COMPARATIVE POPULATION GENETIC ANALYSIS OF FUNGAL ASSOCIATES OF THE MOUNTAIN PINE BEETLE (*Dendroctonus ponderosae)*

by

Lina Farfán

A THESIS SUBMITTED IN PARTIAL FULLFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

in

The Faculty of Graduate and Postdoctoral Studies

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

May 2014

© Lina Farfán , 2014

Abstract

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, has destroyed over 18 million ha of pine forest in Canada since 1999, the largest insect epidemic in recorded history. Fungal symbionts in the Ophiostomales (Ascomycetes) play an important role in the outbreak by reducing the tree defense response following beetle colonization, making its environment more conducive to the insect development. A better understanding of the population genetics attributes of the fungal associates could be important to elucidate their role and to explain epidemic patterns. We investigated the genetic structure of one of those fungal associates, *Leptographium longiclavatum*, sampled from 28 locations in western North America using 11 microsatellite markers developed from the genome of its closest relative, *Grosmannia clavigera*, another fungal symbiont of the MPB. We found that *L. longiclavatum* has a distinctive genetic population structure, and by using Bayesian clustering inference, we discovered the presence of three clusters that are concordant with geographic origin of the samples. In addition, we observed an apparent North-South pattern of genetic diversity consistent with the chronology of the epidemic. Structure output showed one cluster comprised of individuals from Northern Alberta where the beetle-fungus complex has been recently established, and a second cluster composed of individuals originating along the Rocky Mountains, and a third cluster was from populations in BC. High haplotypic diversity was found throughout the range sampled, a surprising result given that sexual fruiting structures have never been observed for this fungus. Consistent with recent introduction history, the least differentiated populations were located in northern BC and Alberta. We

observed a strong correlation in the genetic diversity pattern observed in *L. longiclavatum*, *G. clavigera* and *D. ponderosae*, as shown by a significant correlation in the genetic distance matrices amongst *L. longiclavatum*, *G. clavigera*, and *D. ponderosae*. This correlation and the similar north-south population structure of these interacting organisms suggest a joint population history consistent with the dependence of fungal dispersal on their bark beetle vector. These results can help clarify the roles and close relationships between the beetles and their fungal associates.

Preface

The theses presented here is the independent work by the author Lina Farfan which has not been published.

Table of Contents

List of Tables

List of Figures

Acknowledgements

I would like to thank Richard Hamelin for his patience, encouragement and mentorship in my research.

Many others helped me along the way, in particular the collaborators from the Tria project and Professor Colette Breuil and members of her lab who contributed with the samples. Many thanks to S. Beauseigle and B. Lai for your help in the lab.

To C. Tsui I am very grateful for all your help and for sharing your knowledge and expertise in the field of Fungal Pathology.

To my family, thanks for your support and encouragement, for your faith in me and for your patience to help me come along this way.

1. Introduction

1.1 The Mountain Pine Beetle Epidemic

The mountain pine beetle (MPB *Dendroctonus ponderosae* Hopkins) is a bark beetle that attacks several pine species and has the potential to cause large-scale outbreaks. Population build-ups have been recorded several times over the last century, most notably during the 1960s, 1980s, and 1990s. The latest outbreak is unprecedented in terms of the area covered as well as the latitudinal and longitudinal expansions (Carroll et al. 2003; Kurtz et al. 2008). To date, it is estimated by the BC Ministry of Forests (2013) that about 18.3 million ha of alpine and subalpine forests in BC have to some degree been affected by the beetle. The volume of wood affected by the beetle was approximately 164 million m^3, between 1990 and 2003, with an additional 70 million m^3 of wood volume damaged from 2003 to 2008. The latest projections estimate that between 2008 and 2015 another 30 million m^3 of wood will be affected (Helie et al. 2005).

In addition to these direct economic costs, the current MPB outbreak could also have broad ecosystem-wide impacts. The high rate of tree mortality reduces the levels of carbon fixation and increases future carbon emissions from decaying trees (Kurtz et al. 2008; Beardsley, 2008). Dead lodgepole pines, *Pinus contorta,* also increase the amount of fuel that could result in an increase the risk of forest fire events. Additional forest management challenges include the extensive presence of dead trees, which has a

negative effect on visual forest quality that affects its recreational use for tourism (Pederson 2004; Lee et al. 2005; Mcfarlane et al. 2006; and Stahl et al. 2006).

Losses from the MPB have had a broad economic impact. The recent outbreak of MPB in BC has forced the closure of 16 saw mills, the loss of approximately 11,000 direct forestry jobs and an additional 9,500 jobs that directly rely on the industry (Anonymous, 2011). Furthermore, this has also resulted in losses of between 4.5 and 6.5 billion dollars in stumpage fees that the industry pays to the provincial government in BC (Anonymous, 2011). Another source of economic pressure is for municipalities that reported the loss of 8 million dollars, a significant portion on municipalities' tax-based revenues and a hardship for local economies (Anonymous, 2011).

MPB is a native bark beetle that occurs throughout North America from Northern Mexico across most of the western United States to central British Columbia (BC), southern Alberta (AB), and as Far East as Cypress Hills in southwestern Saskatchewan (Carroll et al. 2004; Safranyik et al. 2010). Endemic MPB populations infest only a few damaged or stressed trees and are relatively harmless. However, given the right conditions, they have the potential to develop into large-scale outbreaks capable of causing tree mortality over vast forest areas (Safranyik and Carroll, 2006b). The MPB outbreak generally last six to ten years (Safranyik and Carroll, 2006b).

Environmental conditions are important and dictate the likelihood of an MPB outbreak. Outbreaks are usually preceded by adverse environmental factors, such as drought during hot and dry summers, which increase tree susceptibility to biotic stresses, including the MPB (Plattner, 2008). Conversely, extreme cold events and resource depletion could significantly aid in the reduction of MPB populations, including high

rates of egg and young mortality, and bring the beetle population back into an endemic phase (Samarasekera et al. 2012). Temperature is an important component; temperatures below –40^{o}C lasting more than three days normally act as a natural population control. Such conditions have not been observed for the past twenty years, possibly explaining one conducing factor to the current outbreak (Safranyik and Carroll, 2006a). Another factor that has favored the MPB epidemic is the availability of large areas of lodgepole pines of highly susceptible age (80-120 years-old). This has been caused in part because of continuous fire suppression which has raised the proportion of old trees that are highly susceptible to MPB attacks (Amman et al. 1990; Plattner, 2008). Furthermore, the historical limitations on harvesting lodgepole pine have increased the density and vulnerability of large forest areas to MPB outbreaks (Konkin and Hopkins, 2009).

During the current epidemic, the MPB has expanded further north and east than previously observed in BC and Alberta, attacking lodgepole and jack pine (*Pinus banksiana*) hybrids (Cunningham et al. 2011) in Alberta. Indeed, in Alberta, the MPB has become well established along the eastern slope of the Rocky Mountains and the west central region of the province. This northward and eastward expansion is an occurrence that has never been observed in recorded history of MPB outbreaks (Taylor and Carroll, 2004; Anonymous, 2012).

1.2 MPB life cycle and host range

Although the MPB can attack any pine species, it has primarily attacked lodgepole pine (*Pinus contorta* Doufl. Ex Loud var. *latifolia* Engelm) in BC mainly because of its profusion. Lodgepole pine in BC's Interior accounts for more than half of the growing

stock of the province (Anonymous, 2003). MPB can also attack a wide range of hosts, including jack pine (*Pinus banksiana* Lamb), western white pine (*Pinus monticola* Dougl. Ex Laws), ponderosa pine (*Pinus ponderosa*) as well as natural hybrids between the lodgepole and the jack pines boreal species (Safranyik and Carroll, 2006a; Rice et al. 2007; Rice and Langor, 2009; Cullingham et al. 2011).

Females select the host tree and bore through the bark into the phloem tissue and are joined by males (Aukema et al. 2009). After mating, females oviposit in niches chewed along vertical galleries emerging larva mine circumferentially around the bole where they develop into four larval instars and overwinter in the trees (Aukema et al. 2009). Pupae form the following spring and transform into adults that emerge in the summer. Emerging adults carry fungal spores in their mycangia (special body structures adapted to transport symbiotic fungi), as well as in their guts and on their exoskeletons. The sticky spores are passively dispersed inside the galleries where they germinate and generate mycelium that colonize the phloem and sapwood (Anonymous**,** 2012). This impedes the transportation of water and nutrients vital for the tree's survival and contributes to the beetle's ability to attack the trees (Anonymous, 2012)

Typically, the MPB overwinter as late-instar larvae before becoming adults during the following spring (Reid, 1962). Beetle development is temperature-dependent, helping with synchronizing the emergence and dispersal of mid-summer adult populations, resulting in massive attacks (Bentz et al. 1991; Powell et al. 2000; Six and Bentz, 2007; Aukema et al. 2009). Finally, new emerging MPB adults accumulate spores in and on their bodies by feeding on the fungi that have previously been vectored (Lee et al. 2006). Although there are numerous fungi that have been found associated with the MPB, some

blue stain fungi belonging to the order Ophiostomatales are believed to be specific MPB symbionts, because they are not found associated with any other beetles (Sinclair et al. 1987).

1.3 MPB fungal symbionts

The importance of the fungal associates has been known for decades, but the role of each of the interacting partner is still under investigation. The MPB has evolved a relationship that is believed to be symbiotic with several species of fungi (order Ophiostomatales, family Ophiostomatacea, genera Ophiostoma and Grosmannia (Safranyik and Carrol, 2006a). The fungi produce sticky spores on the apices of long necked sexual and asexual fruiting structures, and are also present on the galleries and pupal chambers of the beetles. Their adhesive nature and their production inside the beetle galleries make them ideally suited for dissemination by the MPB (Harrington, 1993; Six and Klepzig, 2004). Furthermore, previous accounts mention that there is an ongoing active and passive selection of the spores in the mycangia and exoskeleton of the MPB before they emerge to infect new trees (Harrington, 1993; Six, 2003; Safranyik and Carroll, 2006a).

These relationships are believed to be symbiotic, at least for some of the fungal associates-beetle interactions. One possible role of the fungi is to benefit the beetle's larvae by improving its nutritional food quality (Whitney et al. 1987). In the nutrient-poor wood environment, the fungal mycelium and spores represent an essential source of nutrients for the beetle's development and reproduction (Barras, 1973; Bridges, 1983; Goldhamer et al. 1990). In addition, this association helps to expand its population by limiting the growth of other harmful blue stain fungi to MPB (Barras, 1970; Richmond et

al. 1970; Yearian et al. 1972). The fungi may also help the beetle to overcome tree defense mechanisms (Berryman, 1972; DiGuistini et al. 2007), and to disrupt the tree phloem's chemical and moisture composition (Nelson, 1934; Reid, 1961; Webb and Franklin, 1978; Wagner et al. 1979). In exchange, the fungi can access the phloem and are consistently disseminated by the beetle (Six and Paine, 1999).

The fungi most commonly associated with the MPB are the ophiostomatoid *Grosmannia clavigera* (Robinson-Jeffrey and Davison) Harrington, and *Ophiostoma montium* (Rumbold) Von Arx and a few yeast species (Rumbold, 1931; Robinson, 1962; Safranyik et al. 1975; Whitney and Farris, 1970; Solheim, 1995; Six, 2003; Lee et al. 2005 and Lee et al. 2006). Nonetheless, there are other fungi associated with the MPB such as *Leptographium longiclavatum* (Lee et al. 2003), *Ceratocystiopsis* spp. and Entomocorticium spp. (Whitney et al. 1970; Solheim, 1995; Six, 2003; Lee et al. 2005, 2006).

Grosmannia clavigera is a primary invader of sapwood after beetle infestation (Solheim, 1995) and it is commonly isolated from the MPB mycangia, suggesting some co-evolutionary adaptation between the beetle and this fungus (Six, 2003). In artificial inoculation studies it was shown to be one of the most pathogenic of the fungi associated with the MPB (Solheim and Krokene, 1998; Lee et al. 2006; Rice et al. 2007). *Grosmannia clavigera* can kill mature or young lodgepole pines in the absence of MPB when inoculated at a density similar to that of a beetle mass attack (Yamaoka et al. 1995; Lee et al. 2006). When inoculated, they produce long lesions, occlude large areas, and significantly reduce moisture content in the infected trees after 48 weeks (Lee et al. 2006). Infections appeared to take longer for young trees when compared with older trees

(Kim et al. 2008). The fungus ability to infect and kill conifer seedlings has also been demonstrated in seedlings, although this is not observed in nature (Owen et al. 1987). Pathogenicity tests using different isolates of *G. clavigera* have shown intraspecific variations for characteristics such as lesion length and area occluded (Lee et al. 2006).

Ophiostoma montium is another fungal associate of the MPB that is also commonly isolated from the surface of the beetles (Lee et al. 2006). It causes sapstain in coniferous trees, logs and lumber (Kim et al. 2003); however, it does not seem to be pathogenic and was not found to cause tree death in inoculation experiments (Mathre, 1964; Basham, 1970; Strobel and Sugawara, 1986; Yamaoka et al. 1995). The relationship between *O montium* and the beetle has not been resolved and some have hypothesized that it may actually be an antagonistic relationship (Six and Klepzig, 2004).

Leptographium longiclavatum, another fungus consistently isolated from beetles and infested lodgepole pines was first described by Lee et al. (2005). This new species was first not observed by several other investigators possibly because of the close relationship and the morphological similarities between *G. clavigera* and *L. longiclavatum*. This could have resulted in misidentifications. *L. longiclavatum* can be differentiated from *G. clavigera* by careful examination of its morphological, phylogenetic and temperature growth characteristics (Lee et al. 2005). *Leptographium longiclavatum* has well-developed long conidiophores and elongated conidia that are carried in moist drops at their apices; by contrast, *G. clavigerum* has small, obvate and cylindrical conidia. In addition, *G. clavigera* and *L. longiclavatum* have also been differentiated by DNA multilocus phylogeny which yielded two separate clades on a phylogenetic tree using five independent loci (Roe et al. 2010). Also, their temperature

characteristics help to separate them; *G. clavigera* grows better at 20 and 25 °C than *L. longiclavatum* but neither grows above 30 °C (Rice et al. 2008). The optimal growth for both fungi is between 20-25 °C and they both survive at -20 °C (Rice et al. 2008). The pathogenicity of *L. longiclavatum* was demonstrated on artificial inoculation of lodgepole pine where it causes necrotic lesions around inoculation points both on the phloem and the sapwood (Lee et al. 2006). The length of the necrotic lesions for *L. longiclavatum* were smaller than those of trees inoculated with *G. clavigera,* however, they were also capable of causing infection on trees (Lee et al. 2006). In addition to the pathogenic studies made in lodgepole pine, MPB fungal associates caused lesions in the sapwood of jack pine, lodgepole pine x jack pine hybrids and lodgepole pine in northern AB when artificially infected either alone or in combination (Rice and Langor, 2009). This confirms that these fungi can probably infect these tree species in nature if the beetles were to expand their range beyond the hybrid zones and into pure Jack pine stands (Rice et al. 2007).

1.4 Population genetic studies in the beetle-fungal symbiont systems

The aim of population genetic studies is to attempt to describe the distribution of genetic variability within and among populations of species as well as the microevolutionary processes occurring within them (McDonald and Linde, 2004). In addition, population genetic studies assist in the analysis of genetic changes in populations over time and space (McDonald and Linde, 2004). Various parameters such as gene flow and migration, population size, and extent of sexual reproduction can be measured and quantified. Population genetic studies can provide an insight into genetic

processes such as genetic drift, migration and distribution patterns, which could be affected by the fungal biology and epidemiology and by the fungal-beetle relationship. Therefore, an analysis of the population genetic characteristics of the MPB and its fungal associates provides a very promising approach to further our understanding of this relationship.

Bark beetle species live in close association with their fungal associates since fungi are uniquely adapted to dissemination by insects (Zhou et al. 2007). This suggests that co-evolutionary processes are taking place to ensure that the benefits of the symbiotic relationship will be maintained. The relationship of the beetles and their fungal associates has developed in highly variable and dynamic environments (Bleiker and Six, 2008). Fungal associates have to adapt to this changing habitat and have to ensure their dissemination by sporulating inside the pupal chambers in synchronicity with emerging adults (Bleiker and Six, 2008). Therefore, fungi that are able to extensively colonize phloem will most likely increase their presence on additional pupal chambers, thus improving their chances of being transmitted to new trees by the beetles (Bleiker and Six, 2008). Trees also play an important part in the success of the beetle-fungi interaction since changes in moisture, nutrient level and temperature over time may influence fungal growth and survival (Bleiker and Six, 2008).

MPB populations were clustered into southern and northern populations (Bartell et al. 2008; Samarasekera et al. 2012). This structure is likely the result of post-glacial re-colonization patterns, as well as the climate-driven population dynamics of the current epidemic, which has flattened the population structure in the most recently colonized northern populations (Samarasekera et al. 2012). Importantly, the pattern of the recent

northeastern migration of the beetle was uncovered. The most likely source of the new northward expansion was BC, as indicated by low level of genetic differentiation. Surprisingly, there was no apparent severe loss of genetic diversity that often is the result of population bottleneck following introductions. This indicates that these newly founded populations have the full evolutionary potential of the original populations.

The population structure of *G. clavigera* has been investigated by using amplified fragment length polymorphism (AFLP); the fungus exhibited genetic variability with some population clustering that corresponded to geographic origin (Lee, 2007). Microsatellite markers developed by Tsui et al. (2012) were used to further explore the fungal pathogen genetic diversity across various geographical origins. This study included more populations after the expansion of the beetles further north and across Northern Alberta, but still showed a very similar population structure as observed in Lee et al. (2007). Again, the clusters corresponded closely to the geographical origin but also revealed a high genetic diversity for the *G. clavigera* populations, grouping these populations in four recognizable genetic clusters (Tsui et al. 2012). These clusters roughly correspond to the north-south population pattern of the beetle population structure (Bartell et al. 2008; Tsui et al. 2012). Similarly *G. clavigera* was differentiated in northern and southern clusters using DAN sequencing of multiple genes (Roe et al. 2011). In addition, a competitive exclusion along a latitudinal gradient between *G. clavigera* and *L. longiclavatum* suggests that the latter becomes more common in northern populations; thus fungal community composition patterns could be dynamic and help the development of the MPB in these geographical areas (Roe et al. 2011).

The role of *L. longiclavatum* in the MPB fungal interactions has not been fully established (Lee et al. 2005). However, its phylogeny, morphology and physiological similarity to *G. clavigera* suggest that the relationship between MPB and *L. longiclavatum* could be functionally similar to that between *G. claviera* and MPB (Roe, 2011). In addition, *G. clavigera* and *L. longiclavatum* are affected by changes in latitude since the abundance of the former decreases while the latter increases in abundance with increasing latitude (Roe et al. 2011). Moreover, the study of *L. longiclavatum* population structure may help to understand some of the characteristics of the outbreak, such as population size, founder effects and migration patterns. In particular, it is important to determine if *L. longiclavatum* has similar population parameters and population history as *G. clavigera*. By contrasting their attributes, we may be able to better understand the roles of these fungal associates and their importance in the epidemic.

Microsatellite markers represent an important tool for population genetic studies. They are simple sequences composed of repeats of short 1 to 6 base pairs (bp) nucleotides (Keiper et al. 2006) commonly used as markers as they are codominant, plentiful, and randomly distributed in most eukaryotic genomes (Weber and May, 1989). Microsatellites are prone to be extremely polymorphic due to changes in the number of repeating units (Enkerli et al. 2001). Microsatellite amplification needs a small quantity of genomic DNA utilizing a pair of PCR primers that bind to the microsatellite repeat (Keiper et al. 2006). Likewise, microsatellite markers have several advantages such as they are technically easy to control, small quantities are required for PCR development, they are unambiguously scorable, results are highly reproducible, and they can be used for genome mapping (Weber and May, 1989; Morgante and Oliveiri, 1993;

Gianfranceshi et al. 1998). Finally, microsatellites possess a large number of alleles so that they can give a high probability to differentiate individuals based on their microsatellite profiles. They are among the most widely used markers for the study of molecular ecology and population genetics (Groppe, 1995; O'Connell, 1997; Perez-Lezaum, 1997; Powell, 1995).

There are many analysis tools that have been developed in recent years to analyze genetic data at the population level. Among the most powerful ones, Bayesian clustering methods that use multilocus genotypes have been used to assign individuals into clusters of individuals with similar genetic profiles, thereby illustrating populations (Paetkau, 1995). This approach categorizes random mating groups with very few assumptions with regards to population borders (Pritchard et al. 2000; Dawson and Belkhir, 2001). Methods such as Bayesian clustering make population grouping lessen the Hardy-Weinberg (HW) and gametic disequilibria (GD) within the clusters (Manel, 2003). New approaches can now exploit the spatial coordinates of samples. By having a matrix of samples for which genotypes and GPS coordinates are known, it is possible to infer dispersal distance and pattern for the individuals.

The aim of this study was to investigate the population genetic structure of *L. longiclavatum* using simple sequence repeats to infer population structure, gene flow and migration, distribution, and geographic origin. My hypothesis is that this fungus has a similar population structure as its sister species *G. clavigera* since both are shaped by similar evolutionary and demographic forces driven by the beetle vector. My second hypothesis is that low genetic diversity and high genetic linkages are expected since only the asexual (anamorph) stage of this fungus is known.

2 Materials and Methods

2.1 Sampling

A total of 266 *L. longiclavatum* isolates were obtained over 5 years from 28 locations where MPB was active in British Columbia (BC), Alberta (AB), and USA (Fig 1). Fungi were isolated by placing beetles directly onto agar, or by locating wood disks obtained from MPB galleries within infected pine trees. The plates were incubated for 8 days at 15 °C temperature until fungal growth was evident. Pure cultures were obtained by transferring these cultures onto new plates. Fungal species were identified by culture morphology and by sequencing the ITS gene. Isolates that belonged to *L. longiclavatum* were further cultured to obtain pure single genotype isolates, either by collecting single spores or by transferring single hyphal tips. The sampling location, isolation source (beetles or phloem) and year of collection are reported in Table 1.

2.2 DNA Extraction, PCR, And Microsatellite Genotyping

DNA was extracted from pure cultures of each isolate following the protocols previously published by Lee (2003), and Roe (2010). PCR amplifications were carried out in 10µL reactions for BC samples and 10µL reactions for AB samples. Reaction mixture for BC and USA samples were 0.71 x PCR buffer, 25 mM each dNTP, 0.71 mM MgCL2, 0.71 µM DMSO, 0.71 µM primer, 0.35 µM of each primer (IRDye ^{R} ; LI-COR), 0.35 U of paq5000 ^{TM} DNA polymerase (Stratagene), and 5 ng of template DNA. Reaction mixture for AB samples 1XPCR buffer, 200 µM each dNTP, 1 pmol of each primer, 0.5 µl labeled M13 (IRDye ^{R} ; LI-COR), 1 µ of paq5000 ^{TM} DNA polymerase (Stratagene) and 20 ng of template DNA.

Conditions for PCR amplifications were as follow: 94 °C for 3 minutes, 94 °C for 35 seconds, 58 °C for 30 sec, 72 for 7 min and 34 cycles, 58 °C.

Marker development was accomplished using 60 SSR primers that were previously developed by Tsui (2009) for *G. clavigera*, a closely related species of *L. longiclavatum*. A total of 11 SSR loci were selected because they were polymorphic in a subsample of 14 samples selected from different regions in BC. Genotyping was performed on a LI-COR 4300 DNA analyzer on denaturing polyacrylamide gels with molecular size standards 50-350 bp (IRD-700/800 dye) (LI-COR) and analyzed using the LI-COR SAGA software version 2 (Fig, 2). The strain Kw 1436, a *L. longiclavatum* holotype was used as reference in every PCR to ensure the consistency of genotyping through the study.

2.3 Population genetic analysis

For each isolate, a table of isolates x microsatellite marker was generated. Various measures of genetic diversity were computed for each population, including the number of alleles, allelic frequencies and gene diversity (*ie*, expected heterozygosity) across all loci using GenAlEx6 (Peakall and Smouse, 2005). To quantify the proportion of the population that is clonal, the number of unique haplotypes was obtained and divided by the total number of isolates. After clone identification, the data was clone-corrected by removing all the repeated genotypes from the set by considering the multilocus haplotypes in a geographic population as clones.

We performed an Analysis of Molecular Variance (AMOVA) to estimate the analogs of the fixation indices, F-statistics, as well as the hierarchical partitioning of genetic variability among populations and regions (Peakall and Smouse, 2005). Statistical

significance was tested by performing 1000 permutations of the data and subsequently comparing the observed with the simulated distribution. A principal component analysis (PCA) was completed by using genetic distances amongst populations derived from the multiple loci data; this analysis allows reducing the dataset to vectors and plotting populations along these vectors; it is a powerful approach to identify major patterns within the data (Peakall and Smouse, 2005). Mantel tests permit the analysis for a statistical relationship between the data of two distance matrices. We used it to test for correlation between genetic and geographic distance matrices. This allows a test of the isolation-by-distance hypothesis (Peakall and Smouse, 2005).

In order to test for random mating, a signature of sexual reproduction, the index of association (I_A) was computed using Multilocus 1.3b (Agapow and Burt 2001). The I_A measures linkage disequilibrium among loci within populations by taking into account shared alleles across loci. When there is no linkage disequilibrium the I_A approaches zero for outcrossing populations (Maynard and Smith et al. 1993); and as linkage disequilibrium increases in asexual or inbreeding populations, I_A increases (Hedrick, 2005). The null hypothesis of random mating was calculated by comparing the observed data with 500 randomized data sets (Agapow and Burt, 2001).

2.4 Population Structure

The population structure and genetic clustering of *L. longiclavatum* was analyzed through various approaches to construct models to test for population genetics and ecology processes (Beaumont and Rannala, 2004; Tsui, 2012). A Bayesian analysis that minimizes deviation from equilibrium within clusters was implemented in the software STRUCTURE.

This analysis is useful to identify clusters of individuals independent of their geographic origin. STRUCTURE 2.3, a software that implements a model-based clustering method, was used to analyze the association of individual isolates from different geographical locations to clusters and to test for admixture (Pritchard et al. 2000). The algorithm estimates the allele frequencies in each group and the population relationships for every individual fungi, given the number of clusters (K) and assuming Hardy-Weinberg and linkage equilibrium within clusters (Hubisz, 2009). It uses a Monte Carlo Markov Chain (MCMC) to group individuals into distinct K populations that minimize HW disequilibrium as well as gametic phase disequilibrium between loci within groups by means of including prior information on the geographical location of populations (Pritchard et al. 2000; Hubisz, 2009). Likewise, this model permits the use of correlated frequencies and for individuals to have mixed ancestry (Tsui, 2012). The number of clusters was set from 1 to 15, with each cluster replicated 6 times after a burn in period of 100,000 generations followed by 900,000 generation to check the convergence of likelihood for each K value. The admixture model, in which the fraction of ancestry from each cluster is estimated, as well as the spatial sampling location for each population was tested for every individual sample (Tsui et al. 2009). We used STRUCTURE Harvester (http://taylor0.biology.ucla.edu/structureHarvester) to estimate the optimal value of K by selecting the value with the lowest ΔK (Evano, 2005).

Nei's (1978) unbiased genetic distance was calculated among all pairs of sampled populations among all locations in GenAlex6. The distance matrix was used in two analyses: a principal coordinates analysis (PCoA), implemented in GenAlEx, and a neighbour joining tree implemented in PHYLIP. The tree was built with the *Neighbour* and *Consence* options as implemented in PHYLIP with the aim of evaluating the robustness of grouping among

populations. In addition, a microsatellite analyzer (MSA) was used to obtain one hundred bootstrap pseudoreplicates (Dieringer and Scholtter, 2003). Pairwise F_{st} (Weir and Cokerham, 1984), a measure of genetic differentiation among populations, was calculated and analyzed by using a randomization test with 1000 iterations using Arlequin v. 3. 11 (Excoffier, 2005). These analyses were performed concurrently since they have different prior assumptions and distributions.

2.5 Individual assignment and migration pattern

GENECLASS analysis allows us to allocate or exclude individuals of the study populations for various predetermined genetic criteria on the basis of multilocus genotype profile (Piry, 2004). This was used to calculate the probability of assignment from individuals of the sampled populations. The method of Paetkau (2004) was used to produce 1000 simulated individuals per geographic group and comparing the observed and expected distributions. Standard criterion was calculated using the probability assignment of individuals to all sampled populations (Rannala and Mountain, 1977). The assignment of individuals was completed when a selected group has the highest probability of being the source of this particular individual.

3 Results

3.1 Genetic variation and linkage disequilibrium

There was high gene diversity (H*e*) for the total sample and for each population (219 individuals, after clone-correction of the dataset, and retaining only populations with more than five individuals). Gene diversity ranged from a low of 0.233 in Yoho to a high of 0.471 in Burns Lake (Table 1). In general, BC populations have greater gene diversity (0.471 – 0.317; average=0.372), followed by those from the Rocky Mountains (0.356 – 0.264; average=0.309) and Northern Alberta (0.306 – 0.251; average=0.260) (Table 1). Allelic richness values ranged from 1.636 in Yoho to 2.909 for Merrit and Kamloops, with averages also higher in BC (2.654) than in the Rocky Mountains (2.146) and Northern Alberta (2.091) (Table 1).

The number of alleles per locus varied from three and eleven and gene diversity per locus ranged from 0.748 and 0.017 (Table 2). The proportion of total genetic diversity attributed to differences among populations ranged from 0.086 to 0.320 for the 11 loci, with overall average of 0.0184 (Table 2).

Among the 266 isolates that were studied, we recognized 205 unique multilocus haplotypes. Therefore, the clonal fraction of the population represents 23%. The index of association (I_A) did not statistically deviate from zero in 12 of the 17 populations (Table 1). The other five populations Burns Lake, Quesnel, Williams Lake, Kamloops, Merrit, and Kakwa yielded I_A values significantly different from zero, indicating populations that are not undergoing random mating, possibly due to a clonal expansion or assortative mating.

3.2 Population structure and differentiation

An analysis was performed using STRUCTURE for 235 samples that were included in the clone-corrected data, but included all sampled locations, even those with sample sizes smaller than five. The most likely number of clusters as determined by STRUCTURE harvester was three. There is some association between geographic origin and cluster assignment. Samples from North Alberta formed a clear dominant cluster with little admixture (blue in Fig. 3), while samples from BC and the Rocky Mountains displayed evidence of admixture, with some dominance of different clusters in BC (green in Fig. 3) and the Rocky Mountains (yellow in Fig. 3).

The PCoA was completed using 219 individuals (clone corrected data and populations with more than 5 individuals) grouped the *L. longiclavatum* populations generally along broad geographic lines (see list of population origin in Table 1). The BC populations generally clustered on the upper right quadrant of the first PCoA axis, while the North Alberta populations clustered on the bottom right of the first quadrant of the PCoA axis (explaining 38.90% of the variation). Finally, the six Rocky Mountain populations clustered within the two left quadrants of the first PCoA axis (explaining 23.60% variation) (Fig 3).

A Neighbour Joining tree generated using Nei's unbiased distance matrix also grouped the populations along geographic lines (Fig 4). Interestingly, the population from Valemount, situated on the western side of the Rocky Mountains, is on a branch intermediate between the Rocky Mountains and BC, mirroring its geographic location. A similar position of the Valemount population was observed for the PCoA analysis (Fig. 3)

Overall, the analysis of molecular variance (AMOVA) performed on the 17 populations showed that 10% and 90% of the genetic variation was attributed among and

within populations, respectively (Table 3). The among populations genetic diversity was highly significant. In addition, an AMOVA was performed separately for all populations to further study the differentiation among the clusters identified by the STRUCTURE analysis. The AMOVA partitioned the total variation to 9% among clusters, 3% among locations within clusters and 89% among individual isolates within populations respectively (Table 4).

There were less significant pairwise genetic differentiation (F_{ST}) amongst populations within the BC and the Rocky Mountain clusters (Table 5) compared to other groups. For example, there were a large number of significant F_{st} in pairwise comparisons between populations from BC and Northern Alberta, between BC and the Rocky Mountains and between the Rocky Mountains and Northern Alberta (Table 5). This confirms the general clustering patterns observed in the PCoA (Fig. 3) and NJ analyses (Fig 4). The BC population showing the largest F_{st} values in comparison with other populations was in Kamloops, in the Ponderosa Pine biogeoclimatic zone. The population from Valemount had no significant F_{st} values with any BC populations, but significant differences with Northern Alberta and Rocky Mountain populations.

The populations with the highest pairwise genetic differentiation were Fairview (Northern Alberta cluster) and Yoho (Rocky Mountain cluster) (0.384 P<ß0.01) and the lowest differentiated populations were Quesnel (BC cluster) and Kakwa (Rocky Mountain cluster) (-0.004 P>0.05). Therefore, these results showed similar genetic structural patterns for the studied populations considering that most differentiated populations are opposite located - Fairview in the northern and Yoho in the southern portion of the sampled range. In addition, the least significant differentiated populations were located in the North regions of

BC and AB. As a consequence, we could infer that there is a direct effect of geographical isolation on the genetic diversity for the populations previously described.

3.3 Individual assignment, migration and demographic history

The GENECLASS analysis was conducted with 219 individuals from the 17 sampling location. This analysis assigns individual fungal isolates to populations or the clusters identified by the STRUCTURE analysis (Fig, 5) based on genetic profile expected for the given group. GENECLASS analysis produced a high rate assignment to their original location or to their cluster for populations from BC (Table 4). The overall assignment to the original location was low (26%). Nonetheless, the average rate of assignment to their original population was high for populations sampled in BC (40%) and the Rocky Mountains (34%), but low for the populations from northern Alberta (4%) (Table 4). Almost all individuals from BC were assigned to the BC cluster, while only 38% and 19% of the Rocky Mountains and northern Alberta individuals were assigned to their respective cluster.

Establishing the pathways for gene flow was an important element in understanding the dynamics of the fungus with regards to the beetle expansion during the outbreaks. We calculated pairwise F_{ST} measures (Table 5) to assess the magnitude of gene flow between populations and determine whether gene flow is higher between geographically close populations. Significant population pairwise F_{ST} was common across geographic groupings, suggesting strong genetic differentiation (Table 5). With the exception of populations "Kamloops" and "Sparwood" within the corresponding "BC" and "Rocky" clusters, pair-wise genetic differentiations (F_{ST}) between populations generally were non-significant within the three geographic groupings, supporting their genetic similarities (Table 5). These results

indicate that extensive gene flow occurs among populations within genetic cluster but is restricted between populations in different clusters.

4 Discussion

4.1 Genetic Diversity

Fungi have a mixed mating system, with sexual and asexual cycles occurring in various proportions in different species. Some fungal species, such as *L. longiclavatum*, do not have a known sexual cycle. Such putatively asexual species usually have reduced genetic diversity and are generally composed of clonal lineages. Our study shows high levels of expected heterozygosity amongst the 17 populations of *L. longiclavatum* in BC and Alberta. This high genetic diversity resulted in 205 unique haplotypes out from the 266 individuals sampled, and therefore less than ¼ of the sampled individuals were part of the same clone. For a completely asexual fungus, this proportion should be 100%. Previous studies in MPB fungal associates exhibited similar levels of genetic diversity; for instance, Tsui et al. (2012) reported a total of 250 unique haplotypes in *G. clavigera* with a comparable proportion of clonal lineages.

The gradient of gene diversity observed, from highest in populations belonging to the BC cluster to lowest in the northern Alberta cluster is concordant with this fungus having undergone a loss of diversity during its northward migration. This could result in a lower adaptive potential in the northernmost populations. However, further analysis would be required to properly address this question. The use of additional markers that sample genes with putative adaptive functions would be one way to test this. It is possible that this gradient is transient and only present because of the sudden population outbreak. The high level of gene flow estimated in our study derived from pairwise comparisons would suggest that this diversity gradient could be flattened out by migration.

The Neighbour Joining tree for the 17 populations analysed showed a pattern that is consistent with increassed genetic distances with genographic distances. The tree also displays short internal branches (Fig 4). This suggests low levels of divergence between haplotypes. There were, however, higher levels of divergence for the northern Alberta and the Rocky Mountains populations. This also reflected a congruent trend of reduced genetic diversity declining as the populations advanced into northern Alberta and the Rocky Mountains (Fig 5). This tree is largely congruent with that produced for *G. clavigera*, a fungal associate that shares similar characteristics and the same hosts and vectors as *L. longiclavatum* (Tsui et al. 2013, Lee et al. 2006).

One population that shares intermediate position both geographically and in the genetic analyses is Valemount (this work and Tsui et al. 2012). Both the PCoA and the Neighbour Joining trees placed this population intermediately between the BC and Rocky Mountain clusters. Valemount has been implicated to be the corridor that facilitated *G. clavigera* expansion from BC eastward into northern AB (Tsui et al. 2012). This study demonstrates the presence of high level of genetic relatedness in *L. longiclavatum* populations specifically among Valemount and the populations along the Rocky Mountains Trench. More importantly, this study also identified Kakwa as an important corridor for its high genetic relatedness to eight populations in the recent epidemic populations. This pattern may be also explained by the low barriers to migration that exist along the Rocky Mountains Trench as a result of its proximity to the other passes (Tsui et al. 2012) which could have served as sources for the spread of *L. longiclavatum* to locations north and east of the Rockies.

It should be pointed out that the markers used in this study were generated by screening a panel of individuals for polymorphism. Therefore, we cannot provide estimates of

the genetic diversity across the genome of *L. longiclavatum* or compare it with other fungi or organisms since our markers were selected for polymorphism in a panel of isolates. Other studies (Roe et al. 2011; Tsui et al. unpublished) using sequence-based markers are finding lower genetic diversity in *L. longiclavatum* than in *G. clavigera*. However, microsatellite markers are powerful in generating genetic profiles that yield a high probability of differentiating individuals and assigning them to clusters and for analysis of population structure and demography.

4.2 Sexual reproduction

No significant linkage disequilibrium was observed in 12 out of 17 populations or in the total sampling, which suggests that sexual reproduction occurs in these populations. Sexual reproduction in *L. longiclavatum* has not been reported in the literature since fruiting bodies have not been observed so far in nature or in laboratory experiments (Lee, 2005). Although our analyses found that the signature of sexual recombination can be observed in the data, it is also possible that it occurred in the past and not in the contemporary populations, or that sexual reproduction occurs cryptically and is simply not observable or detected. One possibility is that sexual reproduction has occurred in the ancestral population but has since stopped in the current outbreak populations. An alternative possibility is that there is cryptic sexual reproduction, either occurring at spatial or temporal scales that are distinct from the asexual cycle. The study of sexual reproduction in these fungi has been further investigated by studying their mating type genes. These fungi are believed to be heterothallic (requiring mating between strain with different mating type alleles for sexual reproduction to take place). Two mating type genes are present in *L. longiclavatum* and they occur in different individuals, confirming their heterothallic nature (Tsui et al. 2013). In

addition, the frequency of the two mating type alleles is statistically not different from a 1:1 ratio, indicating a sexually reproducing population at equilibrium. This observation then favors the hypothesis of a cryptic sexual stage, as ancient reproduction would result in mating type gene disequilibrium (Tsui et al. 2013). This is not particularly surprising as similar results were reported for *G. clavigera* in which sexual recombination has been suggested for different populations (Tsui, 2012) but fruiting bodies have been rarely detected or observed in the field or in the laboratory. In addition, the signature of sexual reproduction has been observed in the genomes and at mating type loci in *G. clavigera* (DiGuistini et al. 2011, Tsui et al. 2013).

Although a large fraction of the populations exhibit the signature of sexual reproduction, almost one quarter of the samples were genetically identical and therefore are likely clonally propagated through the asexual cycle. Therefore, asexual reproduction clearly plays an important role in the spread of this fungus and asexual conidiophores producing conidia can be observed in the MPB galleries.

4.3 Population structure

Leptographium longiclavatum in Western Canada has a distinctive genetic population structure. We identified the presence of three clusters that roughly mirror the geographic origin of the populations. These clusters show a highly significant level of genetic differentiation, an indication that they are maintained by barriers to gene flow or that they represent demographic history followed by isolation. The pattern of genetic diversity supports the hypothesis of multiple epicenter of the current MPB epidemic and is not consistent with a single epicenter (Aukema et al. 2009). Given the total dependence of this fungus on the beetle

vector, the population structure of *L. longiclavatum* is expected to be strongly influenced by its vector. Deviation from such expectation could help us understand some of the unique features of the fungal associates.

Analyses of *G. clavigera* reported a very similar population structure, with the presence of four genetic distinct clusters, three of which overlap with those reported in the current study (Tsui et al. 2012; Lee, et al. 2006; S. Lee et al. 2007). Roe et al. (2010) suggested similar levels of population structure and an equal distribution into southern and northern populations for *G. clavigera* and *L. longiclavatum* using multilocus sequence data. In addition, the north grouping reported by Tsui et al. (2012) (similar to Northern AB population in this study, except for Valemount) and the Rocky Mountains population (similar for both populations of *G. clavigera* and *L. longiclavatum*) have a significant differentiation with the reported multilocus data (Roe et al. 2010).

Interestingly, a somewhat similar population structure was reported for the beetle. Samarasekera et al. (2012) reported a significant population genetic structure for the MPB identified by distinctive north-south clustering patterns. However, the finer pattern of clustering observed in *L. longiclavatum* in this and other studies was not found in the beetle populations. This could be the consequence of the mixed mating system found in fungi, a phenomenon that occurs in fungi but not beetles. Asexual clonal expansion by the fungi could be the cause of such fine-level population structure, leading to pronounced genetic drift. It will be interesting to compare the population structure of *L. longiclavatum* and *G. clavigera* with that of *O. montium*, a fungal associate of the MPB that reproduces sexually.

Isolation-by-distance was observed for *G. clavigera* (Tsui et al. 2012), and MPB populations with a clear north-south pattern (Samarasekera et al. 2012). However,

geographical separation reflected in genetic differentiation may indicate obstacles to gene flow influenced by landscape features such as high elevation, climatic barriers, or patterns of past MPB epidemics (Safranyik and Carroll, 2006b; Tsui et al. 2012; Samarasekera et al. 2012). The patterns of genetic diversity observed in the beetle and its fungal associations agree with the hypothesis of postglacial expansion of the beetle and its fungal associates, which predicts that the southern populations are the oldest ones and colonization proceeded northward after the last glaciation event (Abbott and Brochmann, 2003; Beatty and Provan, 2010; Samaradekera et al. 2012). Our analysis is consistent with both ancient colonization of recent expansion. The assignment analysis suggests multiple origins in populations from southern BC, and the Rockies; this could be explained by the postglacial expansion of the beetle-fungal associates complex from several regions into the northwest US and Canada (Mock et al. 2007). But more recent patterns probably related to demographic events are clearly supported by our data. In the recent outbreak MPB experienced a rapid and effective migration into northern Alberta along with its fungal associates. The populations in the northern portion of the range, which have been recently introduced, had the lowest genetic diversity and marked differentiation compared to other populations. Populations from northern BC and northern Alberta were not significantly differentiated and shared patterns of genetic diversity. This low genetic diversity in the northern populations may be explained by the possibility that the MPB had experience a loss of genetic diversity due to its dissemination after a major outbreak as well as its limited mobility, thus restricting its genetic pool to a specific geographic area (Mock et al. 2007 and Berryman 1986). Moreover, the MPB outbreaks in the northeastern regions are believed to be the result of long distance dispersal (Samarasekera, 2012).

These findings coincide with the study on *G. clavigera* (Tsui et al. 2012) and with MPB in northern Alberta, which could indicate a genetic "bottleneck" following its recent introduction (Samarasekera et al. 2012). There was evidence in the MPB that northern BC populations originated from established populations in northern BC. Population sampling in the beetle was far more extensive than was possible for the fungal associates, where isolation and pure culturing makes sampling more challenging. Our current results indicate that the northern Alberta populations were derived, but the actual source is not clearly indicated. It is possible that a complex scenario of admixture is the most likely explanation.

It is possible that additional genetic clusters could have been found with a finer or a more extensive geographic sampling. In particular, southern populations in the US would be expected to generate additional clustering, as was observed in *G. clavigera* (Tsui et al. 2012). An additional cluster was found in southern BC and the most differentiated population for this study was found in Kamloops, southeastern BC, within the BC cluster (Tsui et al. 2013). It is likely that other similar clusters would have been found in other additional southern BC populations. If this were the case, it would provide even stronger support for the multi-center origin of the outbreak.

5. Conclusion

Overall, we found a mirror image of the genetic population structure of *L. longiclavatum* and previously published population structure in *G. clavigera* and their vector the MPB. This structure is consistent with these organisms undergoing similar demographic patterns of expansion and genetic drift. Similar geographic barriers probably shape these populations, in particular along an east-west gradient that comprises the mountain ranges. As well, there is a signature of latitudinal gradients affecting the genetic structure. This has been observed in this study and in previous studies of *G. clavigera* and *D. ponderosa*.

Future research could address issues of adaptation of the fungal associates by sampling genes that may be under selection and by looking at the phenotypes of the fungal associates. This study highlighted that there are different genetic populations of *L. longiclavatum*. However, we still do not know if this translates into differences in adaptation and fitness. The ability of these fungi to sexually recombine and produce novel genetic combinations could lead to selection and adaptation that would increase fitness to varying climate and/or hosts. One of the challenges associated with climate change is the possibility of increasing pest outbreaks. A better understand of the adaptive capacity of beetle-fungal associations will help predicting the outcome of these biological interactions.

6. Tables and Figures

Table 1. Summary information for 17 populations of *Leptographium longiclavatum* sampled from beetles or pines and genotyped at ten microsatellite loci

Sampling location	Origin of isolates	Collected by	Year of isolation	N	Haplotypes	H[a] (s.e.)	Allelic Richness[b]	I_A/P-value[c]
Burns Lake, BC	*Pinus contorta*	Jae-Jim, K.	2004	9	9	0.471 (0.070)	2.818 (0.377)	1.717/0.002**
Prince George, BC	*Pinus contorta*	Jae-Jim, K.	2004	18	17	0.317 (0.084)	2.545 (0.578)	0.087/0.282
Quesnel, BC	*Pinus contorta*	Jae-Jim, K.	2004	12	11	0.365 (0.072)	2.455 (0.390)	0.479/0.038
Williams Lake, BC	*Pinus contorta*	Sangown, L.	2004	22	21	0.371 (0.086)	2.545 (0.390)	0.331/0.002**
Kamloops, BC	*Pinus contorta*	Jae-Jim,K. Sangown, L.	2004	26	22	0.334 (0.074)	2.909 (0.513)	-0.030/0.002**
Merritt, BC	*Pinus contorta*	Kadempour, L.	2009	15	14	0.429 (0.068)	2.909 (0.415)	0.265/0.002**
Fairview, AB	*Pinus contorta* / *Dendroctonus ponderosae*	Boone, C.	2007/2008	15	14	0.251 (0.064)	2.273 (0.407)	0.362/0.11
Tumbler Ridge, BC	*Pinus contorta* / *Dendroctonus ponderosae*	Boone, C.	2007/2008 /2010	21	17	0.278 (0.067)	2.182 (0.325)	0.254/0.07
Grande Prairie, AB	*Pinus contorta* / *Dendroctonus ponderosae*	Rice A. and Roe A.	2007/2008	23	20	0.260 (0.071)	2.091 (0.251)	0.144/0.144
Fox Creek, AB	*Pinus contorta* / *Dendroctonus ponderosae*	Rice A. and Roe A.	2007/2008	17	15	0.251 (0.075)	1.818 (0.263)	0.201/0.098

Kakwa, AB	*Pinus contorta*	Rice A. and Roe A.	2007/2008	19	17	0.306 (0.079)	2.364 (0.509)	0.356/0.042*
Sampling location	**Origin of isolates**	**Collected by**	**Year of isolation**	**N**	**Haplotypes**	**H[a] (s.e.)**	**Allelic Richness[b]**	**I_A/P-value[c]**
Valemount, BC	*Pinus contorta*	Rice A. and Roe A. Boone, C.	2007/2008 /2010	8	8	0.348 (0.069)	2.000 (0.191)	0.355/0.096
Golden, BC	*Pinus contorta* / *Dendroctonus ponderosae*	Rice A. and Roe A.	2007/2008	8	7	0.356 (0.072)	2.091 (0.315)	0.023/0.434
Yoho, AB	*Pinus contorta* / *Dendroctonus ponderosae*	Rice A. and Roe A.	2007/2008	5	5	0.233 (0.084)	1.636 (0.244)	0.810/0.084
Canmore, AB	*Pinus contorta*	Rice A. and Roe A.	2007/2008	12	11	0.302 (0.078)	2.364 (0.491)	-0.043/0.524
Sparwood, BC	*Pinus contorta*	Rice A. and Roe A.	2007/2008	6	6	0.264 (0.095)	1.727 (0.304)	-0.218/0.826
Crow's nest Pass, AB	*Pinus contorta*	Rice A. and Roe A.	2007/2008	5	5	0.356 (0.094)	2.000 (0.302)	-0.267/0.790
Total				241	219	0.323 (0.018)	2.278 (0.093)	1.692/0.002**

[a]expected heterozygosity

[b]average number of alleles

[c]The null hypothesis of random association of alleles in random mating (I_A=0) was tested by comparing the observed value of the statistic with that obtained after 500 randomizations to simulate distribution (*P value <0.05; **P value <0.01).

Table 2. Summary of heterozygosity and F_{st} from each locus for *Leptographium longiclavatum* populations sampled in BC and Alberta and genotyped at 11 microsatellite loci). The clone-corrected dataset using 219 isolates was used**.**

Locus	No Alleles	F_{st}	H-Mean	H-SE
SR7	13	0.153	0.748	0.027
SR10	3	0.086	0.017	0.013
SR14	4	0.180	0.188	0.048
SR16	2	0.185	0.387	0.033
SR24	4	0.204	0.416	0.054
SR36	3	0.120	0.250	0.044
SR45	6	0.265	0.262	0.064
SR47	4	0.212	0.440	0.033
SR52	4	0.125	0.149	0.041
SR53	6	0.320	0.383	0.060
SR57	4	0.175	0.315	0.038

Table 3. Analysis of Molecular Variance (AMOVA) for *Leptographium longiclavatum* populations sampled in BC and Alberta and genotyped at 10 microsatellite loci based on (i) sampling locations and (ii) three genetic clusters pooled according to PCA and neighbor joining analyses

(i)	df	SS	Variance	%	P values
Among all 17 populations	16	318.133	0.902	10	<0.001
Within each of 17 populations	202	1695.164	8.392	90	<0.001
Total	219	2013.297	9.294	100	
(ii)					
Among clusters	3	166.334	0.821	9	<0.001
Among populations within clusters	13	151.799	0.264	3	<0.001
Within populations	202	1695.164	8.392	11	<0.001
Total	218	2013.297	9.477	100	

Table 4. Results of assignment of *Leptographium longiclavatum* individuals genotyped at 10 microsatellite loci using GENECLASS

Cluster/Location	N*	Nhome§	Location (%)ι	Cluster (%)ş	Ratio
BC					
Burns Lake	9	5	55.56	100	3.33
Quesnel	11	4	36.36	100	2.73
Williams Lake	21	11	52.38	100	2.86
Merrit	14	10	71.43	100	3.79
Kamloops	22	7	31.82	100	0.73
Prince George	17	0	0.00	100	0.06
NAB					
Tumble Ridge	17	0	0.00	24	0.00
Kakwa	17	0	0.00	12	0.35
Fairview	14	3	21.43	29	0.57
Fox Creek	15	0	0.00	20	0.00
Grande Prairie	20	0	0.00	10	0.00
Rockies					
Sparwood	6	2	33.33	34	0.33
Yoho	5	2	40.00	40	1.00
Canmore	11	5	45.45	46	0.45
Golden	7	0	0.00	15	0.00
Crowsnest Pass	5	2	40.00	40	0.40
Valemount	8	0	12.50	12	0.13
Total	**219**	**51**	**26**		

*Sample size (N)

§ Number of individuals assigned to original sampling location

ι Proportion of assignment to original location (=N_{home}/N);

ş Proportion of assignment to the genetic cluster

γ Ratio = total number of individuals (out of 219) assigned to the location /sample size of the location (N).

Table 5. Population pairwise F_{ST} (in black) and P-values (in red) among pairs of populations of *Leptographium longiclavatum* genotyped at 10 microsatellite loci and calculated with Arlequin (assessed after 1000 permutations) * p<0.05; ** p<0.01

Clusters	**BC**						**Northern Alberta**					**Rocky Mountains**					
Locations	**BL**	**PG**	**Q**	**WL**	**KL**	**M**	**F**	**TR**	**GP**	**FC**	**K**	**V**	**G**	**Y**	**C**	**S**	**CNP**
Burns Lake	0.00	0.04	0.00	0.04	0.11	0.02	0.08	0.05	0.11**	0.07	0.04	0.091	0.107	0.18	0.11*	0.25**	0.14
Prince George	0.30	0.00	0.03	0.02	0.11**	0.06	0.07	0.05	0.08	0.06	0.01	0.12	0.19**	0.26**	0.08	0.22**	0.21**
Quesnel	0.90	0.54	0.00	0.04	0.07	0.05	0.06	0.03	0.13**	0.02	-0.00	0.07	0.13	0.17	0.08	0.25**	0.19*
Williams Lake	037	0.43	0.32	0.00	0.12**	0.04	0.12**	0.11**	0.20**	0.01	0.06	0.10	0.12	0.20**	0.09	0.19	0.11
Kamloops	0.05	0.00**	0.09	0.00**	0.00	0.05	0.23**	0.13**	0.09**	0.09	0.11	0.11	0.15**	0.17*	0.07	0.23**	0.26*
Merritt	0.72	0.99	0.28	0.15	0.13	0.00	0.14**	0.09**	0.14**	0.17**	0.08*	0.10	0.079	0.22*	0.06	0.21**	0.13
Fairview	0.14	0.10	0.19	0.00*	0.00*	0.00*	0.00	0.02	0.11**	0.08	0.05	0.22**	0.29**	0.38**	0.21**	0.39**	0.32**
Tumble Ridge	0.34	0.18	0.80	0.01*	0.00*	0.01	0.54	0.00	0.09**	0.01	0.01	0.17**	0.20**	0.25**	0.11**	0.32**	0.27**
Grande Prairie	0.23	0.04*	0.16	0.054	0.00*	0.00*	0.01*	0.00*	0.00	0.01	0.03	0.16**	0.16*	0.29*	0.16**	0.34**	0.22**
Fox Creek	0.22	0.21	0.61	0.063	0.00*	0.00*	0.13	0.19	0.57	0.00	-0.01	0.10	0.17	0.27**	0.12**	0.32**	0.24**
Kakwa	0.40	0.60	0.94	0.09	0.02*	0.02*	0.18	0.60	0.27	0.92	0.00	0.08	0.15*	0.23**	0.09	0.28**	0.23**
Valemount	0.31	0.05	0.06	0.06	0.09	0.10	0.00*	0.00**	0.01*	0.13	0.23	0.00	0.09	0.17	0.13	0.24**	0.16
Golden	0.17	0.00*	0.03*	0.10	0.01*	0.31	0.00**	0.00**	0.00**	0.054	0.02*	0.18	0.00	0.20	0.10	0.30**	0.07
Yoho	0.09	0.00*	0.06	0.00*	0.04*	0.01*	0.00**	0.00**	0.00**	0.00*	0.018*	0.07	0.06	0.00	0.18	0.34*	0.23
Canmore	0.03*	0.09	0.14	0.08	0.10	0.20	0.00**	0.01**	0.00**	0.00*	0.05	0.06	0.19	0.08	0.00	0.14	0.10
Sparwood	0.00*	0.00*	0.00*	0.02	0.00*	0.00*	0.00**	0.00**	0.00**	0.00*	0.00*	0.01*	0.00*	0.03*	0.09	0.00	0.20
Crowsnest Pass	0.09	0.00*	0.03*	0.19	0.00*	0.09	0.00**	0.00**	0.01*	0.00*	0.00*	0.09	0.66	0.14	0.20	0.10	0.00

Figure 1. Map showing sampling locations of collections from British Columbia, Alberta and USA

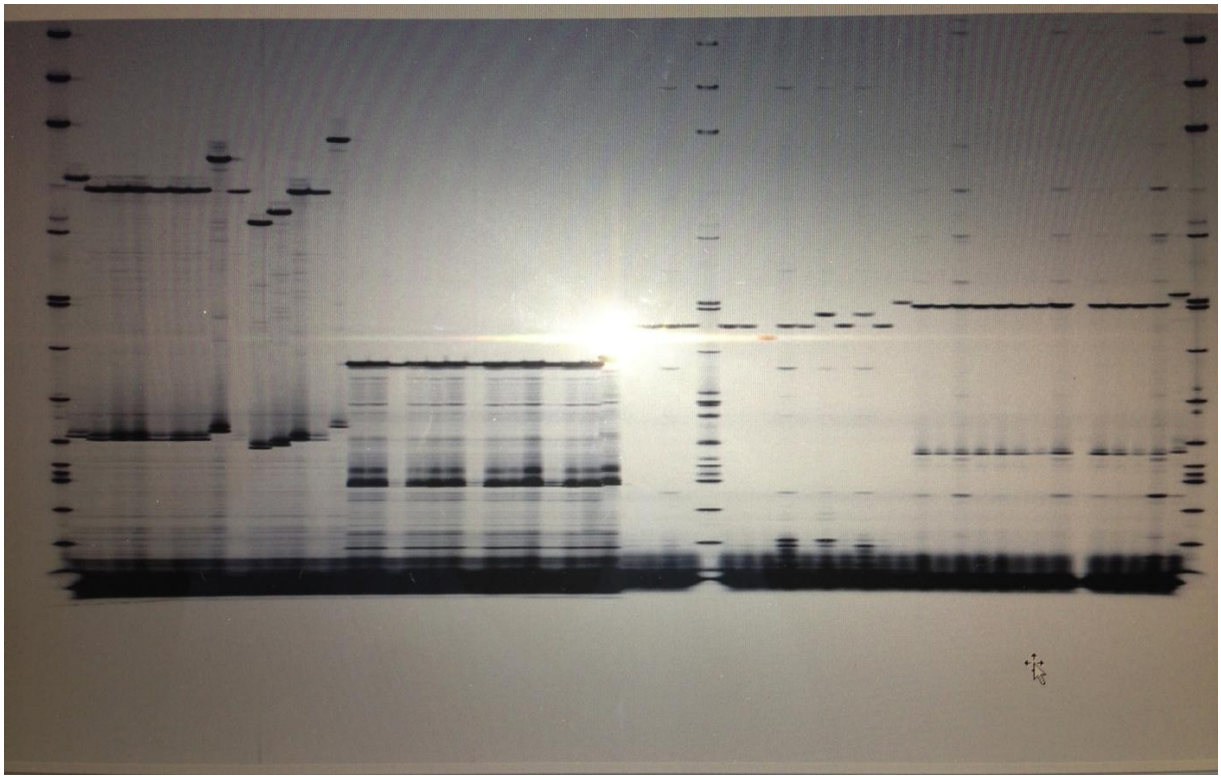

Figure 2. Example of a gel showing the patterns of microsatellite migration of SSR markers using *Leptographium longiclavatum* DNA

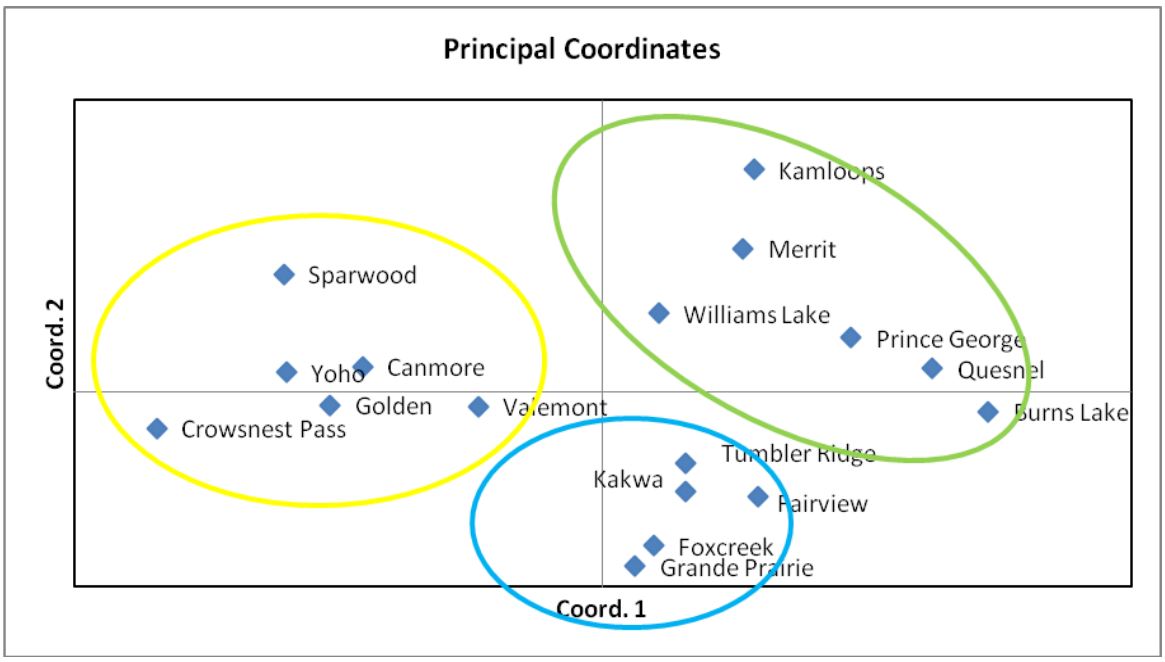

Figure 3. Genetic division of *Leptographium longiclavatum* populations genotyped at 11 microsatellite loci. Principal coordinate analysis (PCoA) is shown using genetic distances as input using GenAlex6.

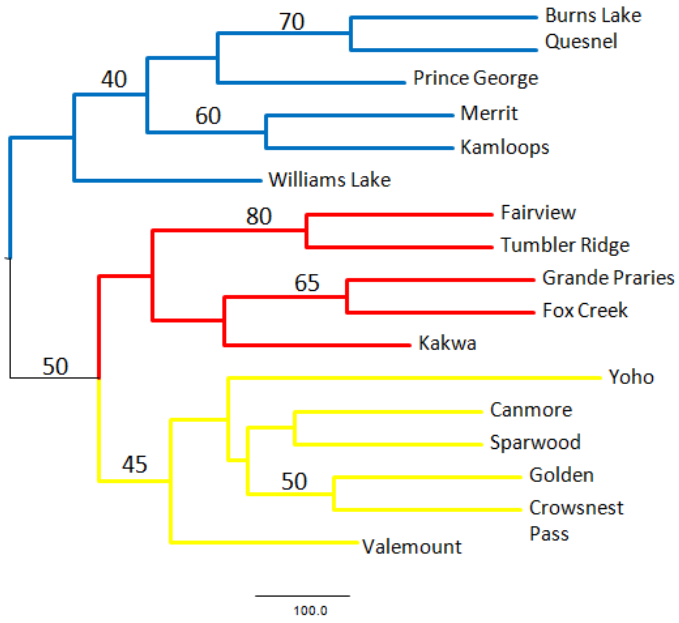

Figure 4. Neighbour joining tree derived from a distance matrix amongst 17 populations of *Leptographium longiclavatum* genotyped at 11 microsatelite loci analysed using PHILYP

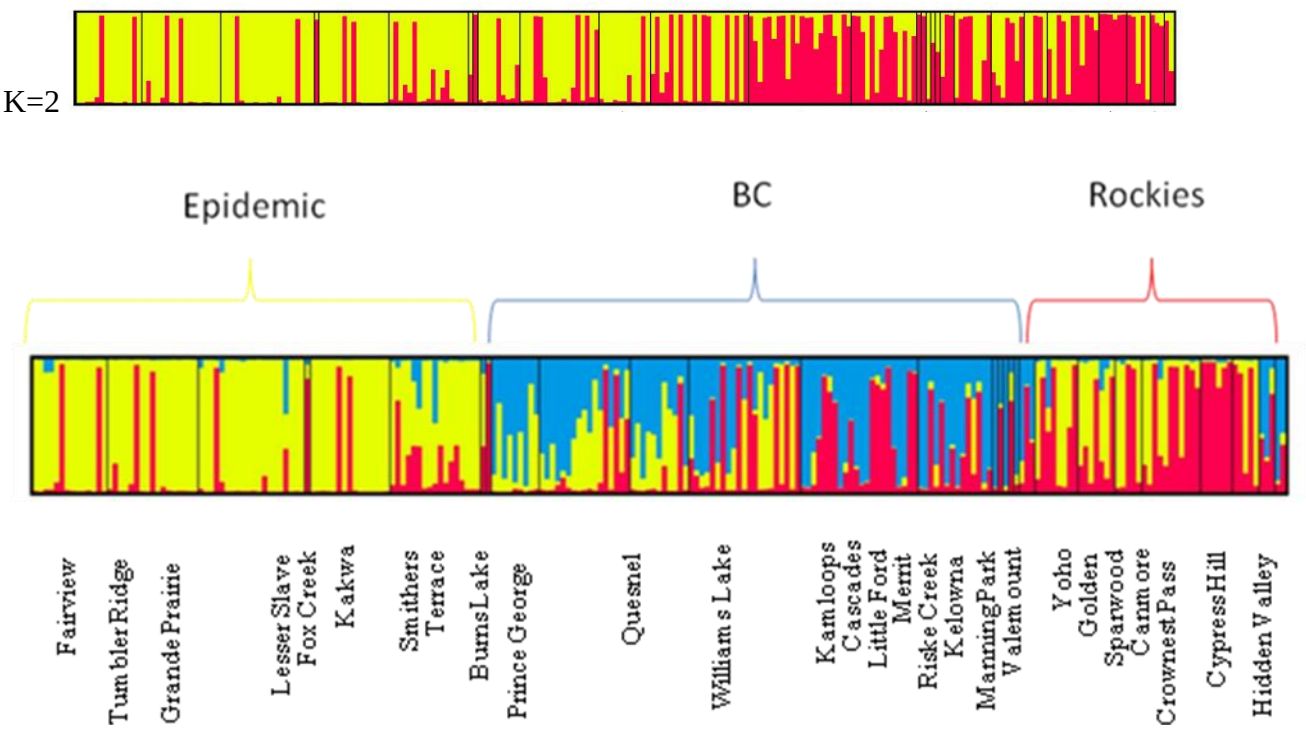

Figure 5. STRUCTURE analysis of *Leptographium longiclavatum* genotyped at 11 microsatellite loci and assigned to K=2 and K=3 clusters

Bibliography

Abbott, R.J., and C. Brochmann. 2003. History and evolution of the Arctic flora: in the footsteps of Eric Hulte. Molecular Ecology 12: 299–313.

Agapow, P.M., and A. Burt. 2001. Indexes of multilocus linkage disequilibrium. Molecular Ecology Notes 1: 101-102.

Amman, G.D., M.D. McGregor, and R.E. Dolph Jr. 1990. Mountain pine beetle forest insect and disease leaflet. US department of agriculture. Retrieved July 12, 2012 from http://www.barkbeetles.org/mountain/fidl2.htm.

Anonymous. Mountain pine beetle (Fact Sheet) Natural Resources Canada. (2012). URL: http://www.nrcan.gc.ca/node/13397

Anonymous. Ministry of Forestry analysis branch. 2003. Timber supply and the mountain pine beetle infestation in British Columbia. https://www.for.gov.bc.ca/hts/pubs/beetledoc_oct29LO.pdf

Anonymous. Southern interior beetle action coalition. 2011. SIBAC. http://www.sibacs.com/wp-content/uploads/2011/05/Feb.2011-update.pdf

Aukema, B. H., A. L. Carrol, J. Zhu, K. F. Raffa , T. A. Sickley. and Taylor. S. W. 2009. Landscape-level Analysis of Mountain Pine Beetle in British Columbia: Spatiotemporal Development and Spatial Synchrony Within the Present Outbreak. Ecography 29: 427-411.

Barras, S.J. 1970. Antagonism between *Dendroctonus frontalis* and the fungus *Ceratocystis minor.* Annals of the Entomological Society of America 63: 1187–90

Barras, S. J. 1973. Reduction of progeny and development in the southern pine beetle following removal of symbiotic fungi. The Canadian Entomologist 105(10): 1295-1299.

Bartell, N.V., B. Lindgren and J.E.K. Cooke. 2008. Using genetic analyses to infer mountain pine beetle population structure and dispersal patterns in British Columbia and Alberta. BC Journal of Ecosystems and Management 9: 140–142.

Basham, H. G. 1970. Wilt of loblolly inoculated with blue stain fungi of the genus *Ceratocystis*. *Phytopathology* 60: 750 – 754.

Beardsley, T. M. 2008. An epidemic with global consequences. BioScience 58(6): 475-475.

Beatty G. P. 2010. Refugial persistence and postglacial recolonization of North America by the cold-tolerant herbaceous plant *Orthilia secunda*. Molecular Ecology 19: 5009–5021.

Beaumont, M., and B. Rannala. 2004. The Bayesian Revolution in Genetics. *Nature Review Genetics* 5 (4): 251–61.

Bentz, B. J., A.L. Jesse, and D.A. Gene. 1991. Temperature-dependent development of the mountain pine beetle (Coleoptera: Scolytidae) and simulation of its phenology. Canadian Entomologist 123(5): 1083.

Berryman, A. A. 1972. Resitance of conifers to invasion by bark beetle-fungus associations. *BioScience* 22: 598 – 602.

Berryman, A. A. 1986. Principals and practices of population management. Plenum Press, New York/ London 279p.

Bleiker, K.P. and Six D L. 2009. Competition and Coexistence in a Multi-partner Mutualism: Interactions Between Two Fungal Symbionts of the Mountain Pine Beetle in Beetle-attacked Trees. Microbial Ecology 57(1): 191–202.

Bridges, J. R. 1983. Mycangial Fungi of Dendroctonus frontalis (Coleoptera: Scolytidae) and their Relationship to Beetle Population Trends. Environmental Entomology 12(3): 858-861.

Carroll, A.L. and L. Safranyik. 2003. The bionomics of the mountain pine beetle in lodgepole pine forests: establishing a context. In Mountain pine beetle symposium: challenges and solutions. Shore, T.L., J.E. Brooks, and J. Stone (Eds.). Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Information Report BC-X-399, Victoria, BC: 21-32.

Carroll, A.L., S.W. Taylor, J. Régnière, and L. Safranyik 2004. Effects of climate change on range expansion by the mountain pine beetle in BC. *In* Mountain pine beetle symposium: challenges and solutions. (2003, October 30-31), Kelowna, British Columbia. Shore, T.L., J.E. Brooks, and J. Stone (Eds.). Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Information Report BC-X-399, Victoria, BC: 223-232

Cullingham, C. I., J.E.K. Cooke, S. Dang, C.S. Davis, B.J. Cooke, and D.W. Coltman. 2011. Mountain pine beetle host-range expansion threatens the boreal forest. Molecular ecology 20(10): 2157-71.

Dawson, K. J., and K. Belkhir. 2001. A Bayesian approach to the identification of panmictic populations and the assignment of individuals. Genetical research 78(01): 59-77.

Dieringer, D., and C. Schotter. 2003. Microssatellite analyzer (MSA); a plataform independent aalysis tool for a large microsatellite data sets. *Molecular Ecology Notes* 3: 167 – 169.

DiGuistini, S., S.G. Ralph, and Y.W. Lim, R. Holt, S. Jones, J. Bohlmann and C. Breuil. 2007. Generation and annotation of lodgepole pine and oleoresin-induced expressed sequences from the blue-stain fungus *Ophiostoma clavigerum*, a Mountain Pine Beetle-associated pathogen. FEMS Microbiology Letters 267: 151-158.

DiGuistini, S., Y. Wang., N. Y. Liao., G. Taylor., P. Tanguay *et al.* 2011. Genome and trascriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*. Proc Natl Acad Sci USA108: 2504 – 2509.

Enkerli, J., F. Widmer, C. Gessler, and S. Keller, S. 2001. Strain-specific microsatellite markers in the entomopathogenic fungus. Mycological Research 105(9): 1079-1087.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An Integrated Software Package for Population Genetics Data Analysis. Evolutionary Bioinformatics Online 47-50.

Gianfranceschi, L., N. Seglias, R. Tarchini, M. Komjanc, and C. Gessler. 1998. Simple sequence repeats for the genetic analysis of apple. Theoretical and Applied Genetics 96(8): 1069-1076.

Goldhammer, D.S., F.M. Stephen, and T.M. Paine. 1990. The effect of the fungi *Ceratocystis minor*, *Ceratocystis minor* var. *barassii* and SJB 122 on reproduction of the southern pine beetle, Dendroctonus frontalis. Canadian Entomology 122: 407–418.

Groppe, K., L. Sanders, A. Wiemken, and T. Boller. 1995. A microsatellite marker for studying the ecology and diversity of fungal endophytes (*Epichloë* spp.) in grasses. Applied and Environmental Microbiology 61(11): 3943-3949.

Harrington, T. C. 1993. Diseases of conifers caused by species of Ophiostoma and Leptographium. Saint Paul: APS Press. 161-172.

Hedrick, P.W. 2005. A standardized genetic differentiation measure. Evolution 59: 1633–1638.

Hélie, J.F., D.L. Peters, and K.R. Tattrie. 2005. Review and synthesis of potential hydrologic impacts of Mountain Pine Beetle and related harvesting activities in British Columbia. Canada, Natural Resources, and Canadian Forest Service Gibson Mountain Pine Beetle Initiative Working Paper, 23p.

Hubisz, M. J., D. Falush, M. Stephens, and J.K. Pritchard. 2009. Inferring Weak Population Structure with the Assistance of Sample Group Information. Molecular Ecology Resources 9(5): 1322–32.

Keiper, F. J., M.S. Haque, M.J. Hayden, and R.F. 2006. Genetic diversity in Australian populations of *Puccinia graminis* f. sp. *avenae*. Phytopathology 96(1): 96-104.

Kim, J. J., S. H. Kim, S. Lee, and C. Breuil. 2003. Distinguishing *Ophiostoma ips* and *Ophiostoma montium*, two bark beetle-associated sapstain fungi. FEMS Microbiology Letters 222(2): 187-192.

Kim, J. J., A. Plattner, Y.W. Lim, and C. Breuil. 2008. Comparison of two methods to assess the virulence of the mountain pine beetle associate, *Grosmannia clavigera*, to *Pinus contorta*. Scandinavian Journal of Forest Research 23(2): 98-104.

Konkin, D., and K. Hopkins. 2009. Learning to deal with climate change and catastrophic forest disturbances. Unasylva 60: 17-23.

Kurtz, W. A., C.C. Dymond, G. Stinson, G.J. Rampley, E.T. Neilson, A. L. Carroll, T. Ebata and L. Safranyik. 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature 452(7190): 987-90.

Lee, S., J. J. Kim, S. Fung, and C. Breuil. 2003. A PCR-RFLP marker distinguishing Ophiostoma clavigerum from morphologically similar Leptographium species associated with bark beetles. Canadian journal of botany 81(11): 1104-1112.

Lee, S., J. J. Kim and Colette Breuil. 2005. Leptographium *Longiclavatum* Sp. Nov., a New Species Associated with the Mountain Pine Beetle, *Dendroctonus Ponderosae*. Mycological Research 109 (10): 1162–1170.

Lee S., J. J. Kim, and C. Breuil. 2006. Diversity of fungi associated with the mountain pine beetle, *Dendroctonus ponderosae* and infested lodgepole pines in British Columbia. Fungal Diversity 22: 91-105.

Lee S., R.C. Hamelin, D.L. Six and C. Breuil. 2007. Genetic diversity and the presence of two distinct groups in *Ophiostoma clavigera* associated with *Dendroctonus ponderosae* in British Columbia and the northern Rocky mountains. Phytopathology 97: 1177-1185.

MacDonald, B. A. and C. Linde. 2004. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology 40: 349-379.

McFarlane, B. L., R. C. Stumpf-Allen, D. O. Watson. 2006. Public perceptions of natural disturbance in Canada's national parks: the case of the mountain pine beetle. Biological Conservation 130: 340-348.

Manel, S., M. K. Schwartz, G. Luikart and P. Taberlet. 2003. Landscape Genetics: Combining Landscape Ecology and Population Genetics. Trends in Ecology and Evolution 18(4): 189–197.

Mathre, E. 1964. Pathogenicity of *Ceratoycystis ips* and *Ceratocystis minor* to *Pinus ponderosae*. Contrib. Boyce Thompson Inst. 22: 363-388.

Maynard-Smith, J., N. H. Smith, M. O'Rourke and B. G. Spratt. 1993. How clonal are bacteria? Proceedings of the National Academy of Sciences of the USA 90: 4384-4388.

Morgante, M. and A. M. Olivieri. 1993. PCR amplified microsatellites as markers in plant genetics. The Plant Journal 3(1): 175-182.

Mock, K. E., B.J. Bentz, E.M. O'Neill, J.P. Chong, J. Orwin, and M.E. Pfrender. 2007. Landscape-scale genetic variation in a forest outbreak species, the mountain pine beetle (*Dendroctonus ponderosae*). Molecular Ecology 16(3): 553-568.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Nelson, R. 1934. Effect of blue-stain fungi in southern pines attacked by bark beetles. Phytopathology 7: 327-353.

O'Connell, M., R.G. Danzmann, J.M. Cornuet, J.M. Wright, and M.M. Ferguson, M. M. 1997. Differentiation of rainbow trout (*Oncorhynchusmykiss*) populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. Canadian Journal of Fish and Aquaculture Science 54:1391-1399.

Owen, D. R., K.Q. Lindahl Jr., D.L. Wood, and J.R. Parmeter Jr. 1987. Pathogenicity of fungi isolated from *Dendroctonus valens*, *D. brevicomis*, and *D. ponderosae* to ponderosa pine seedlings. Phytopathology 77(4): 631-636.

Plattner, A. 2008 Pathogenicity and taxonomy of fungi associated with the mountain pine beetle in British Columbia. The University of British Columbia, M. Sc. thesis.

Paetkau, D., W. Calvert, I. Stirling and C. Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4(3): 347-354.

Paetkau, D., R. Slade, M. Burden and A. Estoup. 2004. Genetic assignment methods for the direct, real time estimation of migration rate: a simulation based exploration of accuracy and power. Molecular Ecology 13(1): 55-65.

Peakall, R and P.E. Smouse. 2005. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

Pederson, L. 2004. How serious is the mountain pine beetle problem? From a timber supply perspective. Public discussion paper.
http://www.cfs.nrcan.gc.ca/pubwarehouse/pdfs/25159.pdf#page=18

Piry, S., A. Alapetite, J-M. Cornuet, D. Paetkau, L. Baudouin and A. Estoup 2004. GENECLASS2: a software for genetic assignment and first generation migrant detection. Journal of Heredity 95: 536-539.

Perez-Lezaun, A., F. Callafell., E. M. Seiekstad., E. Mateu., D. Comas., E. Bosh., and J. Bertranpetit. 1997. Population genetics of Y-chromosome short tandem repeats in humans. *Journal of Molecular Evolution* 45: 265 – 270.

Pritchard J.K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Powell, W., M. Morgante, R. McDevitt, G.G. Vendramin, and J.A. Rafalski, J. A. 1995. Polymorphic simple sequence repeat regions in chloroplast genomes: applications to the population genetics of pines. Proceedings of the National Academy of Sciences 92(17): 7759-7763.

Powell, J. A., J.L. Jenkins, J.A. Logan and B.J. Bentz. 2000. Seasonal temperature alone can synchronize life cycles. Bulletin of mathematical biology 62(5): 977-998.

Rannala, B. and J. L. Mountain. 1997. Detecting immigrants by using multilocus genotypes. Proceedings of the National Academy of Sciences of the USA 94: 9197-9200.

Reid, R.W. 1961. Moisture changes in lodgepole pine before and after attack by mountain pine beetle. Forestry Chronicle 37: 368–375.

Reid, R.W. 1962. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the East Kootenay region of British Columbia. I. Life cycle, brood development, and flight periods. The Canadian Entomologist 94: 531-538.

Rice, A. V., M. N. Thormann, and D.W. Langor. 2007. Virulence of, and interactions among, mountain pine beetle associated blue-stain fungi on two pine species and their hybrids in Alberta. Canadian Journal of Botany 85(3): 316-323.

Rice, A. V., M. N. Thormann, and D.W. Langor. 2008. Mountain pine beetle-associated blue-stain fungi are differentially adapted to boreal temperatures. Forestry Pathology 38(2): 113-123.

Rice, A. V., and D.W. Langor. 2009. Mountain pine beetle-associated blue-stain fungi in lodgepole × jack pine hybrids near Grande Prairie, Alberta (Canada). Forest Pathology 39(5): 323-334.

Richmond, J. A., C. Mills and E. W. Clark. 1970. Chemical changes in Loblolly Pine, *Pinus taeda* L., inner bark caused by blue stain fungus, Ceratocystis minor (Hedg.) Hunt. *Journal of the Elisha Mitchell Scientific Society* 86, no. 4 (1970)

Robinson, R.C. 1962. Blue stain fungi in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) infested by the mountain pine beetle (Dendroctonus monticolae Hopk.). Canadian Journal of Botany 40: 609-614

Roe, A. D., A.V. Rice, S.E. Bromilow, J.E.K. Cooke, and F.A.H. Sperling. 2010. Multilocus species identification and fungal DNA barcoding: insights from blue stain fungal symbionts of the mountain pine beetle. Molecular ecology resources 10(6): 946-59.

Roe, A. D., P.M. James, A.V. Rice, J.E.K. Cooke, and F.A.H. Sperling. 2011. Spatial community structure of mountain pine beetle fungal symbionts across a latitudinal gradient. Microbial ecology 62(2): 347-60.

Rumbold, C. T. 1931. Two blue stain fungi associated with bark beetle infestation of pines. *Journal of Agrycultural Research* 43: 847 – 873.

Safranyik, L., D. M. Shrimpton., and H. S. Whitney. 1975. An interpretation of the interaction between lodgepole pine, the mountain pine beetle, and its associated blue stain fungi in western Canada. Management of lodgepole pine ecosustems symposium proceedings: 406 – 428.

Safranyik, L. and A. L. Carroll. 2006a. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. *In* The mountain pine beetle: a synthesis of biology, management, and impacts on lodgepole pine. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC.3-66.

Safranyik, L. and B. Wilson (Eds.). 2006b. The mountain pine beetle: a synthesis of biology, management, and impacts on lodgepole pine. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, B.C.

Safranyik, L., A. L. Carroll, J. Régnière, D. W. Langor, W. G. Riel, T. L. Shore, B. J. C. B. Peter, B. J. Cooke, V. G. Nealis, and S. W. Taylor. (2010). Potential for range expansion of mountain pine beetle into the boreal forest of North America. *The Canadian Entomologist*, *142*(05), 415-442.

Samarasekera, G., N.V. Bartell, B.S. Lindgren, J.E. Cooke, C.S. Davis, P. James, and B. Murray. 2012. Spatial genetic structure of the mountain pine beetle (*Dendroctonus ponderosae*) outbreak in western Canada: historical patterns and contemporary dispersal. Molecular Ecology 21(12): 2931-2948.

Sinclair, W. A. 1987., Lyon. H., and Johnson, W. T. 1987. Diseases of trees and shrubs. Cornell University Press, Ithaca, New York, USA.

Six, D. L. and T.D. Paine. 1997. *Ophiostoma clavigerum* is the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi*. Mycologia 858-866.

Six, D. L. and T.D. Paine. 1999. Allozyme diversity and gene flow in *Ophiostoma clavigerum* (Ophiostomatales: Ophiostomataceae), the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi* (Coleoptera: Scolytidae). Enzyme 331: 324-331.

Six, D. L. 2003. A comparison of mycangial and phoretic fungi of individual mountain pine beetles. *Canadian Journal of Forest Research*, 33:1331-1334

Six, D. L., and K.D. Klepzig. 2004. Dendroctonus Bark Beetles as Model Systems for Studies on Symbiosis 37: 1-26.

Six, D. L., and B.J. Bentz. 2007. Temperature determines symbiont abundance in a multipartite bark beetle-fungus ectosymbiosis. Microbial Ecology 54(1): 112-118.

Solheim, H. 1995. A comparision of blue-stain fungi associated with the North American spruce beetle *Dendroctonus rufipennis* and the Eurasian spruce bark beetle *Ips typographus*. *In Forest Pathology Research in the Nordic Countries* 1994. *Proceedings from the SNS-meeting in Forest Pathology at Skobrukets Kurssenter, Biri, Norway* 9 – 12 *August* 1994 (ed. D. Aamlid), pp 61 –67. *Aktuelt fra Skogfortsk* 4-95. Norsk Institutt for skogforskning: Norway.

Solheim, H., and P. Krokene. 1998. Growth and virulence of mountain pine beetle associated blue-stain fungi *Grosmannia clavigera* and *Ophiostoma montium*. Canadian Journal of Botany 76: 561-566.

Strobel, G. A., and F. Sugawara. 1986. The pathogenecity of *Ceratocystosis montia* to lodge pine. Canadian Journal of Botany 64: 113-116

Stahl, K., R.D. Moore, and I.G. McKendry. 2006. Climatology of winter cold spells in relation to mountain pine beetle mortality in British Columbia, Canada. Climate Research 32: 13-23.

Taylor, S.W., A.L. Carroll. 2004. Disturbance, forest age, and mountain pine beetle outbreak dynamics in B.C.: a historical perspective. In Mountain Pine Beetle Symposium: Challenges and Solutions. Shore, T.L., J.E. Brooks, and J.E. Stone (Eds). Report BC-X-399, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC. 67-94

Taylor, S. W., Carroll, A. L., Alfaro, R. I., & Safranyik, L. (2006). Forest, climate and mountain pine beetle outbreak dynamics in western Canada. *The mountain pine beetle: A synthesis of biology, management, and impacts on lodgepole pine*, 67-94.

Tsui, C. K. M., N. Feau, C.E. Ritland, A.S. Massoumi, S. DiGuistini, L. Khadempour, and J. Bohlmann, C. Breuil, and R. C. Hamelin. 2009. Characterization of microsatellite loci in the fungus, *Grosmannia clavigera*, a pine pathogen associated with the mountain pine beetle. Molecular ecology resources 9(6): 1500-3.

Tsui, C. K. M., A.D. Roe, Y. El-Kassaby, A. Rice, S.M. Alamouti, F.H. Sperling, J.E.H. Cooke, J. Bohlmann and R. C. Hamelin. 2012. Population structure and migration pattern of a

conifer pathogen, *Grosmannia clavigera*, as influenced by its symbiont, the mountain pine beetle. Molecular ecology 21(1): 71-86.

Tusi, C. K. M., DiGuistini, S., Wang, Y., Feau, N., Dhillon, B., Bohlmann, J., and Hamelin, R. C. 2013. Unequal recombination and evolution of the Mating-Type (MAT) loci in the pathogenic fungus *Grosmannia clavigera* and relatives. *G*3: *Genes*|*Genomes*| *Genetics* 3 (3): 465 – 480.

Wagner, T. L., J.A. Gagne, P.C. Doraiswamy, R.N. Coulson, and K.W. Brown. 1979. Development time and mortality of *Dendroctonus frontalis* in relation to changes in tree moisture and xylem water potential. Environmental Entomology 8(6): 1129-1138.

Webb, J. W., and R.T. Franklin. 1978. Influence of phloem moisture on brood development of the southern pine beetle (Coleoptera: Scolytidae). Environmental Entomology 7(3): 405-410.

Weber, J. L., and P.E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American journal of human genetics 44(3): 388.

Weir, B. S., and C. C. Cokerham. 1984. Estimating F-statistics for the analysis of population structure . *Evolution* 38: 1358 – 1370.

Whitney, H. S., and Farris, S. H. 1970. Maxillary mycangium in the mountain pine beetle. *Science* 167: 54 – 55.

Whitney, H.S., R.J. Bandoni, and F. Oberwinkler. 1987. *Entomocorticium dendroctoni* gen.et. sp. nov. (Basidiomycotina), a possible nutritional symbiote of the mountain pine beetle in lodgepole pine in British Columbia. Canadian Journal of Botany, 65: 95–102

Yamaoka, Y., R.H. Swanson, Y. Hiratsuka. 1995. The ability of *Ophiostoma clavigerum* to kill mature lodgepole-pine trees. Forest Pathology 25: 401-404.

Yearian, W. C., Gouger, R. J., and Wilkinson, R. C. 1972. Effects of the blue stain fungus, *Ceratocystis ips*, on development of *Ips* barl beetles in pine bolts. *Annuls of the Entomological Society of America* 65: 481487.

Zhou, X., T. I. Burgess, Z. W. De Beer, F. Lieutier, A. Yart, K. Klepzig, A. Carnegie, J. M. Portales, B. D. Wingfield, and M. J. Wingfield. 2007. High intercontinental migration rates and population admixture in the sapstain fungus *Ophiostoma ips*. *Molecular Ecology* 16: 89-99.