLAIMER: The movement patterns I have inferred are where beetles are dispersing and attacking suitable host species. MPBs may be dispersing into sinks lacking suitable host species; these movements do not contribute to the propagation of epidemics nor do these movements contribute to the persistence of MPB populations. It is important to explain my rationale in terms of how I inferred general MPB dispersal patterns (Figure 12). I used my population genetic patterns primarily, and Jackson *et al.*'s (unpublished) wind modeling data secondarily, to establish general directions of dispersal (eg. Okanagan Plateau to the Kootenays; Figure 12). I then used the incidence of infestations on outbreak maps to discern finer-scale, landform-influenced dispersal directions when possible. Although stands are referred to as place names (eg. Prince George), they represent the general area indicated by the place name.

Figure 12. Map of inferred historical MPB dispersal routes (solid lines) in BC and AB. These dispersal routes were inferred from: 1) generalized patterns of genetic similarities among 35 MPB stands analyzed at six microsatellite loci; 2) atmospheric wind patterns (Jackson *et al.*, unpublished data); 3) landform influences using a time series of aerial survey maps of the current epidemic. Note that dashed lines are movements that were suggested by genetic data but were not apparent from aerial survey maps of the current epidemic. DISCLAIMER: These "movements" represent an educated best guess using primarily my genetic data. They may have occurred within the current epidemic or sometime in the past; statistical methods cannot discern between recent and historical dispersal at this time.

4.2.1. Spread of the Epidemic – Southern Group

The Southern MPB group was characterized by isolation by distance; this is a genetic pattern that takes thousands of generations to develop (Johnson *et al.*, 2007). Thus, in the Southern group, inferred movements do not necessarily represent recent dispersal but reflect thousands of years of stepwise movements among populations. History lends credence to my assertion that the movements I have inferred in the Southern group are the most likely routes for MPB dispersal during any epidemic.

Geographic barriers seem to have played a major role in "directing" MPB dispersal throughout the epidemic area in Western Canada. Given my genetic data, many MPBs in the Kootenays and southern Rocky Mountains (the Icefields area south to the Lake Louise/Banff region) have probably come from regions to the west. Specifically, MPBs from Lillooet and the Okanagan (Kamloops, Merritt, Falkland) appear to be penetrating through weaknesses (passes) in the Monashee Mountains, in the vicinity of Highway 6, into the Columbia River/Arrow Lake valley. From this valley MPBs are likely simultaneously moving across part of the Selkirk Mountains into the next valley, the Slocan Lake valley, which is represented by the site in Valhalla Provincial Park, as well as moving through the river valley leading to Nelson, as represented by the site in West Arm Provincial Park. The site in Nancy Greene Provincial Park is only ~10 kilometers south of the Lower Arrow Lake valley and of the sites, is the most representative of the MPBs penetrating the Monashees. There is no genetic or outbreak map evidence for MPB movements south through Kelowna and the Okanagan Lake valley, then east through weaknesses in the Monashee Mountains along the BC/US

border (though I did not sample south of Kelowna or along the BC/US border in the Monashees).

From sites in the Monashee and Selkirk Mountains, MPB movements appear to have gone around as well as through the bulk of the Purcell Mountain Range. The former movements likely followed the Kootenay Lake Valley south through Creston, where there is a major pass through the Purcell Mountains into Cranbrook and the Rocky Mountain Trench. However, as inferred from the location of MPB infestations within the current epidemic, many MPBs from the Monashees and Selkirks have penetrated a large weakness in the Purcells, a west-east mountain valley east of the community of Gray Creek on Highway 3A. This valley has an active logging road on it, the Gray Creek and Redding Creek forest service roads, and the road/valley apexes at 2000m above sea level (~6600 feet) before descending eastward to the Trench.

Once in the Rocky Mountain Trench, I suggest that MPBs have moved north from the site in Kimberley (near Cranbrook) to Kootenay National Park, then through a major pass in the Rocky Mountains (which Highway 93 follows) to the Icefields Parkway and north to Lake Louise (but apparently not south to Banff). I also suggest that MPBs have dispersed long distances northwest up the Rocky Mountain Trench, apparently bypassing the Golden site, along Kinbasket Lake to the Valemount area. However, annual MPB outbreak maps do not support recent movements from the Golden area of the Rocky Mountain Trench to the Valemount area. My genetic data may reflect connections between Golden and Valemount that occurred during past outbreaks.

MPBs from the Lillooet and the Okanagan (Kamloops, Merritt, Falkland) area have also apparently dispersed up the Thompson River valley into Wells Gray Provincial

Park and into the Valemount area. MPBs from the Lac La Hache area are suggested to have dispersed eastward and contributed to movements into Wells Gray and eventually to Valemount. Again, concerning Valemount, dispersal inferred using outbreaks maps of the progression of the current epidemic conflict with dispersal inferred from my genetic data. Annual outbreak maps of the current epidemic do not support recent MPB dispersal up the North Thompson Valley past Wells Gray Provincial Park. Thus, historically, MPBs moved up the Thompson River valley north from the Okanagan to the Valemount area. I also infer long distance MPB movements northward from the lower Fraser Valley (Lillooet and Lac La Hache) to the Quesnel/Bowron Lake/Prince George region, where the latter group is apparently the "connection" hub between the Northern and Southern MPB groups.

The Banff site appeared to be relatively isolated from neighbouring stands throughout the Rocky Mountain region. Banff was only genetically similar to two sites in the Kootenays (Nancy Greene and West Arm). Indeed, independent but severe outbreaks have occurred in the Banff area over the past century (Wood and Unger, 1996). This result contradicts population genetic results for the MPBs primary symbiont, *G. clavigera*. In their phylogeographic analysis of *G. clavigera* from MPBs, Lee *et al.'s* (2007) data was strongly indicative of historical one-way gene flow of fungi (and thus MPBs) from the BC Interior to the Rocky Mountains. I hypothesize that historically, native Rocky Mountain MPB populations are isolated from Interior populations; however, periodic outbreaks in and gene flow from the Interior are sufficient to create genetic similarities between Rocky Mountain and Interior stands. This hypothesis is consistent with genetic similarities between Banff and the two stands in the Kootenays.

4.2.2. Spread of the Epidemic – Northern Group

In Northern BC, based on historical outbreak records (Wood and Unger, 1996), Chetwynd/Pine Pass, Tumbler Ridge, and Grande Prairie represent new MPB populations. For example, Grande Prairie arose from a massive MPB dispersal event in the summer of 2006, which witnesses described as "beetle rain." My MPB data indicate that the MPB populations in the Peace River area largely represent an expansion of the Northern group. It should be noted that Grande Prairie (2007) and Chetwynd/Pine Pass (2006) were genetically similar but were sampled in different years (given in parentheses). Thus the beetles represented by Chetwynd/Pine Pass had one year (summer '06) to potentially contribute to 2007-sampled infestations in the Peace River area.

However, it should be noted that there were genetic similarities between both new populations in the Peace River area and the northern populations of the Southern group, specifically in the Quesnel/Bowron Lake region. Thus, the northern portion of the Southern group may have contributed dispersers to the new infestations in the Peace River region. Also, Chetwynd/Pine Pass was the only stand in this study that was genetically similar to the Tatla Lake stand in the Chilcotin region. If the similarity to Tatla Lake was not spurious, then some of the MPB colonists of Chetwynd/Pine Pass came from the Chilcotin region nearly 500 kilometers away (straight-line). This result has implications in terms of the massive Cariboo-Chilcotin MPB outbreak in the 1980's in that this outbreak may have created new MPB populations through long distance dispersal events (up to 500km) to various regions of North-Central BC. In a final note, as shown in Figure 1, there were also genetic similarities from the Telkwa/Houston

region through the Bulkley Valley to the Prince George/Quesnel/Bowron Lake region. However, it is difficult to infer directionality and to date the implied gene flow; these similarities may have arisen through Ice Age recolonization from the Prince George region and/or from gene flow between the regions which did not necessarily occur during the current epidemic.

4.2.3. The Population Genetics of Long Distance MPB Dispersal

Though MPB populations in Chetwynd/Pine Pass and Grande Prairie were newly founded, I did not expect severe founder effects. I expected these populations to retain a considerable portion of their ancestral gene pool/s. My data support these expectations. These stands exhibited levels of genetic diversity comparable to other Northern group stands that are almost certainly native (eg. Houston, Telkwa). As noted, the founding of the Grande Prairie population was an exceptional event that involved potentially billions of MPBs from throughout the north. Chetwynd/Pine Pass was founded less auspiciously.

I hypothesize that the founding of Chetwynd/Pine Pass, in terms of the number of dispersing beetles, is typical of the long distance MPB dispersal event. For long distance dispersing MPBs to establish a population, their most basic requirement (besides suitable hosts) is to land in sufficient numbers. Jackson *et al.* (2008) conducted airplane mounted MPB-capture transects near Prince George and estimated: 1) an average of 4950 beetles "are dumped" per ha per year by long distance dispersal in the Central Interior during epidemics of the current severity and scale; 2) ~577 beetles are required to successfully mass-attack a mature lodgepole pine; 3) in leading stands, a conservative average of 8.6 trees will be infested per ha. This prediction, ~8.6 infested trees per ha, was surprisingly

congruent with the infestation intensities I observed in Chetwynd/Pine Pass and in many of the other 35 sampled stands. Thus, if new MPB populations are founded, the required numbers of MPBs preclude significant founder effects. Although long distance dispersal events may involve one or several source populations, in which the latter involves mixing of population genotypes, I suggest that the numbers required to found a new population also preclude sampling effects as long as a sufficient number of beetles (~50) are sampled.

4.2.4. Concordance with Atmospheric Wind Data

Jackson *et al.* (2008) conducted aerial MPB-capture transects at various altitudes during the dispersal season and determined that MPBs in the atmosphere are found up to ~850 m above ground level. Thus the atmospheric particle modelling that Jackson *et al.* (unpublished data) conducted, where each particle is equivalent to a beetle, used site climatic data up to 850 m above ground level. The wind patterns reported below are generalized from site-specific modelling and are for this most-relevant atmospheric layer. Jackson *et al.*'s (unpublished data) model simulations help explain both recent long distance dispersal events as well as trends in historical movements.

Generally in BC, winds are westerly or southwesterly. Thus, MPBs involved in long distance dispersal events typically move east or northeast. However, there are exceptions to this pattern. At modelling sites in the Cariboo-Chilcotin region bounded to the north at 53°N (approximate latitude of Quesnel), the east by the Fraser River, and the south at 51°N (approximate latitude of Cache Creek), predominant winds are NE and thus transport dispersing MPBs to the southwest. Thus MPB outbreaks in this major

portion of the Cariboo-Chilcotin are expected to be contained and spread to the southwest, into the Coast Mountains.

At a provincial scale, my genetically-inferred dispersal results are consistent with MPB atmospheric transport patterns modelled by Jackson *et al.* (unpublished data). Most movements within both the Northern and Southern groups appear to be to the east or northeast. I argued (see Section 4.2.6) that the stands in the Chilcotin, Farwell Canyon and Tatla Lake, were comparatively isolated based on the number of connections they had with other stands. Indeed, Jackson *et al.*'s (unpublished data) modelling showed that Cariboo-Chilcotin stands are unlikely to act as source populations to neighbouring populations and that neighbouring populations tend to disperse beetles to the east or northeast, away from the Cariboo-Chilcotin. Thus the MPBs in the Cariboo-Chilcotin are unlikely to interbreed with populations outside this region.

In the Fraser Valley, Jackson *et al.* (unpub. data) found that north-south winds along this corridor were rarer than W or SW winds. At Jackson *et al.*'s (unpub. data) sites in the Fraser Valley, less than one out of twenty (~5%) simulated MPBs travelled north or south. However, I suggest that the connections between MPB stands through the Fraser Valley, which I inferred from genetic data, may be maintained by these periodically favourable winds. It should be noted that MPB transport requires a combination of favourable atmospheric conditions as well as an outbreak in that region. The combined probability of these north-south transport events would be much less than 5% in any given year but apparently this is sufficient for the maintenance of genetic similarities ("connections").

4.2.5. Five Genetically Unique MPB Stands

In my analyses I discovered five unique MPB stands; these stands were "unique" in that they were significantly different from every other stand in this study. They were: Mackenzie, Tumbler Ridge, McBride, Willmore Wilderness, and Whistler. I will present different arguments to explain the divergence of these stands. Because these stands were unique and did not resemble source stands, they likely did not arise from long distance transport during the current epidemic (Gaggiotti, 1996). An exception to this statement is the Tumbler Ridge infestation, which as part of the new infestations in the Peace River region, is known to have recently arisen from long distance dispersal (Wood and Unger, 1996; A. Carroll, unpub. data). Severe founder events from long distance transport, as previously mentioned, do not occur and thus did not cause this "uniqueness." Though bottlenecks after the founding of new populations can quickly increase genetic divergence (Hey *et al.*, 2004), I found no evidence for bottlenecks in any stand. The "uniqueness" of these stands also indicates that they have not significantly interbred recently with the sampled stands.

The most parsimonious explanation is that these stands (with the exception of Tumbler Ridge) represent isolated native populations that have diverged by genetic drift over time. Isolation may be complete, causing rapid divergence, or isolation may involve some level of inter-population gene flow, which can slow, balance, or erase divergence due to drift (England *et al.*, 2003; Beebee and Rowe, 2004). The location of three of the five unique stands in the Northern group (four if the boundary population in the Willmore Wilderness is included) is strongly suggestive that a scarcity of inter-

population connections, which is characteristic of the north, promotes the isolation and divergence of MPB populations.

A hypothesis of an "old" founding, isolation from other populations, and bottlenecks can be tested in terms of population outcomes. I looked for evidence of genetic isolation and geographical isolation in each unique stand. I initially compared the mean F_{ST} values of each unique population to the mean across all 35 populations (F_{ST} = 0.040). A higher mean F_{ST} value indicates considerable long-term population isolation.

McBride had a mean F_{ST} value (0.090) much higher than the population mean. This indicates that the McBride stand probably represents a native MPB population that has been highly isolated by mountains, distance, and/or northern population dynamics for a long time and even within the current epidemic (to 2005). McBride was significantly different from Willmore Wilderness, in spite of the ~35km straight-line distance between them that made them the closest stands in this study. Outbreak maps from the past century suggest the McBride stand in the Rocky Mountain Trench is independent of infestations and possibly native populations in the adjacent Morkill and Holmes River valleys (see page 110). McBride was genetically closest to Northern stands (Fort St. James then Houston), suggesting ancestry from these areas. This apparent ancestry may have been enhanced by migrations from severe outbreaks in the Fort St. James region in 1930-36 and 1946-65 (Wood and Unger, 1996).

Willmore Wilderness, in contrast, had a mean F_{ST} value (0.063) 1.5 times higher than the population mean, indicating that this is likely a native population that has been

historically less-isolated than neighbouring McBride. Thus, the Willmore population may have undergone recent gene flow with other populations (Willmore Wilderness was sampled in 2006). Note: for the remainder of this Section, the year given for infestations has been extrapolated back one year to the date of green attack, or the year when MPBs flew into an area and attacked trees.[1]

MPBs have been continuously recorded in the Willmore Wilderness since at least 1997, when pheromone-baited trees were attacked. In contrast, during the current epidemic in regions of BC adjacent to the Willmore Wilderness, MPB infestations first erupted in the Holmes River valley east of McBride in 1998 (red attacks were recorded in 1999). These infestations were relatively constant to 2001 (20-40 spot infestations per year). In 2002 and 2003 infestations in the Holmes River valley declined, but slowly started building again from 2004 to 2006 (red attacks recorded in 2005-07). It is important to note that most of the spot infestations recorded in the Holmes River valley were in its upper reaches, near the BC/AB border at Bess Pass and ~35 km (straight line across several mountains) from the Willmore Wilderness stand.

Northwest of McBride in the Morkill River valley, whose headwaters meet the Willmore Wilderness at the BC/AB border, two spot infestations occurred in the upper reaches of the watershed in 1999. In 2003, several spot infestations occurred at the headwaters of this valley on the BC/AB border (red attacks in 2004). 2004 saw a swath

[1] MPB-attacked trees take about one year to change colour from green to red. Since aerial surveys detect red attacks, outbreak data derived from these surveys exhibits a one year lag. Thus an aerial survey reporting red attacks in 2007, for example, is detecting MPB attacks made from dispersal flights in 2006. Again, the infestation years noted below are for the year of green attack, or the year of flight. In terms of the spread of outbreaks, the year in which red attacks are noted is the year in which these trees act as sources for MPB flights and thus new attacks.

of infestations through the Morkill River valley (2005 red attacks), but these infestations declined annually to 2006. Infestations were located at the mouth of the Morkill River valley in 1998, 1999, and 2001-2006.

Since MPB infestations were detected in Willmore Wilderness in 1997, at least a year before infestations were detected in adjacent regions of BC, combined with my genetic data there is strong evidence for MPB populations endemic to the Willmore Wilderness (as represented by the sampled stand). Up to and including the 2005 flight (since I sampled Willmore Wilderness in 2006), I suggest that the Willmore outbreaks largely arose from native populations supplemented by limited migration from the Morkill (2005) and Holmes River valleys (1999-2002 and 2005-current). Migrations through the Holmes are consistent with the pre-summer of 2006 advance of infestations down the Smoky River valley, which is continuous with the Holmes. The severe 2006 flight likely overwhelmed the Willmore Wilderness and Grande Cache region with migrants from regions of North-Central BC. Thus a majority of the MPBs currently (as of the aftermath of the summer of 2007 flight) overrunning the Willmore Wilderness and the Grande Cache area are likely not descendents of populations endemic to the Willmore. I predict that if beetles were sampled in these regions in 2007-current, then these beetles would be genetically similar to stands in North-Central BC. The Holmes valley route involves crossing Bess Pass at ~1600m (~5200 feet) above sea level but as mentioned previously for the Purcell Mountains, the MPB apparently can disperse to and through such barriers. Though the Willmore Wilderness stand (as of 2006) probably reflected some migration from the Morkill and Holmes valleys, Willmore was

significantly different from McBride. Thus the McBride, Morkill, and Holmes stands most likely are historically isolated native MPB populations.

The ancestry of the MPB population endemic to the Willmore Wilderness is likely more recent compared to its neighbour in McBride. The exact timing of this founding is very difficult to estimate but two approximate times are most likely. Notably, the headwaters of the Morkill River are located less than 10km from the sampled site for the Willmore Wilderness (in Beaverdam Pass). Historical outbreaks in the Morkill River valley could have established a native MPB population in the Willmore Wilderness, specifically in the BC/AB border region. A founding sometime in the past, combined with periodic moderate bottlenecks, would be consistent with Willmore Wilderness's mean F_{ST} (0.063) compared to a population mean F_{ST} of 0.040. Willmore's mean F_{ST} may also be explained by more severe bottlenecks since a more recent founding, specifically from the massive Cariboo-Chilcotin outbreak in the 1980's. Willmore Wilderness was genetically closest to stands near Quesnel and Bowron Lake, which was the north-eastern extent of the 1980's outbreak. Jackson *et al.*'s (unpub. data) atmospheric modelling near these sites suggests that atmospheric winds could occasionally (<5% of the time) move MPBs from Quesnel/Bowron Lake into the McBride/Willmore Wilderness region of the Rockies.

Mackenzie had a mean F_{ST} value (0.105) that was the highest among the 35 populations. This value indicates that Mackenzie represented an endemic (native) population which has been isolated, even within the current epidemic (to 2005). Mackenzie was genetically closest to Tumbler Ridge, then more distantly related to a range of stands in both the Northern and Southern groups. Ancestry was thus difficult to

discern from the Mackenzie stand, which may be very old and may have genetically converged. The Mackenzie population may have been old enough to reach a limit on divergence at these microsatellite loci (Paetkau *et al.*, 1997), after which convergence occurs and the population begins to resemble random populations to which it was/is not connected by gene flow. Because I sampled in 2005, given the large dispersal flights that have occurred to this region during the summers of 2005 and 2006, I suggest that later samples would find that Mackenzie beetles are genetically similar to stands in North-Central BC, as suggested for the Willmore.

Tumbler Ridge had a mean F_{ST} value (0.048) not much higher than the mean across populations. This indicates that the Tumbler Ridge stand was not a native population; indeed, extensive ground surveys have revealed no evidence that the MPB is endemic to this region (A. Carroll, unpub. data). I suggest that Tumbler Ridge was founded early in the current epidemic (perhaps ~5 years ago) and has suffered climate-induced bottlenecks (eg. overwintering mortality) and limited immigration up to 2004/2005 (samples were collected at this site over 2 years in 2005 and 2006).

The first instance of MPB infestations in the Peace River region during the current epidemic occurred as a few spot infestations between Moberly Lake and Chetwynd townsite in 1999 (red attacks in 2000). These infestations disappeared by 2000 (no red attacks in 2001), when the lone infestation in the Peace River region was a single spot infestation about 30km NE of Tumbler Ridge townsite. In 2001, there were a few spot infestations ~40km west of Chetwynd, a spot infestation ~60km south of

Chetwynd, and a single spot infestation in roughly the same area as recorded NE of Tumbler Ridge in 2000. By 2002 all of these spot infestations had disappeared (no red attacks in 2003).

There was a large MPB dispersal event in the summer of 2003. Indeed, in 2003 there were large aggregations of spot infestations 40-80km south of Chetwynd, a large infestation ~35km south of Tumbler Ridge, and isolated spot infestations nearing 20km from Tumbler Ridge (detected as red attacks in 2004). In 2004 the spot infestations occurred in the same areas as for 2003 but the number of infestations shrunk considerably. Thus there were no significant long distance MPB dispersal flights to this region observed in the summer of 2004. In 2005 spot infestations recorded in 2004 grew, probably due to localized increases; this implies that no significant MPB flights to this region occurred in 2005. However, in 2006 a massive MPB dispersal event (as mentioned earlier) involving most of North-Central BC swept over the Rocky Mountains and inundated the entire Peace River region with continuous, not spot, infestations (recorded as red attacks in 2007).

In sum, over the nine most recent years of MPB "green attack" infestation data from the Peace River region (1998 to 2006), infestation-causing immigration events only occurred in five years (1999, 2000, 2001, 2003, and 2006). Of these events, only the 2003 and 2006 immigration events were considerable. Immigration events in 1999, 2000, and 2001 produced only a few spot infestations. There was also a pattern of newly-founded spot infestations shrinking, or in most years, disappearing by the next year in the Peace River region; these contractions or extirpations of new MPB populations occurred in 2000, 2002, and 2004, could not have occurred in 1998 (no

infestations) or 1999 (infestations just founded), did not occur in 2005 (I believe there were local population increases), and in 2001, 2003, and 2006 population declines could have been masked by significant immigration. Thus conditions in the Peace River region, as well as very significant control efforts, caused MPB declines or extirpations in three of seven years, and in a maximum of six of seven years if not for large immigration events.

I have noted that the first MPB infestation in Tumbler Ridge, during the current epidemic, was a spot infestation 30km NE of the townsite in 2000. This infestation was not part of the MPB stand I sampled in 2005/2006. Indeed, the Tumbler Ridge site was actually 22km SW of the townsite and thus ~50km from this spot infestation. As detailed above, in 2003 there was a major MPB dispersal event that created, among other infestations, spot infestations ~20km SW of Tumbler Ridge in the same area as the sampled stand. By 2004 spot infestations at the stand location had disappeared, or been extirpated, probably because of the winter of 2003/2004. The year 2005 saw the localized expansion of infestations further SW of Tumbler Ridge; these infestations apparently survived the winter of 2004/2005 in large numbers and immigrated into the stand location ~20km SW of Tumbler Ridge.

With regard to the hypothesis regarding the origin of the stand in Tumbler Ridge, the earliest this stand was founded within the current epidemic was 2003. This population may have survived an abrupt transition to endemic levels in 2004, a year in which no red-attacks were spotted in the area. If so, this 2003-founded population would have received immigration in 2005 from outbreaks further south of Tumbler Ridge, which were also founded in 2003. Based on annual provincial MPB outbreak maps, no

significant long distance immigrations of MPBs into the Peace River region occurred in 2004 or 2005; thus immigration from outside of the Tumbler Ridge region did not affect the beetle offspring I sampled in 2005 and 2006.

I thus speculate that the stand in Tumbler Ridge was founded in 2003, during the current epidemic, and further speculate that moderate bottlenecks in 2004 and 2005 were only slightly offset by immigration from less-severely-bottlenecked stands ~10km to the SW in 2005. I imply that in the stand I sampled, the '04 and '05 bottlenecks were only moderate, perhaps reducing the founding population in this stand from 10,000's to 1000's of MPBs in a single year. This conflicts with aerial survey data, as in the sampled stand, insufficient numbers of MPBs apparently survived the winter of 2003/2004 to mass-attack and kill a single tree in 2004 (no red attacks from this stand reported in 2005). However, as all of my collections were of beetle brood, my 2005 collections of 36 of 51 individual MPBs represented beetles that were descendants of the 2003-founders of this stand. Considerable immigration to the sampled stand likely occurred in 2005, but because I only sampled beetle brood, immigration only influenced the 15 beetles I sampled in 2006. The MPBs in this stand, which were apparently extirpated in 2004 (based on aerial surveys), attacked trees that yielded these 2005 results: five red attacks had four or more brood-containing galleries while remaining trees were largely green strip attacks (all sampled galleries had large numbers of brood). Thus, the majority of the beetles I sampled from Tumbler Ridge were descendants of the original 2003-founded population. The 15 individual MPBs sampled from this stand in 2006 probably had ancestry with the founding population and with dispersers from larger infestations

~10km SW of the stand. The above explanation is congruent with Tumbler Ridge's mean F_{ST} value, which was only slightly higher than the mean value.

It is also possible, though unlikely, that Tumbler Ridge was genetically unique because I failed to sample its source stand. It is also possible that the multi-year sampling at Tumbler Ridge caused bias. As previously mentioned, 33 beetles were sampled in 2005 and 15 in 2006. These two beetle samples were largely similar. Comparing the allele frequencies of the 2005 and 2006 beetles revealed that frequencies were similar, despite large discrepancies in sample size. An AMOVA also found that these two stands were not significantly different (Arlequin – $F_{ST} = 0.014$; $P = 0.104$; 10,100 permutations). Although data supported my decision to include the 2006 beetles in my analyses, if they were excluded, then Tumbler Ridge lost its "unique" status. Tumbler Ridge became connected to Houston, Fort St. James, Lac La Hache, Quesnel, and Chetwynd/Pine Pass. I suggest that these connections were a direct result of reduced power caused by a sample of only 33 beetles; if 48 beetles were sampled from Tumbler Ridge in 2005, then Tumbler Ridge probably would have remained genetically unique.

In terms of ancestry, Tumbler Ridge was most closely related to Chetwynd/Pine Pass, which suggests their respective foundings in 2003 and 2005 involved similar regions of North-Central BC.

Finally, based on historical outbreak data, MPBs are endemic to the Whistler region (Wood and Unger, 1996). However, Whistler's mean F_{ST} value (0.044) was not much higher than the mean across populations. This suggests that the Whistler stand, the only unique population from the Southern group, was native but has not been very

isolated either historically and/or currently. This lack of isolation opposes dispersal trends predicted by prevailing wind patterns (Jackson *et al.*, unpub. data). Atmospheric winds during the MPB dispersal season tend to isolate MPBs in Whistler. Winds are easterly at Whistler, keeping it from acting as a source to neighbouring populations in the Fraser Valley and Okanagan. Winds from these neighbouring populations are from the west, preventing Whistler from acting as a sink. I can, however, hypothesize that since large outbreaks have occurred in the Whistler region over the past century (Wood and Unger, 1996), and because Jackson *et al.* (unpub. data) found that in perhaps one out of every ten instances, westerly winds occur at Whistler, occasional dispersal of MPBs to neighbouring populations to the east may be enough to maintain Whistler's relatively low level of genetic isolation.

In sum, though this is a large extrapolation, if these wind patterns are historically representative of the Whistler region, then Whistler is most likely to act as a source of beetles. Occasional gene flow to neighboring MPB populations may maintain Whistler's low average genetic distance to other populations.

4.2.6. Ten Comparatively Isolated MPB Populations

Based on the new outbreaks in the Peace River region, I suggest that populations connected by significant immigration should show many instances of genetic similarity. The corollary is that expanding native populations that have been connected by little immigration before and/or during the current epidemic should have few instances of similarity. I suggest that these latter populations are isolated given a comparative regional context where neighbouring stands had many (~10) connections. Fort St. James (4), Francois Lake (2), Tatla Lake (1), Farwell Canyon (5), Mount Robson (5), Golden (4), Banff (2), Manning Park (4), Kelowna (3), and Argenta (4) were all native populations based on their comparative isolation (connections in parentheses). Of these, mean-F_{ST} values suggested that MPB populations in the Chilcotin region (Tatla Lake and Farwell Canyon), Mount Robson Provincial Park, Manning Park, Kelowna, the northeast Kootenays (Argenta), Banff, and Golden were the most isolated.

A stark contrast is found in Lake Louise, a "hub" stand where as previously mentioned, migrants that have apparently originated in the Okanagan have moved through the Kootenay and Rocky Mountains. A major source for migrants to Lake Louise has probably also been nearby Golden and Yoho National Park.

In the Northern MPB group, Telkwa and Houston apparently were the major source stands and Fort St. James and Francois Lake were less important sources. Connections between the Northern and Southern MPB groups were apparently mediated via a "hub" involving Prince George, Quesnel, and Bowron Lake. This hub was connected to Farwell Canyon, Lac La Hache, and Lillooet in the Southern group.

4.2.7. MPB Population Structure Versus Spread of the Epidemic

In the Southern MPB group there was a "mess" of connections (AMOVA) between stands. I thus expected to find little evidence for significant population structure within the Southern group. The mass movement of beetles among populations during epidemics is expected to mask or even erase between-stand differences as populations interbreed and become genetically similar. However, the between-stand connections I found seemed to follow a pattern of MPB dispersal through weaknesses in barriers, primarily mountain ranges; this dispersal may have occurred during the current epidemic, the recent past, or the distant past. Most between-stand AMOVA comparisons found significant differences. Moreover, isolation by distance (IBD) was strongest within the Southern group. This highly significant IBD relationship indicates ongoing gene flow and divergence among populations. Thus in the Southern group, stepwise inter-population breeding, where nearby populations are most likely to interbreed (and become genetically similar) compared to distant populations, was dominant. These results, in sum, suggest that many of the connections between stands arose from stepwise inter-population breeding. The corollary is that endemism and population structure are being maintained in the Southern group, despite the expectation that epidemics should mask or erase population identities. Thus, the spread of the epidemic in the region encompassed by the Southern group has been largely influenced by the stepwise spread of native populations.

In the Northern MPB group there were fewer connections (AMOVA) between stands. I found significant population structure within the Northern group (AMOVA). However, a finding of non-significant IBD indicated that long distance dispersal events

were dominant in the north. I suggest that the comparative lack of geographic barriers, specifically mountain ranges, in the north versus the south has facilitated the dominance of long distance dispersal in the north. As previously mentioned, a dominance of long distance dispersal would disrupt the IBD correlation between genetic and geographic distance between populations. Movements in the north are likely to involve large "jumps" among populations, with little correlation with distance between populations. High rates of genetic drift, consistent with historical Northern MPB population dynamics, have also probably contributed to a lack of IBD in the north. Thus, the spread of the epidemic in the north has been largely influenced by long distance MPB dispersal, facilitated by a lack of geographic barriers.

4.3. MPB Population Structure and the Geographical Distribution of Climatic Suitability

Over the past century, the distribution of regions that are climatically suitable, in terms of Climatic Suitability Classes (CSC) for the MPB, has expanded across BC and especially in the North (Figure 9; Carroll *et al.*, 2004). My results suggest that the Northern group and the northern-most portion of the Southern group are the sources of recent climatically-induced MPB range expansion into the Peace River region. However, the MPBs current North-South population genetic structure (2005/2006) has a stronger concordance with a historical 1921-1950 as opposed to a recent 1971-2000 CSC distribution (Figure 9; Carroll *et al.*, 2004). My results indicate that the genetic structure of the MPB in Western Canada has not shifted in response to recent expansions of climatic suitability. I previously argued that the existence of a Northern and Southern group was a partial consequence of differential climate-driven MPB population dynamics; Southern BC is typified by high to extreme climatic suitability for the MPB while Northern BC is markedly less suitable (Carroll *et al.*, 2004). Over the 20th century, in addition to expansions of climatic suitability in the Peace River region, there was a major expansion of high to extreme climatic suitability into the regions west of and including Prince George. Range expansion by the Southern group into this adjoining region would be expected. However, current MPB population structure did not reflect this change.

I propose two explanations: 1) If the Fraser Valley was an active corridor for MPB movements between the Northern and Southern groups, then the Southern group should have shifted into the newly-suitable region west of Prince George. Since this shift

did not occur, the Fraser Valley probably represents a postglacial recolonization route and/or a little-used corridor. The Northern and Southern groups are probably effectively separated. The corollary is that MPB responses to climate-induced range expansions will most likely occur within groups. An expansion of climatic suitability in the Southern group will probably involve range expansion by the Southern group, and vice versa. 2) It is also possible that my sampling in the region surrounding the CSC shift located west of Prince George was insufficient to detect a range shift. In the near future, annual population genetic surveillance of MPB populations will hopefully become a reality. This surveillance would hopefully occur at a high enough resolution to detect MPB range shifts in response to climate shifts.

4.4. A Model for MPB Population Genetic Dynamics

Based on my literature review and my results regarding the dynamics of MPB population structure, I propose a model for MPB population genetic dynamics. In terms of divergence, MPB populations become differentiated by drift and selection during isolation in the endemic phase; divergence is slowed, balanced, or erased by inter-population migrations during the epidemic phase. In terms of population genetic diversity, MPB population dynamics, which are characterized by temporally-dominant small (endemic-phase) populations and punctuated by widespread population explosions (epidemics), may be neutral with respect to genetic diversity maintenance. In other words, mutation/migration and genetic drift averaged over the phases of the MPB population cycle may be in equilibrium. This model for population genetic dynamics, where divergence and losses of genetic diversity during the endemic phase are balanced by convergence and gains in genetic diversity during the epidemic phase, may be characteristic of all eruptive herbivorous insects, such as locusts (Ibrahim, 2001).

During the endemic phase, local selection is significant. More importantly, genetic drift should be very heavy as these widely distributed populations have small effective sizes. Indeed, the MPB endemic phase is characterized by ~five to ten beetles per stand that are outcompeted by other bark beetles in blown-over and suppressed trees (Safranyik and Carroll, 2006). Thus, during the endemic phase, MPB individuals and populations are extirpated at high rates due to competition and demographic and environmental stochasticity, although some extirpated regions are likely recolonized by migrants.

During epidemics, there is enormous generation and retention of mutations. These mutations are widely distributed by long and short distance migrations (Petit *et al.*, 2005). Populations are connected by levels of gene flow correlated with geographic barriers (distance and/or mountain ranges), such that nearby populations become genetically similar while distant populations remain differentiated. When epidemics decline, most new genetic variation is likely maintained in myriad small MPB populations that are distributed over the landscape. Epidemics may not only span previously occupied regions but may also contribute migrants to distant regions. These migrants may survive and become endemic-level populations that retain considerable genetic diversity.

4.5. MPB "Range Expansion"

The Pleistocene has been characterized by major ice ages (glacials) on a ~100kyr cycle that are interrupted by warm interglacials as per current conditions (Hewitt, 1996). The transitions between these climatic extremes are characterized by gradual warming or cooling and punctuated by frequent short and severe oscillations. For example, during the Eemian interglacial (135-115kyr BP), oscillations involved 10-12°C changes in average temperature over just 5-10 years (Hewitt, 1996); this climate endured for 70-5000 years depending on the event. Such rapid climatic change would extirpate most or all of a species over its northern range (sudden cooling) or its southern range (sudden warming). Under Hewitt's (1996) model of range oscillations, only "central" populations ultimately survive. Thus, if Idaho/Utah approximates the MPBs core (ancestral Pleistocene) range, then during glacials this would be the northern-most (and likely smallest) population and during interglacials it would be a central or south-central (larger) population as per its current location within the MPBs range.

I hypothesize that the current northward range expansion of the MPB, into "historically climatically-unsuitable areas," is part of a trend of interglacial expansions and glacial retreats that have occurred over the past several million years (the Pleistocene). In other words, the MPBs current range expansion, while novel with reference to the MPBs range over the past century, is a normal event that has recurred many times over the Pleistocene. When interpreting range shifts, defining an appropriate frame of reference is critical. The gradual changes and severe oscillations of both the Pleistocene climate, and of the present Holocene climate, have caused and continue to

cause obvious shifts in the geographic distribution of climatic suitability for all organisms. Retreats during glaciations and expansions during interglacials have occurred for millions of years (Hewitt, 1996; 1999; 2000), long before humans significantly influenced the Earth. These cycles will most probably continue long after we are gone.

During this interglacial, like any other, climatic suitability will continue to shift northwards and the MPB will continue to expand north, reaching an apex somewhere in the Yukon (Logan and Powell, 2001). Perhaps in a century or two this prediction will come to fruition. When the next glacial begins, these northern MPB populations will be extirpated and the MPB will again, as I hypothesize, persist in Idaho/Utah until the next interglacial.

4.6. Potential Research Limitations and Biases

I identified eight potential areas of limitation or bias in my research: sample sizes; genetically-independent sampling; adjusting significance levels for the number of tests; inherent microsatellite limitations; the estimation of recolonization or divergence times; limitations of the program Structure; potential influences from outbreaks in the United States; potential anthropogenic influences on dispersal patterns.

Sample sizes were an improbable source of bias for my study. All sample sizes referred to in this paragraph represent final sample sizes, or the number of individual beetles that were fully genotyped. In my study, I collected more individual beetles for every site than were represented in the final sample size. All of my sample sizes were in the range of 43-49 beetles per site (mean = 46) with the exceptions of McBride (n=24), Grande Prairie (n=30), and Chetwynd/Pine Pass (n=55). The potential n-induced bias associated with McBride and Grande Prairie has been previously mentioned. The magnitude and restricted range of my study's sample sizes is robust, especially in comparison to the three previous microsatellite-based studies of Scolytids. Kerdelhué *et al.* (2006) sampled only 30 beetles per site, an average n over 50% smaller than ours. Sallé *et al.* (2007) sampled an average of only 23.0 beetles/site (28 sites) and their sample size distribution was bimodal, with most sites having an n=30 or n=20. The most recent of the three Scolytid microsatellite studies had the most potential for bias. Maroja *et al.* (2007) sampled an average of 39.7 beetles/site (14 sites) but there were notable outliers in Alaska (n=115), Utah (n=73), Montana (n=23), and Washington (n=22). The magnitude and range of my study's sample sizes were robust.

I found low levels of significant linkage disequilibrium distributed evenly across stands and loci. This was probably a consequence of the fact that the infestations I sampled likely arose from endemic populations. Endemic populations are characterized by very few beetles per stand. A direct consequence of small population sizes is strong bottlenecks and inbreeding, both of which cause significant linkage disequilibrium. Bottlenecks and inbreeding during the endemic phase most likely play a large role in the dynamics of all bark beetle populations.

My samples were genetically-independent. I define genetic independence as the fact that genotyped beetles had different parents. An argument can be made that such genetic independence caused us to over-represent genetic variation in sampled stands. However, I feel that my sampling regime is the most robust of any population genetic study of a Scolytid beetle. As inferred from their methods sections, the past studies previously mentioned have treated sampling regimes as relatively unimportant. Protocols for beetle sampling are rarely found. The potential for bias due to the methodological unknowns of these studies is great. Moreover, the direction and magnitude of such bias is unknown. I strenuously advocate the use of known, "lesser-evils" (methodologies with known small biases) in place of unknown, "greater-evils" (methodologies with potentially huge biases).

Genetically-independent samples, moreover, are strongly preferable to pseudoreplicates in population genetic research. Genetic independence maintains sample sizes while pseudoreplication involves sampling of redundant genotypes, lowering effective sample size (eg. Yoon Chung *et al.*, 2005). With genetic independence, one can accurately characterize population genetic diversity and determine population structure

using a much lower sample size than studies affected by pseudoreplication. Indeed, the widespread reporting of no population structure among many Scolytid studies may be attributable to a failure to correct for pseudoreplication. My sampling regime, however, was not perfect. MPB populations should be sampled randomly but this poses technical challenges. Sampling beetles during flight (pheromone trapping) probably does not represent effective dispersal. Thus brood must be sampled and this can only be done under the bark of infested trees. How MPBs can be sampled randomly in reference to a total MPB population, whose geographical boundaries may not exist, is a problem I leave to future studies.

A fundamental question in my research was whether to be more-conservative or less-conservative when assessing the genetic similarity between stands. Specifically, the adjustment (lowering) of significance levels for multiple comparisons has been haphazardly applied across ecological disciplines (Cabin and Mitchell, 2000). No formal criteria exist in any scientific fields for when to use such corrections and for which of the many available corrections to use (Nakagawa, 2004); indeed, the application of multiple testing procedures is a subjective practice (Perneger, 1998; Moran, 2003). Although I used a standard Bonferroni correction in my analyses of Hardy-Weinberg Equilibrium and linkage disequilibrium, I chose not to make any adjustments to the significance level (a=.05) for the AMOVA analyses. Nearly 81% of 595 pairwise between-stand AMOVA tests rejected the null hypothesis (H_0: stands are genetically similar) and thus found stands were significantly different. Compared to the HWE and linkage disequilibria results, which showed no consistent patterns, the AMOVA results were strongly suggestive that I detected something important (Moran, 2003; Verhoeven *et al.*, 2005).

Bonferroni corrections would have reduced the rate of Type I error (rejecting a true null hypothesis) at the expense of a higher rate of Type II error (accepting a false null hypothesis). In my study, more Type II errors would mean more tests found non-significant differences, or genetic similarities, between stands. I considered the assessment of genetic similarities, or "connections," within the current epidemic to be of critical importance and chose to minimize Type II errors by keeping the significance level at 0.05.

We conducted a sequential Bonferroni correction (Holm, 1979), which reduced the effective level of significance to 0.000206 (0.05 / ~243), to determine how correcting for multiple comparisons would have affected the AMOVA-derived "connection" map (Figure 1). The sequential Bonferroni correction resulted in 242 non-significant tests (41%), as compared to only ~19% of tests at a 0.05 level of significance. Revising Figure 1 for Bonferroni-correction (not shown) resulted in more connections within the Northern and Southern groups, as well as more connections between the groups through the Fraser Valley. At a stand-level, Bonferroni corrections caused "hub" stands to have many more connections, comparatively isolated stands to become "hubs," and four of the five genetically-unique populations to become connected. Whistler became connected to Manning Park, Lac La Hache, and Kamloops; McBride became connected to Fort St. James and Houston; Willmore became connected to Quesnel; Tumbler Ridge became connected to Houston, Fort St. James, Lac La Hache, Quesnel, Chetwynd/Pine Pass, and Grande Prairie. In all cases, these four unique populations became genetically similar to the stands to which they were most related. Mackenzie remained significantly different from all sampled stands. These additional "connections" confounded relationships

among stands. In sum, by keeping a 0.05 significance level and minimizing Type II errors, I have identified the most important historical and/or recent relationships among stands.

Microsatellite analysis has inherent sources of error. Null alleles, which are caused by a point mutation in the primer annealing site (Jarne and Lagoda, 1996; Dakin and Avise, 2004), can result in non-amplification of a sample at a locus. Although null alleles are typically restricted to just a few individuals in a study, their effects can be widespread and difficult to detect. Short allele dominance (Wattier *et al.*, 1998), where the smaller sized allele of a heterozygote is preferentially amplified, can also be prevalent. Both types of errors can cause heterozygotes to be scored as homozygotes, reducing apparent population differentiation (Dakin and Avise, 2004). I minimized these errors by establishing all alleles at all loci, across all populations, prior to manual scoring of genotypes; this ensured that valid peaks were recognized as true alleles. Permutation tests confirmed that most populations did not have excess homozygotes and were well within Hardy-Weinberg equilibrium.

It would be impossible to estimate recolonization or divergence times from my microsatellite-derived data set. Such estimates are influenced by levels of population genetic diversity, fluctuations in population size since divergence/recolonization, the age of populations, recombination rates at each locus, the number of repeats at each locus, the location of microsatellite loci on chromosomes, and many other factors. Calculating and determining the cumulative effects of these influences on the mutation rates of my six loci could not be done with any precision. Future studies that aim to calculate the recolonization and divergence times of MPB populations should employ sequencing

analyses that preferably involve both the nuclear and mitochondrial genomes. There is a considerable body of statistics devoted to estimating various timelines from sequence data and highly accurate estimates are possible (Marshall *et al.*, 2002).

The statistical program, Structure, is limited in that it works optimally for data sets with small numbers of discrete populations. Moreover, data that are characterized by isolation by distance, where there are allele frequency gradients among populations, is not ideal. Under isolation by distance, MPB genotypes imply membership in multiple clusters and population structure is increasingly obscured at higher cluster values. This pattern of population structure dilution at increasing values of K occurred in my analyses; thus, the lowest tested value of K=2 yielded the strongest structure.

There is low likelihood that recent MPB outbreaks in the United States have acted as significant sources of long distance dispersers to the current Western Canadian epidemic. Maps of outbreaks in the states of Washington, Oregon, and Montana over the past decade indicate that both the scale and severity of outbreaks pale in comparison to the Canadian outbreak. While the MPB epidemic newly infested 10.1 million ha at various mortality levels in 2007 in Western Canada, equivalent infestation areas in the United States were notably lower. Washington State recorded 103,000 ha of all pine beetle mortality in 2006 (Nelson *et al.*, 2007a). Most of this mortality was MPB-caused and was focused in the remote northern parts of the state, primarily in the North Cascades (adjacent to Manning Provincial Park in BC) and west of Franklin D. Roosevelt Lake in NE Washington State. MPB infestations in Oregon totalled 144,000 ha in 2006 and were focused on the eastern slope of the Cascade Mountains (Nelson *et al.*, 2007b). In Idaho, infestations covered 124,000 ha in 2006 (Idaho Forest Health Protection, 2007) but an

outbreak location map was not readily available. Based on host tree distributions, much of northern Idaho contains suitable host species for the MPB. Lodgepole pine, ponderosa pine, and western white pine are abundant in northern Idaho and all are ideal MPB hosts. Of the states mentioned, Montana sustained the largest area of MPB infestations in 2006 at 329,000 ha; most of these outbreaks were in west-central and south-west Montana (Gannon *et al.*, 2007).

Overall, only Washington and possibly Idaho have had recent MPB outbreaks that are proximate to and could possibly act as source populations for Canada. Given the small relative size of these outbreaks, contributions to the Canadian epidemic, if any, were and probably continue to be minimal. Given the scale and severity of the current Western Canadian MPB epidemic, it is more likely that Canadian infestations have been sources for point infestations across the border in the United States. It is unfortunate that my sampling did not involve sites in northern Washington State and Idaho State. My prediction is that infestations at these sites would be genetically contiguous with sites in southern BC. This prediction awaits a future study that examines the range-wide population genetic structure of the MPB at a resolution, in terms of distance between sampling sites, that is as fine as or finer than ours.

My results may have been biased by anthropogenic translocations of MPBs between normally isolated populations through MPB-infested timber. Such biases are not uncommon in studies of Scolytids (Nilssen, 1984; Stauffer *et al.*, 1992). Indeed, the international trade of pine timber may be a contributing cause of apparent gene flow among isolated populations of *T. piniperda* (Faccoli *et al.*, 2005). Moreover, the operation of pine plantations may involve movements of possibly infested trees since this

beetle does its maturation feeding in shoots (Horn *et al.*, 2006). Faccoli *et al.* (2005) genetically corroborated that, as previously asserted by forest monitoring, their sampled stand of *T. destruens* in northern Italy represented a population that was introduced with a plantation from Greece about 50 years ago. Moreover, about 2000 years ago a system of plantations was created during the Roman era to defend the Adriatic coast and may have resulted in much translocation of beetles (Faccoli *et al.*, 2005).

MPB-infested trees, stacked in lumber yards, can produce dispersing MPBs. However, no stands were within 80 km of a log yard. Imports of MPB-infested wood for campfires were only potentially significant in Lake Louise, which sourced MPB-killed timber from adjacent Yoho National Park (Banff National Park Warden Lee Smith – personal communication). However, inspection of the gravel pit where MPB-infested timber was stored revealed no successful MPB attacks on nearby pines.

4.7. Recommendations for Future Research

Future studies of MPB population structure should use more genetic markers and sample at an equivalent or finer spatial resolution than ours but extended over the MPBs North American range. To maximize potential gains in knowledge, such a study should also extensively sample stands of *D. jeffreyi*. A combined approach would not only yield a high-resolution picture of the population genetic structures of each species, with information on rates of gene flow and historical movements, but a combined approach may also elucidate the mode of speciation between these sister species. Based on their differential host-use and geographical allopatry (Wood, 1982), I hypothesize that *D. jeffreyi* diverged from a MPB-like common ancestor, which was a host generalist (Kelley and Farrell, 1998), via isolation during the Pleistocene. Though there is debate over the role of Pleistocene glaciations in divergence, many studies support Pleistocene-mediated speciation (Knowles, 2001). The ancestor of *D. jeffreyi* may have been isolated from the eventual MPB by the Cascade, Klamath, and Sierra Nevada Mountains as well as the Great Basin and Mohave Deserts in a California refugium, and responded altitudinally (up and down mountains) rather than latitudinally (Hewitt, 1999), during the frequent advances and retreats of the past two millions years of glaciations (the Pleistocene epoch). However, due to Pleistocene range dynamics, species distributions may have changed greatly since the time of speciation (Knowles, 2001). A related explanation is that *D. jeffreyi* arose during the Pleistocene in a refugium adjoining California, from which it achieved its current distribution.

In a case study of post-Pleistocene recolonization patterns across taxa, Hewitt (1999) found that populations in the eastern Mediterranean were not a source of colonists

to northern areas. As Horn *et al.* (2006) indicated for the beetle *T. destruens*, major geographical barriers (distance and mountains) and/or lack of suitable hosts played a large role in this across-taxa pattern of isolation. Thus, if *D. jeffreyi* was established during the Pleistocene, geographical barriers and a fragmented host distribution probably limited its range to California, even during interglacial periods, and prevented northward expansions and interbreeding with the common ancestor of the MPB, resulting in allopatric speciation. Indeed, Horn *et al.* (2006) estimated that the split between western and eastern groups of *T. destruens* in the Mediterranean occurred during the Pleistocene, with a mean estimate of 430 000 years bp (range 350 000–1 200 000 years). Although these groups have not diverged enough to justify the genetic designation of a new species, *T. destruens* has two dispersal flights per generation compared to one flight per generation among *D. ponderosae* and *D. jeffreyi*. Thus speciation could have occurred at a faster rate for *D. jeffreyi* and could have occurred during Pleistocene events. The current sympatry between *D. ponderosae* and *D. jeffreyi* (Kelley *et al.*, 2000) would represent recent secondary contact due to southward recolonization along the West Coast that I suggest occurred post-Ice Age for the MPB.

Future studies that examine the range-wide population genetics of the MPB at a high-resolution should simultaneously examine the following hypotheses of post-Ice Age recolonization: 1) post-Pleistocene recolonization was associated with successive founder events (reduced genetic diversity) away from a single refugium (the "core range" in southern Idaho and in Utah), as I previously hypothesized; or 2) regional population structures and hybrid zones exist, suggesting multiple MPB refugia (Knowles, 2001).

Importantly, if future landscape-scale studies of MPB population genetics employ more genetic markers (Rosenberg *et al.*, 2001), then they will be able to explore the presence of sub-clusters within the Northern and Southern MPB groups. The programs Structure and SAMOVA should be employed. The hierarchical analyses of population structure outlined by Rosenberg *et al.* (2001) would entail first testing for clusters using all individuals, then testing within groups found by the previous analysis, and onwards until population structure is not apparent at increasingly finer geographic scales.

Future studies should examine the temporal dynamics of MPB population genetics by sampling stands over consecutive years and determining how genetic diversity and allele frequencies change with population status (endemic to incipient-epidemic to epidemic to post-epidemic). Ideally, sampled stands would contain native MPB populations that were highly isolated from influxes of migrants. Information from this research could likely be accurately extrapolated to other Scolytids, including the Jeffrey pine beetle (*D. jeffreyi*) and the Douglas-fir beetle (*D. pseudotsugae*), as population dynamics are similar across many bark beetle species.

A related recommendation regards employing more marker loci on a similar number of samples per stand to allow for powerful bottleneck detection. Though I did not detect bottlenecks among stands, my negative result was consistent with studies employing low power (Luikart *et al.*, 1998; Spong and Hellborg, 2002; Busch *et al.*, 2007). Bottleneck detection will be particularly important when explaining the population structure of recently founded stands in Alberta, especially if these stands are significantly different from all others. However, caution must be used in bottleneck detection during MPB epidemics. Even low levels of immigration can erase bottleneck

signatures within a few generations (Keller *et al.*, 2001). Because of massive amounts of mutations and the stochastic distribution of these mutations over the landscape via migrations, bottlenecks may appear to be diffuse. Diffuse bottlenecks allow much allelic and genetic diversity to be retained, making bottlenecks difficult to detect (England *et al.*, 2003).

Future studies should also, as a first step, conduct a preliminary investigation of the comparative pathogenicity of Rocky Mountain and Interior *G. clavigera* via MPB-derived fungal culturing and stand inoculation trials. If a difference in pathogenicity is detectable, then the study should be conducted comprehensively. The F_1 generation of MPBs (surviving brood; not colonizing adults) could be sampled from several stands in the Interior and the Rocky Mountains. *G. clavigera* could be cultured from an appropriate number of the beetles collected per stand. Inoculation trials of both groups of *G. clavigera* (with various individuals) could be conducted at various inoculation intensities at multiple sites in both the Interior and the Rocky Mountains. If my hypothesis is correct and there is stronger, or less-constrained, selection for pathogenicity in the Interior, then significant differences in *G. clavigera* pathogenicity between regions should be apparent.

Future studies should examine the characteristics of weather systems associated with known times for and known results of MPB dispersal flights during the current epidemic. The massive summer of 2006 flight in northern BC deserves special attention. Such research may allow for the prediction of especially favourable conditions for long distance MPB dispersal.

Aukema *et al.* (2008) have developed a model that incorporates the spatio-temporal and climatic influences on the spread of MPB outbreaks. Given inputs of outbreak locations in one year, this model has a very high predictive accuracy for forecasting the location of outbreaks the next year, at a cell resolution of 12km x 12km. However, the authors found that the accuracy of two and three year forecasts declined rapidly as severe climatic events (autumnal cold snaps) caused significant MPB mortality. Thus Aukema *et al.*'s (2008) model works well under normal conditions for MPB outbreaks but stochastic events involving extreme summer or winter climate, and/or long distance dispersal, can invalidate this model. Hopefully, future MPB models will incorporate increasingly detailed data on MPB population structure, as well as data on stepwise and long distance MPB dispersal.

A major gap in MPB research is the lack of detailed understanding of the location of MPB populations endemic to BC. In the past, outbreaks have occurred in extremely isolated areas that are decades and/or hundreds of kilometers removed from the last outbreak. For example, the MPB is endemic to Vancouver Island and outbreaks were partially responsible for the decline of mature white pines on the island from the 1940's to the 1960's (Westfall, 2006). The next infestation on Vancouver Island occurred in 1984, encompassing 30 trees, and 10 trees were MPB-killed in 2005 about 10km southeast of Sayward (Westfall, 2006). These dates are synchronous with previous large-scale MPB outbreaks that have occurred in BC over the past century.

In contrast, in many regions it is not known if there are extant MPB populations. MPB outbreaks have been persistent in the New Hazelton region of north-western BC for the past half-century. Native MPB populations across BC have simultaneously expanded

in the current epidemic and one would expect the same in New Hazelton. However, there is no evidence for localized expansions in this region in the past decade; recent outbreaks have been caused by long distance dispersal flights. The presence of native MPBs in a region can be exceptionally variable and difficult to detect. It is possible that MPBs were extirpated from the New Hazelton region following the outbreak in the mid-1990's, that MPBs in this region simply have not responded in a similar fashion to other native MPB populations in BC, and/or that in this region endemic-caused damage has occurred at low levels and has not been detected.

4.8. Executive Summary

I met my objective: to determine the phylogeography of the MPB in Western Canada and to examine patterns of MPB population structure at varying scales to infer MPB dispersal. At the largest scale, my data indicated that the current epidemic consists of a group of populations connected by varying degrees of gene flow. Thus the current epidemic has arisen by the expansion of a number of native MPB populations. As these populations have expanded, they have provided migrants at a mostly regional scale which has caused the rapid escalation of severity ("fill-in" between geographically-separated outbreaks) within the epidemic area. Though the expansion of native populations has been largely synchronous, some populations (eg. Kamloops, Nancy Greene, North-Central BC) seem to have expanded more rapidly and have become significant sources of both local and long distance dispersers.

4.9. Management Recommendations

Outbreaks are a major factor influencing the evolution of the MPB as they maintain/increase the genetic diversity upon which evolutionary forces act. Thus, there is a positive-feedback loop in which climate change, by increasing the frequency and severity of MPB outbreaks, in turn generates higher levels of genetic diversity upon which MPB adaptation to a changing climate may occur. There are many other, probably more-important, factors that will influence the MPBs success in the 21st century: the distribution of host trees of suitable size and species; climate-induced MPB mortality; the effects of climate change on host tree resistance in various regions of BC and AB; whether or not the MPB has successfully colonized the jack pine forests of Canada. From an evolutionary and management perspective, it may be slightly beneficial to limit the frequency and severity of MPB outbreaks to limit MPB adaptations to a changing climate. It is also important to realize that the MPB exhibits considerable variation in life history traits, such as number of generations per year and cold tolerance, over its North American range; the MPB may exhibit high levels of phenotypic plasticity and strict adaptations to a changing climate may not be required.

The existence of genetic diversity gradients across the MPBs range is probably unimportant for MPB management. The reduced genetic diversity of MPB populations at the northern and southern portions of their range implies that these populations have reduced fitness (Reed and Frankham, 2003). Low genetic diversity can constrain the evolution of local adaptations that are important for the long-term persistence of populations. However, many species' success has not been constrained by reduced genetic diversity (eg. Frankham, 2005; Puillandre *et al.*, 2008). Indeed, the MPB has

high phenotypic plasticity and likely possesses much adaptive variation. Genetic diversity gradients in the MPB likely do not imply fitness gradients.

Climate change-induced range shifts may be very similar to Pleistocene patterns of range expansion. I suggest that studying the indicators and patterns of postglacial range shifts is of maximum predictive value for understanding how species will respond to a changing climate. For the MPB, by understanding the processes of postglacial recolonization in terms of dispersal trends and population genetics, we may be able to predict the range expansion and evolution of the MPB under a changing climate.

Landscape-level seral diversity, in combination with tree species diversity, is probably the best measure for preventing widespread MPB outbreaks. In the near future, complex landscape level models should aid forest managers in creating such diversity. In contrast, allowing landscape succession to take its course may allow non-pine understory species such as spruce, subalpine fir, and Douglas-fir to dominate many stands and create MPB-resilient landscapes (Burton, 2006). However, given my finding of a Northern and Southern MPB group, I have management recommendations specific to each group. In southern BC, by identifying the scale and magnitude of MPB dispersal, my results are probably best used in planning for the containment of outbreaks to regions.

In southern BC, the majority of MPB dispersal occurs over relatively short distances and most long distance dispersal seems to be funneled through weaknesses in geographic barriers. It is thus most pertinent to focus stand management (diversity management) at regional scales to prevent not only the expansion of outbreaks but also movements into susceptible stands in adjoining regions ("regional containment"). In a similar thread, outbreak management in southern BC should also be regionally-focused

in terms of strategic harvesting to contain outbreaks to a region. As an example, the Gray Creek valley that bisects the Purcell Mountains should be a major management focus in case of outbreaks in the Nelson region. Thus in southern BC, the general dispersal patterns I have identified are priority areas for containing outbreaks to regions.

In northern BC, creating management recommendations is made difficult by the apparently widespread occurrence of long distance dispersal events. Such widespread long distance dispersal is undoubtedly an artifact of the unprecedented scale and severity of the current epidemic. I suggest that planning for seral and species diversity across the northern BC landscape (not regionally) is probably the best option as geographic barriers and specific dispersal routes are relatively nonexistent in the north. Even in the north, seral diversity has the best chance of containing outbreaks to regions and preventing widespread epidemics.

MPBs are exceptionally difficult to manage (Safranyik *et al.*, 1999). Unfortunately, there is much evidence for historical MPB epidemics, in the obvious absence of human interference, whose scale is unknown relative to current epidemics (Schmid and Mata, 1996; Alfaro *et al.*, 2004). My study, by identifying MPB "dispersal highways," may facilitate better management of MPB epidemics; however, it is likely that MPB outbreaks will occur for the foreseeable future.

My study demonstrates that a population genetic approach to researching long distance MPB dispersal is highly efficient and effective (Kim *et al.*, 2006). I recommend that the British Columbia provincial government fund an annual standardized MPB genetic monitoring program. Samples could be collected using a standard protocol in the course of regular Ministry of Forests and Range forest health duties, adding value to

current programs (Nelson *et al.*, 2007c). Sampling entails two to three hours of labour per site. The genotyping costs of annual population genetic surveillance of MPB populations would range from $70 to $100 per sampling site. One hundred sites would provide excellent surveillance across the province. The annual cost for one hundred sites, including genotyping and labour for typing and data analysis, would be under $20,000. Such data would not only meet applied (management) objectives in terms of monitoring MPB population trends and dispersal, but would also provide internationally-unprecedented data for advancing theories of population genetics and evolution.

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