Local population structure of white pine weevil (*Pissodes strobi* [Peck]) in interior and Sitka spruce stands in British Columbia

by

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B.Sc., University of Manitoba, 1999
B.Sc., University of Winnipeg, 2003

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

MASTER OF SCIENCE

in

The Faculty of Graduate Studies

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April 2011

Abstract

White pine weevil is a pest of interior and Sitka spruce species in British Columbia. It is native to eastern Canada, and migrated to the west. To date, control of the weevil has been ineffective even with the combined use of several control methods. Current research has focused on breeding resistant trees for use in plantations to overcome attack from *Pissodes strobi*. Knowledge of the weevil genetic structure on a small-scale stand level is extremely important in developing strategies that decrease the possible development of tolerance in *P. strobi* populations to resistant trees. To understand the population structure of *P. strobi* 15 microsatellite markers were used to investigate local population structure. Genetic structure of local weevil populations differed over stand age in both interior and Sitka spruce plantations. The younger and older plots had more single populations associated with individual trees than did middle aged plots. Middle-aged plots had increased beetle movement regardless of the number of weevil larvae per leader, increased number of females ovipositing per tree and less weevil genetic differentiation between trees. Understanding reproductive dynamics of *P. strobi* will help develop strategies for planting resistant trees to decrease the development of insect tolerance and further our knowledge of the possible co-evolutionary dynamics of this system.

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Acknowledgements

I would like to thank my thesis committee and the Treenomix lab for all their help and critiques. Special thanks goes to Dr. Kermit Ritland for his kindness in giving me the opportunity to work with him and his guidance throughout my project. To Dr. Carol Ritland and Hesther Yeuh for their undying support and belief in me, I would never have made it this far without your support.

To my family, for their support throughout this experience and my entire life, I could not have made it here without their love. Hazel and Callum, you helped maintain my smiles, thank you for the wonderful thesis breaks.

To Simon, for keeping me sane, taking me biking and forcing me to focus in times when I needed that extra push.

Finally to Dr. Terry Galloway at the University of Manitoba, the reason I went into entomology, you are the entomologist I strive to become. You opened up a whole new world to me and for that I will always be grateful.

To my parents, my inspiration

1.0 General Introduction

1.1 White Pine Weevil Ecology

Pissodes strobi (Peck) belongs to the family Curculionidae that is comprised of 598 species in Canada and Alaska (McNamara 1991). Of the 12 known species of *Pissodes* in Canada, 11 are recorded in British Columbia and of these, four belong to the *Pissodes strobi* species complex (*P. strobi*, *P. nemorensis*, *P. terminalis* and *P. schwarzi*) (Boyce et al. 1994, Langor and Sperling 1997).

Pissodes strobi is native to North America and distributed across Canada (Figure 1) (Turnquist and Alfaro 1996, Liewlaksaneeyanawin et al. 2002, Laffin et al. 2004). In eastern Canada the weevil primarily attacks white pine (*Pinus strobus*), while in western Canada it primarily attacks species of spruce (Table 1) (Alfaro 1994, Turnquist and Alfaro 1996).

White pine weevil (*P. strobi*) was first described in 1817 by William Dandridge Peck on white pine (*Pinus strobus*) in eastern North America (Wallace and Sullivan 1985). In later descriptions, species from western North America were described based on different host associations. *Pissodes sitchensis* was found on Sitka spruce and *P. engelmanni* was found on Engelmann spruce. Later these two “new” species were synonymised with *P. strobi* (Turnquist and Alfaro 1996, Laffin et al. 2004). Of the four members of this species complex, *Pissodes strobi* causes extensive damage to spruce within the province of British Columbia. This species can cause major growth defects that decrease timber values (i.e. forks), and in serious infestations, tree death can occur after repeated attacks (Alfaro 1994).

White pine weevil is univoltine. Eggs are deposited below the apical buds of the terminal shoot (leader) of the tree from late April to early June in the punctures made by female weevils (Silver 1968, Alfaro 1994, Turnquist and Alfaro 1996). Each egg puncture is covered over by a faecal plug. Larvae burrow into the phloem of the terminal shoot feeding downward, eventually girdling and killing the leader (Silver 1968, Alfaro 1994, Turnquist and Alfaro 1996). If an infestation is large, larvae can also mine beyond the year-old leader and into lateral shoots (Silver 1968, Alfaro 1994, Turnquist and Alfaro 1996). By midsummer larval development is complete, and pupal cells (chip cocoons) are constructed in the pith or on the surface. Adults emerge in late August to early September, feed on terminals and branches and then overwinter in the duff layer. The following April or May adults crawl or fly to the top of the spruce leaders, where they feed, mate and resume their life cycle (Silver 1968, Alfaro 1994, Turnquist and Alfaro 1996). The life cycle varies depending on local climatic conditions. In colder areas portions of populations may overwinter as pupae, mature larvae, or even as teneral adults (Alfaro 1994). Optimal temperatures for oviposition range between 20°C and 25°C, and after egg deposition; weevils require 785 growing degree-days on white spruce and 888 growing degree-days on coastal Sitka spruce above 7.2°C for adult emergence (Spittlehouse et al. 1994, Turnquist and Alfaro 1996).

Weevils can live up to four years, and the females can multiple mate during each year. Females are able to store sperm in and between years and do not need to mate during the current year to produce offspring (Liewlaksaneeyanawin et al. 2002). Re-attack of the same tree the following year is known to occur in high attack

densities where oviposition can occur on the main stem under the previous year's damage or in lateral branches (Alfaro 1994). Original attacks cause forks in the trees and other various stem defects. If the infestation is large, and attack on the tree repeated year after year, tree death can occur.

1.2 White Pine Weevil Epidemiology

In natural spruce stands the occurrence of *P. strobi* is quite low, probably due to the successional dynamics of naturally occurring stands in which spruce trees regenerate in the shaded understory (Wallace and Sullivan 1985, Alfaro 1994). Taylor et al. (1994) found that the weevil is negatively affected by shade. Sullivan (1961) also found differences in the feeding oviposition behaviour of weevils in white pine stands shaded by Red Oak (*Quercus rubra*). Weevils in shaded stands did not confine the majority of their feeding and oviposition punctures to the upper portion of the leader. Weevils under shade fed and oviposited on the main stem of the tree encompassing the last 4-5 years growth. There were also fewer oviposition plugs that contained eggs (Sullivan 1961). The lack of adequate heat for the leader under large overstory trees may account for the low occurrence of weevils in natural stands. Also when trees are grown under shade, the leaders tend to be thinner, shorter and generally weaker looking compared to open grown spruce (Sullivan 1961, Taylor et al. 1994).

In these natural stands weevil populations increase when canopies open due to windfall, natural succession or large-scale events such as fire (Alfaro 1994). This natural cycle is altered when trees are harvested from an area and replanted in

large-scale monoculture plots. When this occurs, there is adequate sun and a large number of trees for the white pine weevil to successfully grow in population size, and potentially damage many trees in the plantation. Lack of overstory increases heat in the stand and can increase leader temperature by up to 5°C (Spittlehouse et al. 1994). This makes conditions ideal for white pine weevil development and subsequent outbreak.

In Sitka spruce plantations weevil attacks generally begin when trees are 5 years old (Figure 2). The weevil population can then grow exponentially until the stand reaches 15 years when it enters into an equilibrium phase that can last 10 –20 years. The initial rapid increase in number of weevils is mainly due to the abundance of resources. As stated before, once the weevils attack and kill the leader, lateral branches become dominant, which causes the presence of more than one 'leader' per tree. This creates more resources for the weevils to infest and the population can grow for years. After the stand reaches 15 years, the population tends to stabilize as resources become reduced and natural weevil mortality occurs. When trees reach the ages of 30-40 years, weevil populations decline. As the tree growth slows, fewer resources are available to support large numbers of weevils. As the canopy closes weevils may move out of the stand to other available areas (Alfaro 1994).

1.3 White Pine Weevil And Stand Dynamics

In order for the weevil to invade new habitats, long distance recognition of host species is required. Not only do they need to land on the correct species but

also the habitat/host has to be adequate for larval reproduction (i.e. warm, sunny, thick leaders). Locating newly forested plantations might be easier for the weevil as in natural stands it would have to search through many tree species (conifer and non conifer) to locate a suitable host. Alfaro (1994) examined beating records collected during insect disease surveys and noted that *P. strobi* was rarely reported on non-host trees. This suggests that the weevil possess high optical and/or chemical acuity to locate trees. Mehary et al. (1994) showed that traps baited with cut Sitka spruce leaders attracted more weevils than non-baited traps. They also showed that more female spring weevils were in traps baited with cut spruce leaders, whereas spring male weevils showed no preference. Males did prefer traps baited with mated females. This suggests that weevils are most likely able to detect host trees in a plot before they land on the host and once they land, share closer tactile and sensory cues with the host tree.

Chemical recognition is important, but visual cues are also important for weevil survival. *Pissodes strobi* prefer to attack the longest leaders suggesting that attack by the weevil is not random (Silver 1968, Gara et al. 1971, Alfaro 1994, Sahota et al. 1994). Silver (1968) noted that leaders greater than 16 inches in length were preferred, while those greater than 23 inches were always attacked. VanderSar and Borden (1977a) found that weevils preferred vertical leaders with thicker bark and a diameter of 3cm or greater. Visual recognition also insures adequate food for larval survival. Visual and chemical recognition are important in long distance travel and are extremely important to females who tend to disperse further in the fall than the males (Mehary et al. 1994). Females must find new host

trees, to establish their offspring the following spring, and they must do this before the cold weather restricts their movements.

Alfaro (1988) reports that weevils were more successful on the tree species from which they originated. Weevils were collected from both Sitka and Engelmann spruce leaders and were forced to oviposit on Sitka spruce, Engelmann spruce and Lodgepole pine. They found that more eggs were laid on the host species from which the population originated and adult weights were greater on these leaders. Another consequence to weevils forced to feed on other host species was a change in development time. Sitka-reared weevils completed development faster on Sitka spruce verses other hosts provided. VanderSar and Borden (1977) also found that weevils that were forced on different species did not reproduce as successfully, and weevils from eastern Canada can be more flexible than western Canadian individuals and successfully reproduce on a greater number of host species. They collected weevils from Sitka spruce and used Sitka spruce leaders, Douglas-fir, Western Hemlock and Red Cedar in the force-feeding experiments. They found that the weevils preferred Sitka spruce but also would feed on Douglas-fir and Western Hemlock whereas Red Cedar was excluded by both males and females. Female weevils appear to be able to discriminate between the four tree species in this study, whereas males did not distinguish between Sitka spruce and Douglas-fir.

1.4 White Pine Weevil Control

Weevils are extremely damaging and difficult to control, because unlike many forest pests, the weevil stays in the forest stand once it infests a forest plot (Alfaro

1994). The initial attack density may start off small, but grows in size over time since the weevils tend not to kill the tree. Spruce trees grow new leaders, and the attack continues over time, until the tree is killed or is no longer an acceptable host for the weevil which may take on the order of thirty years or more (Alfaro 1994, Hall 1994).

Trees possess natural mechanisms to resist attack by white pine weevil. Mechanisms can involve deterring the adult from feeding or ovipositing on the tree. Alfaro and Borden (1982) tested conifers, broadleaved trees and ferns against weevils found in Sitka Spruce trees. They found that all the conifers contained some type of feeding deterrent that was absent in the other plants tested. Resistance may also directly affect egg maturation and ovarian development. The number of mature eggs in weevils feeding on resistant trees was significantly less than those feeding on susceptible trees and ovarian development was also negatively affected (i.e. the more resistant the less ovary development) (Sahota et al. 1998). Resinosis is a major factor in tree defences against white pine weevil. The resin floods the larval galleries killing the insects in the leader. Trees considered to be resistant to the weevil possess a greater number of outer resin canals and larger canals (Tomlin and Borden 1994). Even though trees possess natural defences, not all trees are able to successfully overcome weevil attack.

Different management strategies have been attempted to help control *P. strobi*: annual applications of insecticides, clipping leaders or planting resistant tree species (Hall 1994). Early chemical trials showed good results using dieldrin, DDT and endosulfan (Silver 1968). Silver (1968) furthered chemical testing with varying concentrations of the chemicals. He found that 5% phosphamidon was adequate in

deterring all weevils from the leaders, and good results were also obtained in DDT concentrations of 5 and 1 percent. Aerial applications of insecticides are a major concern because of effects on non-target organisms especially fish and stream wildlife (Fraser and Szeto 1994). To solve this problem Fraser and Szeto (1994) applied Metasystox-R (oxydemeton-methyl) and Dimethoate (dimethoate) using a Lancet to inject these chemicals into the tree. They found this application could provide coverage for up to two years. De Groot and Helson (1994) discuss the history of chemical applications to control white pine weevil, which to date have not been one hundred percent successful.

Silvicultural methods have also been applied to control white pine weevil. These methods take advantage of the fact that the weevils require warm temperatures for growth (heat sum accumulation). Planting overstory trees blocks out the sun and alters the temperature of the leader. Mclean (1994) tested the effectiveness of overstory shading using red alder on Sitka spruce growth and weevil control. I visited the plots used to test overstory shading, and although few leaders were attacked, the Sitka spruce grown under shade was achromatized and attenuated. Bellocq and Smith (1996) found high overwintering weevil mortality due to the depth of the duff layer and suggest that silvicultural methods paired with site maintenance may decrease weevil populations.

Leader clipping has also been used to control *P. strobi*. Rankin and Lewis (1994) tested the effectiveness of leader clipping over a five-year period. They found that clipping did not reduce infestation levels significantly especially when

considering the cost to execute annual clipping (e.g. average cost over the five years $250/ha).

Like other insects, weevils are subject to predation and parasitism (Table 2) neither of which seem to be sufficient to control weevil populations. *Lonchaea corticis* has been shown to be an important parasite of white pine weevil, but has not been unsuccessful in controlling the weevil (Alfaro 1994). Laboratory and field assessments of *L. corticis* show that this dipteran parasite preferred prepupae, pupae and unhealthy weevil larvae (Alfaro and Borden 1980, Hulme 1989, Hulme 1990). *L*. corticis require a threshold (0.3 *Lonchaea* per weevil pupal cell) to successfully reduce weevil populations in a leader; Hulme (1989, 1990) found that this threshold could vary with weather conditions. In cool summers two larvae per weevil pupal cell are required for adequate control whereas warmer weather required 5 or more *Lonchaea* per pupal cell. This is a major problem when using *Lonchaea* for biological control, as the numbers needed to properly control weevil populations are so high that small releases are inadequate. There is also no guarantee that the fly will stay within the desired plot for control, and since the larvae show a preference for later immature stages, tree damage will likely have already occurred from the actively feeding larvae inside the leader. The combination of *L. corticis* with other control methods might be more successful.

To date, no chemical or biological, or a combination of them, have been successful in decreasing weevil pressure in spruce stands across Canada (except avoidance which is not beneficial in the long run). Much of the current interest is focused on the discovery of ‘resistant’ trees.

Understanding tree resistance to weevil attack is important, but equally important is the understanding of weevil population genetics. The co-evolution between the weevil and its host trees is a very intricate interaction, and both sides need to be fully understood before any control strategies are implemented. Genetic studies are important in discovering the mechanism of evolution in this system, but before we can delve deeper, a further understanding of weevil population genetic structure should be in place. Whether the beetle is specific to a tree or landscape greatly shapes future genetic/genomic research and control of this insect.

1.5 White Pine Weevil Genetics

Pissodes strobi exists in a series of distinct populations across its range with much genetic variation exhibited throughout its host range (Lewis et al. 2001, Laffin et al. 2004). Previous population genetic studies focused on long distance differentiation of *P. strobi*. Lewis et al. (2000) used isozymes to investigate population structure of *P. strobi* across Canada and found that one population occurred in eastern Canada (including Manitoba) and that three distinct populations existed in British Columbia (north-central coast, Vancouver Island and the Interior). An analysis using Random Amplified Polymorphic DNA (RAPD) showed three populations: Vancouver Island, Interior BC and North Coast BC/Ontario (Lewis et al. 2001). Laffin et al. (2004) used mitochondrial DNA to detect genetic structure, with similar results to the previous two studies.

To date these genetic studies have shown that weevil genetic diversity decreases from eastern to western Canada (Phillips and Lanier 2000, Lewis et al.

2001, Laffin et al. 2004). Contrary to a study by Langor and Sperling (1997) that found one widespread haplotype for a cytochrome oxidase I (COI) region, Laffin et al. (2004) found that haplotypes from a different COI region were restricted to a few populations. Levels of genetic variation within a population could determine evolutionary responses in *P. strobi* populations to different host species and resistant trees (Liewlaksaneeyanawin et al. 2002).

Although most genetic studies on insects involve mitochondrial DNA, microsatellite DNA is also proving useful in entomology. Studies with boll weevils (*Anthonomus grandis* Boheman) have successfully shown the migration of weevils from Mexico into the US, as well as the population differences that occurred as the weevils migrated from east to west. These differences allowed the researchers to track the source from which populations of weevils were reintroduced (Kim et al. 2006).

Subramanian and Mohankumar (2006) found that *Helicoverpa armigera* (Hübner) (cotton bollworm) populations associated with cotton differed genetically to populations found on other host crops. Studies on the lepidopteran *Cydia pomonella* (Linnaeus) (codling moth), used 9 microsatellites to investigate insect population differentiation among different host plant species and among insect populations on the same host (apple) (Chen and Dorn 2010). They found that populations differed amongst the different host species as well as among insects on the same host. Populations less than 10km apart were genetically distinct and the geography of Switzerland did not account for the differences (Chen and Dorn 2010).

Microsatellites, also called simple sequence repeats (SSRs), variable number of tandem repeats (VNTRs) or short tandem repeats (STRs) are tandem repeats of DNA that can be 1-6 nucleotides in length. The entire length of the microsatellite can vary from 5-40 repeats in length or greater. Microsatellites vary in repeat sequence length through proofreading errors and slippage during DNA replication (Schlötterer 2000, Selkoe and Toonen 2006). Dinucleotide, trinucleotide and tetranucleotide repeats are most commonly used in genetic studies compared with mononucleotide repeats. Located on either side of the microsatellite are DNA regions called flanking regions. These areas allow researchers to develop primers to amplify specific microsatellite regions in the genome.

Microsatellites garnered much favour because they allowed researchers to use small tissue samples, which is important for researchers who use non-invasive sampling or smaller organisms such as insects (Selkoe and Toonen 2006). Using multiple loci with high allelic diversity can give higher resolution than other techniques providing individual identification and parentage analysis (Schlötterer 2000, Sunnucks 2000). Microsatellites are also species-specific therefore problems with cross-contamination are minimal. This is important for many insect species that ingest plant matter or other organisms, or insects that have parasites associated with their life cycle (Sunnucks 2000, Selkoe and Toonen 2006).

Although microsatellites offer advantages from previous molecular methods, they also should be used with caution. To isolate microsatellites in different individuals, flanking regions must be identical with no mutations. If there are flanking region changes, then the primer will not bind and the microsatellite will not amplify.

Failure to amplify can also be due to a low quality template. Alleles that consistently do not amplify are considered null alleles. Null alleles can cause an overestimation of homozygotes and F_{ST} values (Selkoe and Toonen 2000, Dakin and Avise 2004).

In 2008 Carlsson used the program Structure to test the effects of null alleles. Through his simulations he found that null alleles had very little effect on population allocation in Structure, and only inflated fixation index (F_{ST}) values slightly.

Research has mainly been focused on the use of resistant trees to overcome weevil population pressure. This research has shed some light on the ability of the tree to adapt and overcome pressure from the weevils. However, very little research has focused on the weevil, their population structure, and the implications of population level variation on the adaptation potential of the weevil to resistant host trees. Large-scale studies have shown that *P. strobi* has some host preferences but when forced to they can feed on alternate tree species (Soles et al. 1970, Vandersar et al. 1977). Underlying these host preferences may be a genetic component to weevil host adaptation. Large-scale population differences have already been shown via karyology, allozymes, and mtDNA studies (Phillips and Lanier 2000). Thus far, no genetic studies have addressed the question of local weevil population structure within a stand or a tree.

1.6 Objectives

The ability of weevils to associate with different tree species may also indicate a genetic component to population structure, which allows weevils on different tree species to adapt to use the host. Weevils can oviposit on non-host tree species but their success is greatly diminished on these non-host trees.

Mark and recapture studies suggest that *P. strobi* does not fly very far within plantations especially during reproductive periods (Alfaro 1994, Mehary et al. 1994, Sahota et al. 1994, Laffin et al. 2004). In plantations, weevils remain close to the point of release, creating population aggregations (Alfaro 1994, Sahota et al. 1998). In 1975 Harman conducted a mark and release study and found the majority of weevils placed in a plot change trees only once during mating and oviposition, and the majority terminated flight 12m from their starting point with a maximum distance of 90m. Overhulser and Gara (1975) found that weevil movement differed between the spring and fall, which accounted for discrepancies in reports of weevil movement. Fall weevils did not disperse as much as spring weevil populations.

McIntosh et al. (1996) noted significant weevil movement within white spruce plantations throughout the day. Weevil flight is highly affected by temperature, as during the high daytime temperatures they tend to move to laterals or duff in July or the laterals and stem in August. During the evening weevils are more prone to sit on the tree more often on either the terminal or lateral shoots in both July and August. According to this study weevils do not move further than the adjacent tree throughout the season and movement during the night is negligible.

Female movement and flight will drive the structure of populations of *P. strobi* within a stand. Structured populations within a stand can lead to genetic variations in weevil ability to adapt to resistance hosts. Studies of local genetics will lead the way to further understanding of these interactions and help to developed better models and strategies to control *P. strobi*.

The objectives of the present study are to further understand the population genetics of *P. strobi* by 1) assessing *Pissodes strobi* population structure using microsatellite DNA, 2) estimating the number of female parents in spruce leaders, 3) assessing possible movement of weevils in each plot, 4) assessing the influence of differences in stand age on *P. strobi* population structure, and 5) assessing the differences in *P. strobi* populations between Sitka and interior spruce host species.

2.0 Materials and Methods

Appropriate research locations were chosen following consultation with staff of the Pacific Forestry Centre and Western Forest Products Ltd. Six sites were recommended for the research (Figure 3), based on site availability, tree type, known presence of infestation, and an approximate age class.

Age classes were chosen based on weevil infestation dynamics as discussed in section 1.2 and shown in Figure 2. The first age category selected was in stands ranging from 5-10 years old, the second age category was selected from the plateau age range from 15-25 years of age, and the last age category was chosen from plots aged 25+ years of age.

The tree species were chosen based on the economic importance of the two species and the amount of research previously conducted on these two host species. Population markers were also chosen based on previous work by Liewlaksaneeyanawin et al. (2001). Microsatellites have the benefit of being; codominant, locus specific, highly polymorphic, PCR-based and they can reveal fine scale phylogenies (Selkoe and Toonen 2006).

2.1 Data Collection

In 2007 samples were collected from three planted, interior spruce plots. All plots were located near Prince George, BC. The Church Road (CH) (54°07.996, 121° 49.152), plot was located ~67km NNE of Prince George BC and was 5 years old. John Elmsley Woodlot (WL) (53° 55.120, 122° 53.527) located ~ 8 km West of Prince George BC, was 13 years old. PGTIS (PG) (53° 45.867, 122° 44.303)

located ~ 18 km South of Prince George BC, was 23 years old at the time of harvest (Figure 4).

Samples were also collected from three planted Sitka spruce plots. All plots were located on Vancouver Island, BC. Grafton Road (GR) (49° 16.771, 124° 29.561) located ~ 14 km WSW of Parksville BC, was 8 years old at harvest, Camp 4 (CF) (50° 05.514, 125° 22.061) located ~ 10 km NE of Campbell River BC, was 13 years old and DM1400 (DM)(44° 44.750, 124°55.779) located ~57 km South of Port Alberni BC, was 23 years. All plots were single species plantations (Figure 4).

2.2 Field Methodology

Tree height (cm), Diameter at Breast Height (DBH) and Global Positioning System (GPS) decimal degree co-ordinates (eTrex®, Garmin® Ltd), were recorded for each tree to ensure individual tree morphology was consistent within each plot and did not confound weevil population inferences (see Appendix 1 for sample tree layout in each plot). Leaders were harvested with pole and hand pruners. Each leader was labelled, placed in an individual mesh bag and transported back to the lab where they were stored at 4°C until further processed.

2.3 Laboratory Methodology

In the Treenomix laboratory (University of British Columbia, UBC), leader length and top, middle, and base diameters were measured in centimetres. Feeding punctures and oviposition plugs were counted. Weevils were removed from the leader, counted, placed in individual vials, flash frozen in liquid nitrogen and stored

at –80°C until processed for DNA extraction (Table 3). To insure statistical significance in testing, six leaders from each plot were chosen and twelve individual larvae from each leader were used for molecular testing.

Total genomic DNA was extracted from a small segment of abdominal tissue from each larva. Individual segments were minced with a razor blade before extraction. The Phenol/Chloroform (Sambrook et al. 1989) method was used to extract DNA from 429 larvae (CH=69, WL=72, PG=72, GR=72, CF=72, DM=72). Primers for SSR analysis were from previous studies (Liewlaksaneeyanawin et al. 2001) and developed in this thesis using EST sequences (Table 2). PCR protocol (see section 2.5) was the same for all primers with the exception of We3-14, which included the addition of 50mM $MgCl_2$. DNA was quantified on a spectrophotometer and diluted to 10ng/μl for PCR. Some samples (~100) were checked for DNA degradation on 0.8% agarose gels.

2.4 Primer Development

Five SSR primers were selected from Liewlaksaneeyanawin et al. (2001) based on their ability to consistently amplify from the only available primers directly associated with *Pissodes strobi*. Ten primers were developed in the Treenomix lab (University of British Columbia) using EST sequences.

An EST library was established using DNA from both *Pissodes strobi* adults and larvae. Twelve larvae were collected from one tree in a Sitka Spruce plantation (Grafton Road) on Vancouver Island. A normalised full length enriched directionally cloned library was constructed using total RNA pooled from all larvae. Twenty adult

weevils were collected from two BCFS plantations in Campbell River (Keeling pers. com). Total RNA was extracted and pooled together with the larvae. EST sequences were aligned at UBC, by the Treenomix laboratory. Microsatellites were found from the first build using MicroSAtellite identification tool (MISA) (Thiel et al. 2003). A total of 5690 sequences were examined and 88 microsatellites were found of which 53 were chosen for further testing. The primers for the new 53 microsatellites were designed by eye and PCR conditions were optimized. Two known parental crosses were provided by Liewlaksaneeyanawin et al. (2002) to test the final 10 sets of cDNA microsatellite primers for the presence of null alleles. Each parental cross test included eight female and eight male offspring. In total 32 offspring and 4 adults were tested. Results were quantified as above in section 2.5 paying close attention to the presence of null alleles.

2.5 PCR Protocol

The PCR reactions were performed in a total volume of 10μl using 10ng/μl genomic DNA, 5U/μl Paq reaction buffer (Stratagene, La Jolla, CA), 2mM dNTP's (New England BioLabs Inc., Beverly, MA), 10mM Forward and Reverse primer (Eurofins MWG Operon, Ebersberg, Germany), 2mM Paq (Stratagene, La Jolla, CA), 10μM M-13 Primer (Li-Cor® Bioscience, Lincoln, NE) and dH_2O. Amplification was performed in an MJ Research thermal cycler (Waltham, MA) under the following conditions; one cycle for 5 minutes at 94°C, 35 cycles of 1 minute at 94°C, 45 seconds at respective their annealing temperatures, and 45 seconds at 72°C, followed by an extension cycle for 5 minutes at 72°C. PCR products were stored at –20°C until further analyses were performed.

Samples were denatured at 90°C for 3 minutes before being loaded onto 5% polyacrylamide gels. A Li-Cor 4200 automated sequencer (Li-Cor® Biosciences, Lincoln, NE) was used for electrophoresis. A 48 well comb loaded with 1.0ul of sample and four standard markers (Li-Cor® Biosciences, Lincoln, NE) were used on each gel. Microsatellite data were scored on SAGA™ (Li-Cor® Biosciences, Lincoln, NE), which assigned molecular weight values to each allele on the gel.

2.6 Analysis Protocol

Statistical programs were chosen to infer parental genotypes of the weevil larvae and show shared genotypes to elucidate the question of how many females oviposit on a single leader as well as possible distances travelled by weevils for reproduction. The question of how genotypes are dispersed among trees and plots will lead to a better understanding of weevil host choice and preference. The larvae were also grouped into populations to ascertain whether population structure changes as the host stand ages. Data were also tested to insure they follow the assumptions used in each program: linkage equilibrium and Hardy-Weinberg equilibrium.

The allele size results obtained from visual analysis in SAGA™ (Li-Cor® Biosciences, Lincoln, NE) were then formatted for analysis for use in: Colony for parentage analysis (Wang 2004); Structure (Pritchard et al. 2000) and Tess (Chen et al. 2007, Durand et al. 2009) for population structure inference; Genepop (Raymond and Rousset 1995) and MSA (Dieringer and Schlötterer 2003) for F-statistics and Rcmdr (Fox et al. 2009) for general statistical analyses.

Colony (Wang 2004) is a maximum likelihood method designed to infer parentage/sibships amongst sampled individuals. Offspring are assigned to a parental male and female genotype. The assumptions are as follows: markers are in linkage equilibrium; markers are in Hardy-Weinberg equilibrium; and offspring are full sibs, half sibs or unrelated. Colony also includes the ability to assign error values to each locus (dropout rates and other genotyping errors). In this study, parental genotype refers to the parental male as a single genotype and parental female as a single genotype. Full sibs implies the offspring share both a parental male and a parental female, while half sib implies either a single parental male or single parental female is shared between offspring.

Structure (Prichard et al. 2000) is a model-based program used to infer genetic structure by clustering genotype data. Individuals are assigned to each cluster probabilistically and can therefore be assigned to more than one genetic cluster. The assumptions of the model are: 1) there is K unknown populations each characterized by a genotype; 2) all loci are in Hardy-Weinberg equilibrium; 3) all loci are in linkage equilibrium. Results from structure were further processed using the Evanno method to estimate a more accurate number of genetic clusters from data provided by structure (Evanno et al. 2005).

Tess (Chen et al. 2007, Durand et al. 2009) uses a Bayesian clustering methods to assign individuals to genetic clusters. This program was used to ensure that results from Structure were consistent.

Genepop (Raymond and Rousset 1995) provides exact tests for Hardy-Weinberg equilibrium, population differentiation and disequilibrium. This program

uses the Markov chain method for Hardy-Weinberg Equilibrium (HWE) tests. F_{ST} values obtained from this program were interpreted using Wrights guidelines: 1) 0-0.05 range indicates little genetic differentiation, 2) 0.05-0.15 indicates moderate genetic differentiation, 3) 0.15-0.25 indicates great genetic differentiation and 4) >0.25 represents very great genetic differentiation (Hartl and Clark 1997). F_{ST} ranges from 0 (equal allele frequencies) to 1 (all subpopulations are fixed for different alleles). F_{IS} is a measure of departure from Hardy-Weinberg equilibrium, which ranges from -1 to 1. A value of -1 indicates a population is completely outbred, 0 means the organisms meet HWE, and a value of 1 show they are inbred completely. F_{IT}, the inbreeding coefficient ranges from -1 to 1 where negative values express excess heterozygosity and positive number indicate inbreeding or the presence of null alleles. Microsatellite Analyser (MSA) (Dieringer and Schlötterer 2003) was used to verify results obtained from Genepop.

Rcmdr (Fox et al. 2009) was used for test of normality, ANOVA, t-tests, and non-parametric statistics. The program used the Shapiro-Wilks tests for normality, multi-way ANOVA, one sample t-tests and the Kruskal-Wallis tests for non-parametric analyses.

3.0 Field Data Results

A total of 100 leaders were collected from all six plots. Fifteen leaders were collected in Church Road (CH), eighteen leaders were collected from the Woodlot plot (WL), nineteen leaders in total were collected from PGTIS (PG), Grafton Road (GR) had a total of seventeen leaders collected, Camp 4 (CF) had a total of 17 leaders, and DM1400 had 14 leaders collected from the plot. The initial intention was to collect 12 trees per plot. Extra trees were collected to insure a minimum number of 12 leaders contained weevils for further molecular investigations. The data collected were analysed using statistical software to elucidate differences between leaders in each plot, between different age plots for each host species and differences in weevil populations between different host species.

3.1 Differences In Measured Variables Between Leaders In Plots

In this study a total of 135 larvae emerged from 1037 (13%) oviposition plugs in Church Road, Woodlot had 500 from 1436 (35%), while PG had 216 larvae from 1462 (14%) oviposition plugs. In Grafton Road 487 weevils emerged from 2168 (22.5%) oviposition plugs, 650 weevils emerged from 3391 (19.2%) plugs in Camp 4 and 1000 weevils emerged from 1446 (69%) plugs in DM1400.

Church Road had only one leader of the 15 collected, with no weevils present and a range of 1-23 weevils collected per leader. Woodlot had a range of 1-85 weevils present in a leader, and four trees had only one larva in the leader. In PG there were three leaders with no weevils, and a range of 0-33 weevils present per leader. Grafton Road had one leader with no weevils, and a range of weevils

present per tree of 0-65. The Camp 4 plot had only one tree with no weevils present and the number of weevils present ranged from 0-121. Of the total trees collected from DM1400 only one had no weevils present and a range of weevils per leader of 0-142.

The Sitka and interior spruce plots shared the same height and diameter characteristics. The youngest plots had the smallest values on average for height (CH: 117.1, GR: 306.5cm) and DBH (CH: NA, GR: 11.7cm) and the oldest plots the largest values on average for height (PG: 432.5cm, DM: 842.6cm) and DBH (PG: 7.3, DM: 13.3) (Table 4).

The youngest plots of both interior and Sitka spruce plots had the lowest values on average for leader length. Church Road had an average leader length of 35.2cm and Grafton Road had an average leader length of 61.0cm. The oldest plots PG and DM had the longest lengths, of 53.7cm and 71.8cm respectively (Table 4).

Diameter values also followed the expected results with the younger plots in both host species having the smallest values, and the oldest plots with the largest leader diameter values on average (Table 4).

In both interior spruce and Sitka spruce plots the middle age class had the highest average number of feeding punctures (Table 4). The highest number of feeding punctures in the interior spruce plots was found in Woodlot with on average 19.3 per leader. In Sitka spruce plots, Camp 4, the middle-aged host group, had the highest average number feeding punctures per leader (33.8).

The lowest average number of oviposition plugs (69.3) in the interior spruce plots occurred in Church Road (youngest plot). The lowest average number of oviposition plugs (103.3) in the Sitka spruce plots occurred in DM1400 (oldest plot).

In all of the three plots in each host species the lowest average number weevils present were in the youngest plots. Church Road had on average 9 weevils per tree while Grafton Road had 28.7 per tree. The highest average number of weevils per tree occurred in Woodlot (27.8), the middle-aged interior spruce plot, and in DM1400 (71.4), the oldest Sitka spruce plot (Table 4).

In the fifteen trees sampled in Church Road, six were positive for the presence of *Lonchaea corticis*. The single leader in this plot that had no weevils present contained no *L. corticis*. Of the six trees sampled from CH for this thesis CH05, CH08 and CH12 were positive for the presence of *L. corticis*. These three leaders also had some of the highest numbers of total weevils present (11, 11 and 23 respectively).

Of the 18 trees collected in Woodlot six trees were recorded for the presence of *L. corticis*. Of the six trees used for further testing, none contained the parasitic fly.

Of all the trees collected from PGTIS, four contained *L. corticis*. These four leaders had a range of 4-29 weevils present, which were some of the lowest values for weevils present in this plot.

Grafton Road was positive for *L. corticis* in sixteen of the seventeen trees. All of the six trees used for further testing were positive for *Lonchaea* and had a range of 37-65 weevils present which were some of the highest values in this plot. Camp 4

had all seventeen trees positive for the presence of *L. corticis* but no trees in DM1400 contained the fly larvae.

3.2 Differences In Measured Variables Between Plots

Statistical tests on differences between plots of interior spruce and Sitka spruce revealed that there were significant differences in tree height between the plots within each host species. Diameter at breast height was also significantly different between each host species plot. In both the interior and Sitka spruce plots, the youngest plots were the shortest and thinnest, while the oldest plots were the tallest and thickest.

There was a significant difference between leader top, middle and base diameter between plots within each host species. Significance values ($p< 0.05$) for the interior spruce plots for top, middle and base diameter were: $F_{2,49}$=19.79, $F_{2,49}$=31.89 and $F_{2,49}$=28.90, respectively. Significant values ($p<0.05$) for the Sitka spruce plots for top, middle and base diameter were: $F_{2,45}$=18.51, $F_{2,45}$=23.86 and $F_{2,45}$=13.15 respectively.

This also held true for leader length measurements. ANOVA showed a significant difference ($p<0.05$) between the interior spruce plots ($F_{2,49}$=26.92) and between the Sitka spruce plots ($F_{2,45}$=30.89). The smallest diameters occurred in CH and GR, while the largest were found in PG and DM.

Feeding punctures did not differ significantly between interior spruce plots ($F_{2,49}$=0.445, $p>0.05$), but they did differ between the Sitka spruce plots ($F_{2,45}$=5.80,

$p<0.05$). This highest number of feeding punctures occurred in WL and CF, while the smallest amount of feeding punctures occurred in PG and DM.

The oviposition plugs between the interior spruce plots showed no significant differences in mean values ($F_{2,49}=0.137$, $p>0.05$). The Sitka spruce plots did show a significant difference in the mean number of oviposition plugs present in the three plots using Kruskal-Wallis significant tests ($X^2=9.59$, df=2, $p<0.05$). Church Road had the smallest number of plugs while Woodlot had the highest value for the interior spruce plots. In the Sitka spruce the lowest number of oviposition plugs occurred in DM while the highest number was found in GR.

The mean number of weevils did not differ significantly between plots in the interior spruce ($X^2=0.174$, df=2, $p>0.05$) but did differ significantly in the Sitka spruce ($X^2=8.28$, df=2, $p<0.05$) plots using the Kruskal-Wallis test. The fewest weevils present were from Church Road and Grafton Road, while the most weevils collected were from Woodlot and DM1400.

Numbers of feeding punctures and oviposition plugs were greater in both Woodlot and Camp 4. The most weevils present for the interior spruce were in the Woodlot plot (middle age) but in Sitka spruce, the most were in the DM1400 plot (oldest age).

3.3 Summary of Age Group Data

Tests for significant differences between the same plot ages between the different host species revealed that for Church Road vs. Grafton Road there were

significant differences between mean numbers of all measured values except feeding punctures. All measured variables were normally distributed.

t-tests comparing means for the middle age groups (WL and CF) indicated that there were significant differences between all measured variables except the number of weevils present between these two host species plots. The weevil presence data for this age category was not normally distributed, therefore a Kruskal-Wallis rank sum test was used to compare means.

All variables except for feeding punctures were significantly different between the two host species in the oldest plot age category. Leader base diameter and oviposition plugs were the only variables that were not transformed. Leader length was not normally distributed and the Kruskal-Wallis test was applied to these data.

3.4 Differences In Measured Variables Between Interior And Sitka Spruce

In both the interior and Sitka spruce plots, average height increased with the age of the plot. This was also true for leader length. Average leader lengths were larger in the Sitka spruce plots than in the interior spruce plots. PGTIS, the oldest interior plot had on average leader lengths of 54.0cm while the youngest Sitka spruce plot averaged 61.2cm (Figure 4).

Leader diameter followed the same pattern with the oldest plots having the thickest leaders. There was some overlap of values with the oldest interior spruce plot having slightly smaller diameter leaders than the Sitka middle aged plot (CF).

More feeding punctures occurred on average in the Sitka spruce plots. Both host species had the most punctures recorded in the middle age classes. None of

the values overlapped between the host species plot (Figure 5). Oviposition plugs were also more prevalent in the Sitka spruce plots which sometimes had mean values twice the amount of the interior spruce plots (Figure 5).

In all age classes fewer weevils were present in the interior spruce plots. Mean values were higher in all three age classes for Sitka spruce (Figure 5). No strong relationships existed between any of the measured variables and weevil presence. All values for R^2 were relatively low (Appendix 4).

4.0 Local Population Structure Using SSR Markers

4.1 Null Allele Test

Null allele tests on the 10 new microsatellite primers did not reveal the presence of any null alleles. Null alleles for the primers provided by Liewlaksaneeyanawin et al. (2002) showed that We2-19 and We3-16 had values of 0.235 and 0.335 respectively. However, Carlsson (2008) found that the presence of null alleles marginally affected population assignment even with null allele values of 0.913. The new EST-derived primers developed had null allele values calculated in Genpop (Raymond and Rousset 1995) of less than 0.300.

4.2 Parentage Differences

There were 13 different full sib groupings in Church Road (CH) (Table 5). Two trees in Church Road (CH02 and CH12) had the highest number of full sib families (3) for this location, while CH02 and CH12 had the most individuals (4) in one of their respective full sib groupings. In Woodlot (WL) there were a total of 10 different full sib groupings (Table 5). WL13 had the highest number of different full sib family groupings (3) at this location and WL07 had no full sibships. PGTIS (PG) had a total of 13 different full sib family groups (Table 5). The largest full sib family groupings contained 3 individuals and were found in PG01, PG02a, PG10 and two in PG17. PG17 also had the most full sib families per tree (3). PG05 and PG09 also shared two different full sibships.

In the Sitka spruce plots, Grafton Road had a total of 13 different full sib families (Table 5). GR10 had the highest number of full sib families (4) present on one tree while GR09 had the largest full sib family with four individuals. Camp 4 had 13 full sib families (Table 5) with C409 having the most number of full sib families. There were full sibships shared between trees in Camp 4; one family was shared between CF03 and CF08, one family was shared between CF09 and CF14; one group was shared between CF09 and CF11; a second different group was again shared between CF09 and CF11 and the last full sib family grouping occurred between CF11 and CF14. CF03 had no sibships present within this leader. DM1400 had 7 full sib families (Table 5) with two of these families occurring between trees. DM04 and DM08 shared one full sibship while DM05 and DM11 shared a different full sibship. The largest family in this plot had 4 individuals and occurred in DM01 while DM01 also had the highest number of different sibship families present (2).

In Church Road 50 different parental genotypes were assigned to this plot, among which 35 were unique. The fifteen remaining parental genotypes were shared between the trees. Of these fifteen genotypes, two occurred on three (one on CH03, CH08, CH12 and one on CH02, CH08, CH10) out of the six trees. Woodlot had a total of 62 parental genotypes were inferred in the plot, of these 41 were unique. Twenty-one parental genotypes were shared between trees, with one parent appearing in four of the six trees (WL02, WL07, WL10, and WL12). PGTIS had 48 inferred parental genotypes, thirty-two of these were unique and sixteen parental genotypes were shared between trees. A single parental genotype

occurred more often and was shared between four of the six sampled trees (PG05, PG09, PG10 and PG17) (Table 5).

In the Sitka spruce plots (Table 5), Grafton Road had forty-seven inferred parental genotypes with 27 of these being unique. Twenty parental genotypes were shared amongst the trees sampled in the plot. One parental genotype was found in 4 (GR01, GR10, GR11, GR15) trees. Camp 4 had forty-eight inferred parental genotypes with 20 unique and twenty-eight of those shared amongst the 6 sampled trees. One parental genotype occurred more often in four of the six trees (CF08, CF09, CF11, and CF14). DM1400 had forty-five inferred parental genotypes, of those eighteen were unique and twenty-seven were shared throughout the six sampled trees. Four different individual parent genotypes occurred more often between trees (occurred amongst four different tree combinations). Two single parental genotypes were present in the same groups of trees: DM01, DM05, DM08 and DM11. The third most common single parental genotype occurred in DM01, DM04, DM08 and DM11, while the final most widespread single parental genotype was found amongst DM01, DM04, DM11 and DM14.

The most unique parental genotypes in Church Road (8) occurred on CH05, WL13 had ten, PG17 had eight, GR09 had six, CF15 had seven and DM14 had seven. DM11 is the only tree that had no unique parental genotypes. The tree in Church Road with the most shared parental genotypes was CH03 with seven. WL07 shared 13 parental genotypes and both PG05 and PG17 had nine. GR10 had eleven shared parental genotypes, CF03 had fourteen and DM08 had sixteen (Table 5). The average number of unique parental genotypes per tree in Church Road,

Woodlot, PGTIS, Grafton Road, Camp 4 and DM1400 were 5.83, 6.83, 5.50, 4.50, 2.83 and 3.00, respectively.

CH02 and CH03 were the closest (3 metres apart) trees in Church Road and only shared two parental genotypes. The closest trees in Woodlot were 0.46 metres apart and shared two parental genotypes. In PGTIS, PG09 and PG10 were 2 metres apart and shared three. Three metres separated the closest trees in Grafton Road, which shared four parental genotypes. Camp 4 shared two parental genotypes in trees 13 meters apart, while DM1400 shared two genotypes between trees 32 metres apart.

The trees furthest apart (75m) in Church Road shared no common weevil parents. Woodlot shared three parents between trees 63m apart and PGTIS shared one between trees 78m apart. Grafton Road shares four parents with trees 94 metres apart, Camp 4 trees 59m apart share only one, while DM1400 trees 532m apart share six parents.

Church Road can be used as a model of weevil populations in leaders as all but one of the 6 sampled leaders had complete population enumeration. In Church Road there was minimum range of 4-6 females per tree with an average of 5.67-5.50 females per tree. Each female weevil in Church Road produced an average of 2.05-2.14 larvae per leader. Woodlot had 5-11 parental females per tree with an average range of 7.00-7.33. On average one female from Woodlot can produce 0.87-1.78 larvae. PGTIS had 4-9 parental females per tree with an average of 5.33-6.33. In the samples collected from PGTIS females on average produce 2.04-2.35 larvae. Grafton Road had 4-8 females per leader with an average of 5.00-7.50 females per

tree. Each female successfully produced on average 1.60-2.55 larvae in each leader. Camp 4 had a range of parental females of 6-9 with on average 6.80-7.20 females per tree and each female produced 1.69-1.82 larvae per tree. The parental females in a single leader in DM1400 ranged from 3-11. Each tree supported on average 7.50-7.70 females that produced on average 1.60-1.90 larvae. All the numbers presented above were calculated from the larval enumeration on each leader as well as the count of inferred number of female genotypes from Colony (Wang 2004).

4.3 Population Differences Between Plots

Results from population structuring programs inferred 6 populations in Church Road, five populations for Woodlot, four for PGTIS, five for Grafton Road, four for Camp 4 and three for DM1400. Structure outputs are shown in Figure 8.

Based on results calculated using the program structure, CH08 and CH05 formed two distinct population clusters. A third distinct population cluster was formed with 67% of the individuals from CH02, while the rest formed a fourth cluster with CH03. Ten of the twelve CH12 offspring clustered in their own population, forming the fifth population while the last structure cluster contained two individuals from CH12, one from CH03 and all of CH10 (Figure 6).

In WL16 nine of the 12 individuals belonged in one population. All of WL10 cluster into a second population with three members from WL07, two from WL12 and two from WL13. The last three clusters are made of mixtures of various individuals from the six trees. Two of the remaining clusters contain individuals from

5 of the trees, while the last cluster contains individuals from only WL02 and WL07. Not all individuals were strongly assigned to only one population cluster; some individuals had low probability scores or similar scores placing them in more than one population cluster. No individuals were partitioned between more than two population clusters (Figure 6).

PG01 was in one population cluster. A second population cluster contained all individuals from PG05, five from PG02, six from PG09 and one from PG10. The third population cluster contained all of PG17 plus one PG10, and six from PG09. The last cluster had eleven of the PG10 individuals with seven from PG02. The majority of the individuals had high probability assigned to each cluster.

The first population cluster contained 9 individuals from GR01, 1 from GR09, 7 from of GR10 and 2 from GR11. All of GR02 clustered into a second population with 1 from GR10. One individual from GR01, 9 from GR09 and 1 from GR10 clustered in a third population. Nine individuals from GR11 clustered into a fourth population. All individuals in GR15 grouped in the final fifth population. Low cluster assignment probabilities (<0.5) were not included. Five individuals were closely associated with more than one population (Figure 7).

Seven individuals from CF03, four from CF08, and three from CF15 clustered into population one. Seven from CF09, five from CF11 and nine from CF14 clustered together in population two. Three individuals from CF03 and nine individuals from CF15 clustered into population three. One from CF08, three from CF09, five from CF11 and three from CF14 clustered in population four. Individuals

not listed are either shared between two or more populations or their probability values were below 0.5 (Figure 7).

Population cluster one contained 6 individuals from DM04, 1 from DM05, 7 from DM08, 3 from DM11 and 9 from DM14. All of DM01, 5 from DM04, 3 from DM05, 2 from DM08 and 1 from DM11 and three from DM14 formed cluster two. Eight from DM05, 2 from DM08, and 7 from DM11 formed population cluster three (Figure 7).

4.4 Inbreeding Coefficient Within And Between Populations

The Church Road plot had an inbreeding coefficient that indicated a deficit in heterozygosity and an F_{ST} value that showed good genetic structuring according to Wright (see section 2.6). The number of immigrants per generation in Church Road was less than the other interior spruce plots (Table 6). Woodlot had the largest deficit in heterozygosity in the interior spruce plots, and the highest number immigrants per generation (Table 6). The values for PGTIS were in between Church Road and Woodlot. PGTIS also had good genetic structuring according to Wright (Table 6).

Grafton Road had the lowest inbreeding coefficient for the Sitka spruce plots and the highest F_{ST} (Table 6). Camp 4 and DM 1400 had very similar inbreeding coefficients and F_{ST} values (Table 6). Camp 4 had the highest number of immigrants per generation while Grafton Road had the lowest (Table 6).

All plots are in heterozygosity deficit and every plot except Camp 4 had equal allele frequencies. Private alleles occur between interior spruce plots and more than one private allele may occur in one locus. Church Road had 5 private alleles, Woodlot had 9 and PG had 3.

Private alleles in CH were in the following loci: PST02K14 (1 allele), PST04C09 (1 allele), PST04L04 (1 allele) and We2-19 (2 alleles). The Woodlot private alleles occur in PST0D01 (3), PST02K14 (1), PST02P01 (2), PST04E11 (1), PST04L04 (1), We2-19 (1). PG had three private alleles: PST02L13 (1), PST04E11 (1) and We2-19 (1).

Private alleles were present when comparing all three Sitka spruce plots. Grafton Road had 15 private alleles, Camp 4 had 10 private alleles, and DM1400 had 36 private alleles. In Grafton Road the private alleles occurred in: PST02D01 (1), PST02L13 (2), PST04C09 (1), PST04L04 (1), We2-19 (2), We3-14 (5), We3-16 (3). In Camp 4 the ten private alleles occurred in: PST02J22 (1), PST02J24 (2), PST02P01 (1), PST04E11 (1), PST04L04 (1), We2-19 (1), We3-14 (2) and We3-16 (1). In DM1400 private alleles occurred in: PST02J22 (1), PST02L13 (1), PST02P01 (6), PST04C09 (2), PST04E11 (2), PST04I09 (1), PST04L04 (3), We2-19 (4), We2-7.2 (2), We3-14 (6), We3-16 (3) and We3-18 (5).

In general there were more private alleles per plot in the Sitka spruce than in the interior spruce. The interior spruce plots had more private alleles in Woodlot (middle age class) where DM1400 (oldest age class) had the highest number of private alleles.

When comparing private alleles between interior and Sitka spruce plots Church Road had three private alleles: one in PST02K14, one in PST04C09 and one in We2-19. Woodlot had 5 private alleles: two in PST02D01, one in PST02K14, one in PST02P01, and one in We2-19. PGTIS had no private alleles. Grafton Road had 8 private alleles: two in PST02L13, one in PST04C09, one in We2-19, three in We3-14, and one in We3-16. Camp 4 had 6 private alleles: one in PST02J22, two in PST02J24, one in PST04L04, and two in We3-14. DM1400 had no private alleles.

5.0 Discussion

This study was designed to further understand the genetics of white pine weevil populations in interior and Sitka spruce stands in British Columbia. This initial assessment of population genetics has shown differences in weevil populations exist throughout the age of a stand and warrant further investigation. Currently at the University of British Columbia, the Treenomix laboratory is investigating the genomics of spruce. This weevil study shows that weevils may respond to more than tree morphology and further investigation of the possible genetic connection between host and pest is required.

5.1 Population Biology Findings

Interior spruce trees support fewer weevils than Sitka spruce host trees. This may be due to the larger size and vigorous growth of the Sitka spruce tree and its leader. In both host species, middle aged plots yielded the most oviposition plugs and punctures, but differed in which age class yielded the highest number of larvae. In the interior spruce plots, the highest number of larvae was observed in the middle age class (WL) while the oldest Sitka spruce plot (DM) yielded the highest number of larvae. However, when regression analysis was applied to all measured variables against number of larvae retrieved from the leaders, no strong relationships were evident (Appendix 4). It was not expected that the two host species would support the same number of offspring, given the differences in length and diameter of the leaders between the two host species (i.e. larger leaders of the Sitka spruce should support more larvae).

My results concur with the conclusion reached by VanderSar and Borden (1977) who found that *P. strobi* preferentially infests long, large diameter leaders. When plot selection was undertaken for this study, the graph by Alfaro (1994) was used as a guideline to obtain trees in the beginning of the population growth cycle, the equilibrium region and in the population decline region (Figure 2). However, our data for the oldest Sitka spruce did not include the population decline phase, which accounts for the higher abundance of weevils in DM1400. The graph by Alfaro (1994) is not an absolute and local variations in this population cycle can and do occur as evident in my study.

The eggs of the white pine weevil require a minimum leader bark thickness of 0.8cm to support development (VanderSar and Borden 1977a). Measurements of bark thickness were not taken, but could account for the larger number of larvae supported in the middle age and older age classes, as bark thickness increases with tree age (Sullivan 1961, Manville et al. 2002, Manville et al. 2004). However, as the tree ages the composition of the tree changes which make it less attractive to ovipositing female weevils and this may override any benefit of the thicker bark (Alfaro 1994). More recently, Robert and Bohlmann (2010) found that bark thickness was not significantly different between susceptible and resistance Sitka spruce trees and therefore was not a major factor in the different feeding patterns exhibited in their study. Trees in my study were relatively young, natural stands of Sitka can live 700-800 years (Farrar 1995), and would not account for the larger differences in bark thickness and weevil activity of the previous studies.

The number of weevils emerging is not consistently estimated from the number of oviposition plugs present on a leader. In this study, in both host tree species, the number of larvae collected did not reflect oviposition plugs recorded. Originally female weevils were first thought to deposit only one egg per oviposition plug, however, studies have shown that more than one egg can be laid in each plug (Trudel et al. 2001). However, in all trees, in all six plots, the number of larvae collected was less than the number of oviposition plugs. This may result from egg failure, the recorded presence of *L. corticis* (Alfaro and Borden 1980, Hulme 1989, Hulme 1990) in leaders in each of the study plots, or possible competition between larvae. I also noted that some eggs were laid in a clustered pattern.

Eggs were deposited in a cluster near the top of some leaders and also deposited further down the leader with substantial space between the two oviposition sites. In open grown stands egg deposition occurs at the top of the leader and is generally evenly distributed, whereas shaded trees can produce irregular oviposition patterns (Dixon and Houseweart 1983). Deposition of eggs in an irregular manner in open grown trees, may decrease competition between the clusters, assuming resources between the clusters are adequate or it could be the result of competition; one female deposits early on to ensure survival and the later laying female must oviposit lower on the leader. If this type of oviposition pattern was due to competition, then one underlying assumption of this system is that some type of chemical control/signal occurs between adult female weevils.

Currently chemical signalling between female weevils has not been shown, but occurs readily in insects. One example of female oviposition-deterring

pheromone occurs in *Monochamus alternatus* Hope (Japanese pine sawyer) (Coleoptera: Cerambycidae). After the female deposits her egg in a scar in the bark of the host tree, she secretes a jelly-like substance into the tree wound then rubs the wound with her abdomen. Other females of the species inspect the tree scar by touching their antennae and mouthparts to the area, and if the pheromone is discovered the female leaves the area and does not deposit an egg (Anbutsu and Togashi 2001).

The investment in offspring production is one of the key driving forces for population adaptation. If females place all their reproductive effort into one single tree, and if that one tree dies, all her future investments are lost. Female reproduction is also important for the potential deployment of resistant trees as females must lay eggs in trees that assure the survival of the offspring. The number of females reproducing per tree can give an indication of how quickly resistance to a host tree may accumulate in the weevil population. If females are able to reproduce in a greater number of trees they may come into contact with trees of various gradations of resistance allowing the possibility of the female to produce offspring that are also capable of infesting a greater range of host resistance types.

If females were to invest time in ensuring the survival of their offspring in one leader then one or very few females would occupy a single leader for reproduction. Although not all weevils were assayed on each branch (see Appendix 2), if Church Road was used as an example, we can see that CH10 had 10 larvae with 6 inferred mothers and fathers; this means that each mother produced 1.67 successful larvae on this single leader. As parentage is inferred from Colony (Wang 2004), we can

estimate the average number of larvae produced per female per tree and plot (per 12 larvae sampled). No tree in our experimentation contained a single female per leader. On average females will lay twice as many eggs per tree in the younger and older aged stands than during the more dynamic middle-aged stands regardless of host species. As weevil movement within a plot increases, so does the female's chance to spread her genetic investment and possibly insure greater success when competition for leader resources are so strong.

5.2 Genetic Findings

The genetic clusters calculated by Structure confirm the movements I inferred using the Colony (Wang 2004) results. This change in structure can be used to estimate not only how to use resistant trees within a plot, but to help apply combinations of other methods as the stand ages to decrease/control weevil damage.

The population structure of *P. strobi* changes as host stands age. Younger plots in both interior and Sitka spruce, show more genetic clusters, which could be due to the initial colonization of the plot. There is an abundance of resources for the weevil as all leaders in a plot should be available at initial population establishment. If there are enough resources, females may tend toward staying in/around one tree and invest more time and effort in egg deposition (i.e. increased egg deposition on a single leader equals increased reproductive investment on that single leader). Resources for a female white pine weevil include adequate sun on a leader to ensure optimal temperature for larval survival, sufficient leader size for larval survival/competition and adequate leaders for adult feeding (Sullivan 1961, Silver

1968, VanderSar and Borden 1977a, Taylor et al. 1994). This is clearly shown in both the younger interior spruce and Sitka spruce plots, where females produce more offspring per tree.

The genetics of the weevil population on each tree in the younger plots would therefore differ as weevils from other areas immigrate and colonize each tree with their unique genetic makeup. The genetic structure of weevils in the younger plots is linked to the genetic structure of the individuals arriving into the plot. Female weevils could be previously mated and only deposit eggs on leaders. Mating with males currently inhabiting the plot may not occur, which would make each tree a distinct population or deme.

As the host plot ages and movement within the plot increases, we find that mating occurs between more members in a plot and more leaders share common parents. Toward the end of the attack dynamic cycle (Figure 2), the populations become more defined (less individuals shared between multiple trees) but may be less in number as the trees become less desirable to most weevils and emigration occurs. What occurs in the older plots is the possible return to the population structure of the long-term residents of the plot. Alfaro (1994) suggested that weevil populations have a resident population that maintains a low infestation level in natural stands, which may also occur in plantations.

One major missing link to understanding population structure within a tree or host stand is a clear understanding of the chemical signalling between the weevil and host, as well as between individual weevils. It is clear that vision (VanderSar and Borden 1977) and close range feeding stimulants (VanderSar and Borden

1977a) play a role in weevil host preference, but it is still unclear to what extent and what are the definitive chemical(s) or mechanism(s) driving the local genetic structure of the white pine weevil. What this study shows is that local *Pissodes strobi* genetic structure within a plot and between trees changes throughout the age of the host tree but the driving force for this structure is unclear.

As weevil immigration and movement into the middle-aged host stands increase there is a definite increase in the number of male and female parental genotypes detected. As the weevils move around the plot they encounter more possible mates, and can produce more variable offspring, which would increase the number of parental genotypes detected (male and female genotypes are considered two separate genotypes) by Colony (Wang 2004). The increase in movement and mating was also confirmed by the decrease in unique parents (a single male or female parental genotype recorded only once in a sample plot) in the middle-aged stands of both host species. Less individual unique males and females should be found as increased movement leads to more genotype dispersal and females invest less time per tree depositing eggs. The overall higher number of individual male and female parental genotypes in the interior spruce plots may also be due to the hybrid nature of the host and the hosts' geographic location. Trees used in this study were hybrids (*Picea engelmannii* x *Picea glauca*) located on plantations around Prince George, BC. The area surrounding Prince George is part of the natural hybrid zone between white and Engelmann spruce (Meidinger et al. 1991). Since the interior spruce host is a hybrid, weevils that were successful on either white spruce or Engelmann spruce might have an adaptive advantage in colonizing its hybrid. If

weevils moved from eastern to western Canada (Phillips and Lanier 1985, Lewis et al. 2000, Lewis et al. 2001, Laffin et al. 2004) then, weevils able to colonise Sitka spruce might have been rare, leading to a smaller founding population in Sitka spruce.

Currently there is no consensus on how far this insect can travel in a plot during one season, or its lifespan. Prior to this study, the maximum distance recorded for *P. strobi* travel was 1.2km recorded for one individual weevil (McMullen and Condrashoff 1973). Godwin et al. (1957) found that weevils can travel up to 125m during spring flight, which is the key time for dispersal of this insect, and McIntosh et al. (1996) found they moved no further than to the adjacent tree. Data presented in the current study has shown that weevils will move further than previous estimates (Silver 1968, Harman 1975, McIntosh et al. 1996, Tomlin et al. 1997) and geographically close trees do not necessarily share similar genetic structure.

Weevil dispersal was greatest in Sitka spruce plots with 590 metre dispersal distance observed in one season. This large dispersal distance was accounted for by 9 male and female parental genotypes in DM 1400 travelling a minimum distance (one tree to one tree, as the crow flies), as individual mothers and fathers could have travelled to more than one tree in the plot, increasing the actual total distance travelled. In this study, distance travelled was estimated for male and female parental genotype as assigned by Colony (Wang 2004). It is also important to consider that females could multiple mate and carry male sperm long distances; males therefore would not have necessarily travelled the long distances. This large

distance travelled was not observed in the interior spruce plots, but this may be an artefact of sampling, as no trees used for the analysis were >100m apart. It is possible that the weevil could travel farther distances in one season than recorded in this study, but the distance between sampled trees limited our conclusions regarding maximum distances travelled. It is also highly possible that long distance travel, either by flight or wind, may occur more often then originally hypothesized and total distance travelled by a weevil in its four year life span is greater than previously suggested. However, it has been noted that weevil flight decreases during windy periods (Harman 1975) but this does not mean it will not occur in a small number of weevils. Weevils in the current study were either extremely strong fliers or walkers. They may have used wind as a means of dispersal or there were very few windy days in the plots. Weevil movement has important implications on detecting future outbreaks, as previously fertilized females can potentially travel farther to start new populations in other near-by spruce stands.

The microsatellite data presented here has shown that genetic structure of the weevils changes throughout the age of the stand and differs between host species and between individual trees. It has also been shown that large-scale differences in weevil population genetics occur (Phillips and Lanier 1985, Lewis et al. 2000, Lewis et al. 2001, Laffin et al. 2004). These studies further delineate British Columbia populations into Interior, coastal and northern populations, which along with our population differences might lend credence to the deme formation hypothesis occurring in white pine weevil.

The deme formation hypothesis (Edmunds and Alstad 1978) theorizes that insects will evolve specific traits in response to life on individual host plants. Insects should be more successful on their natal plants than conspecifics in the same area or novel areas. Traits can evolve at greater rates in phytophagous insects that spend most of their lifecycle in close association with their host species (Mopper 1996). Using allozyme data, Alstad and Corbin (1990) found different genetic structure in scale insects on different branches of the same tree. Research into deme formation has mainly relied on sessile insects. In 1995 Mopper et al. used *Stilbosis quadricustatella* (Chambers), a lepidopteran leaf miner, to test local deme formation on a mobile insect. Through measuring mine initiation/completion they were able to show that this insect was more successful in natal than novel sites; mines were initiated at a higher rate on natal host plants and the insect had higher initial success on natal trees. Even though this species is highly mobile in the adult stage, it showed a preference for site, host and individual trees (Mopper et al. 1995). Differing local genetic structure has also been shown in the Cerambycidae (Coleoptera). *Tetraopes tetraophthalmus* (Forster) (red milkweed beetle) lives entirely on one host plant, *Ascelpias syriaca* (milkweed). This beetle rarely disperses between patches and allozyme studies have shown that patches of milkweed only a few kilometres apart support different local genetic composition (McCauly and Eanes 1987). Sturgeon and Mitton (1986) found that the highly mobile *Dendroctonus ponderosae* Hopkins (mountain pine beetle) (Coleoptera: Curculionidae) populations on adjacent trees differed in their allozyme composition to the same degree as differences between host sites. Their study showed that

highly mobile insects like the mountain pine beetle can show preference to individual trees.

In the current study, populations of weevils were not similar on trees located close together in a plot. Depending on the age of the plot, trees contain more or less isolated populations. Larvae in trees located far apart in a plot can share more parental genotypes than those located close together. Further evidence of the deme formation hypothesis in *P. strobi*, is the presence of private alleles in the data set. These alleles could be an indication of local adaptation to an individual tree or tree host. Sahota et al. (1994a) found a 75% reduction in oviposition punctures on resistant trees. Resistant trees have different genetic adaptations to deal with weevil pressure and the ability of the weevils to feed and oviposit on resistant trees (Sahota et al. 1998) presents an interesting problem for forest managers and shows the potential for weevils to develop specificity for tree genetics. Studies on feeding preferences in the weevil have been conducted and lead to one general conclusion, i.e., that natal hosts are more attractive to the weevil, and produce more successful broods, again suggesting local adaptation in accord with the deme formation hypothesis (VanderSar and Borden 1977, Alfaro 1988, Phillips and Lanier 2000).

The differences in this study between oviposition plugs and larval survival could be a function of local weevil adaptation to their host tree, or the trees' adaptation to the weevil, ultimately decreasing weevil survival. The Sitka spruce trees in our study have more private alleles in all three age classes than the interior spruce plots. This could be due to their genetic isolation from mainland populations or the beetles' evolution to better cope with this different host species. To further

separate the interior spruce plots from the Sitka spruce plots, the private alleles were further analyzed for differences between the host species. The interior host plots had in total eight private alleles that were not recorded in the Sitka spruce populations (none occurred in the oldest plot PG), while there were 13 private alleles in the Sitka spruce plots that were not found in the interior spruce plots (DM1400 also had none). We can clearly see a difference between the two host plant populations based on microsatellite private alleles, and population structure. The larger distances travelled by this insect could point indirectly to insect specialization on host plants. The lack of genetic similarity of the beetles between trees located close together may imply that the beetle is moving in a plot to find a suitable habitat, which is why such great distances are travelled in one season. Although there are weevil migrants into all of the host plant plots, their effect on gene flow is not enough to exclude local structure, and as such, all plots remain in an overall heterozygote deficit.

This is the first study of the local genetic structure of white pine weevil in British Columbia and therefore information on the underlying genetic structure of the weevil population is lacking. The underlying heterozygote deficit of these weevil populations could be the result of the founding population. A large review on North American refugia was undertaken by Shafer et al. (2010) and two major refugia were noted; the northern Beringia (Alaska and Yukon, and North West Territories) and Pacific Northwest (south of British Columbia). These refugia may also explain why white pine weevils appear to be less heterozygous regardless of host species and host stand age. There is no evidence that either of the two refugia were used by the

white pine weevil to recolonise British Columbia however, another closely related species *D. rufipennis* (Kirby) (spruce beetle) which inhabits similar habitats, colonized British Columbia from refugia. The lack of strong heterozygosity may also be explained by the current mtDNA data suggesting that the weevil originated in eastern Canada. As the weevil moved west, only those adapted to the new host species (*Picea*) could continue migrating westward. As fewer weevils moved, fewer individuals were able to colonize the new host and the genetic pool for the populations in the west was quite small. Whatever the underlying cause of low heterozygosity, these weevils are adapting locally to their host trees.

Overall microsatellite data were less polymorphic in weevils from Sitka spruce than interior spruce populations. Interior spruce had a total of 165 alleles and Sitka spruce had 136 alleles. The total number of alleles in the weevils of each host species differs by 29 alleles, but when looking at the pictures of the gels, there are fewer polymorphic individuals in the Sitka spruce weevil populations. If the weevil moved east to west, then the Sitka spruce should be the last spruce population invaded by the weevil (in its natural coastal distribution). This means that their genetic structure should be more bottlenecked, monomorphic, and have less population structure as the founding population for Sitka is relatively “newer” than that on interior spruce.

All of this data shows that weevils have genetic structure within a plot, and it may be closely linked to specific trees in a plot. Weevils may be showing some preference for trees in a plot as shown by the enormous distances weevils travel for egg deposition and trees that are closer together in a plot do not share many

common parents. Robert and Bohlmann (2010) also showed that weevils move more frequently in the spring and that female weevils tend to orient to and remain on susceptible hosts whereas males would initially orient to susceptible trees but after a time randomly disperse regardless of tree genotype. Alfaro (1988) found that weevils reared on Sitka Spruce and Engelmann spruce preferred to oviposit on their respective host trees. When weevils were allowed to oviposit on other species, weevil development time increased. VanderSar (1978) also found that weevils would feed on other species but only oviposit in the original host. In both of these studies weevils are showed a host preference for the tree species they were reared on, showing further evidence of deme formation. Robert and Bohlmann (2010) found that a small number of weevils will oviposit on resistant trees. These females produce no successful offspring but still carry mature eggs. These females with mature eggs have the potential to oviposit in other susceptible trees in a plot producing offspring that may also have the ability to feed and oviposit on resistant trees. This could potentially lead to weevils co-evolving in this system to surpass tree resistance defences and produce viable eggs and offspring. The results presented in this thesis confer that weevils can show host and possible tree preferences. These results may call into question studies that augmented weevil populations with insects that originated significant distances from the test area. If weevils show population differentiation at the stand and tree level, responses of weevils in augmented studies may not adequately reflect the local natural system.

6.0 Conclusion

An understanding of population genetics may be key to further understanding the white pine weevil and interior and Sitka spruce. Increased knowledge will lead to the creation of better population prediction models, planting strategies and control methods. Genetics of the white pine weevil allows us to take a closer look at the interaction between this insect and its hosts than was previously possible and increase the knowledge of white pine weevil genetics toward the level of that which is currently known of its host species. There are more than one female per leader, weevils (both male and female) can move further in a plot than previously reported and genetic clusters may be tree specific. Care must be taken when simulation models of weevil population dynamics (especially those using movement as a variable) or augmentation for studies are being used. It is recommended that augmentation studies that use weevils to test tree resistance use insects that are directly associated with the host plot area being manipulated. Models of insect movement and outbreak pattern need to be further refined as genetics catches up with insect pest species. Insect movements can inferred using microsatellite markers and their measurement can help to refine current understanding of weevil movements on a local and stand level.

Although both host tree species generally follow the same broad genetic structure, it is the fine structure that leads to population differentiation. All plots show increased weevil movement in the middle age categories, but the microsatellites in the weevils from the Sitka spruce trees are less polymorphic than those from the interior spruce weevils. Generalisations on white pine weevil and its

ability to adapt to resistant trees should used with caution and further genomic work is needed to understand the intimate connection between host and insect.

This research is the first stepping-stone to understanding the population genetics of *P. strobi*, and when future genomic research is conducted and correlated to the spruce genomic research a greater understanding of the co-evolution in this system will help to guide more ecologically sound control decisions.

Table 1. Non hybrid host species with records of white pine weevil (Turnquist and Alfaro 1996)

Western Canada	**Eastern Canada**
Picea sitchensis	*Pinus strobus*
Picea glauca	*Pinus resinosa*
Picea abies	*Pinus banksiana*
Pinus contorta	*Pinus sylvestris*
	Picea glauca
	Picea rubens
	Picea mariana
	Picea abies
	Picea pungens

Table 2. Some insect insect natural enemies associated with *Pissodes strobi* (Williams and Langor 2002)

Order	Genus and species
Diptera	*Lonchaea corticis* *Lonchaea furnissi* *Medetera apicalis* *Medetera vidua*
Hymenoptera	*Bracon nanus* *Bracon pini* *Calliephialtes comstockii* *Coeloides pissodis* *Coeloides rufovariegatus* *Coeloides secundus* *Coeloides vancouverensis* *Cubocephalus occidentalis* *Dolichomitus terrebrans nubilipennis* *Doryctes spp.* *Eubazus calyptoides* *Eubazus crassigaster* *Eupelmus pini* *Eurytoma cleri* *Eurytoma picea* *Eurytoma pissodis* *Helcostizus albator rufiscutum* *Helcostizus contortae* *Helcostizus subrectus* *Heydenia unica* *Labena grallator* *Spathius brachyurus* *Spathius sequoiae* *Rhaphitelus maculates* *Rhopalicus pulchripennis* *Rhopalicus tutela* *Trigonura tarsata*

Table 3. Microsatellite loci used for *Pissodes strobi* population study

Locus	Primer Sequence (5'-3')	Ta (ºC)	Repeat Motif	Size Range (bp)	Observed Allele Numbers	
We2-7.2	F: AATGCTTGCGTAAGTAACGA R: GCCCACTTTTATGAATGGGA	55	$(GATA)_{11}$	210-242	11	Liewlaksaneeyanawin et al. (2001)
We2-19	F:GGCCCCAATATAGTATATTATC R: GGTCTTCCGTTTAAATGTAC	60	$(CTAT)_{20}$	159-239	21	
We 3-14	F:GTTTGTTAATGGAGTCTTGCTGC R: CGCACTCTTGCCCTACTACA	60	(TG)>43 …$(TG)_2$	418-511	40	
We3-16	F: GGCATCAGATTAATGAAGGTTC R: GCGRCACAATTTGGTCCTATTC	60	(TG)20	279-347	26	
We3-18	F:GCTATCCTATGCAAGAATGTATC R: TCGGTTGTGATGGGAAATTC	53	$(AC)_{23}$	63-136	18	
PST02D01	F: GTCATATTCATTGAGTCGAG R: GTCCGGCGCATGTAGATACAC	56	$(AC)_6$	192-224	7	Wytrykush et al. 2011 (In prep)
PST02J22	F: CATTGAGTCGAGAAGGGAGC R: GGAGCGTATCTACATGCACC	58	$(AC)_6$	202-214	4	
PST02J24	F: ACTACTTGAAGGTCTAGGCC R: GCTCAAAGTACTCTAGTCCTTC	58	$(GAT)_5$	333-357	6	
PST02K14	F: AGACGTGCGAAGGACTGGAC R: CATTCTACTTCTACTCGACC	52	$(GTT)_5$	168-189	5	
PST02L13	F: CATTGAGTCGAGAAGGGAGC R:GACTCAGTTCCTCGACGGCAGC	56	$(AC)_6$	247-259	8	
PST02P01	F: GCTTCATGATCATGTCTCGC R: TCAGGGTAAGGAATTCGGCG	58	$(CT)_{18}$	233-269	11	
PST04C09	F: CAGTATCATTAGCATCTGGCTC R: ATCTCCGCCACTTGCTACGTC	58	$(CAT)_5$	256-271	7	
PST04E11	F:GCGTGATAGTCCATATGAACGTC R: AATACGTGTCCGCACTGTGG	56	$(ACT)_5$	339-354	14	
PST04I09	F: GGCAGCCAACCTTTCCTC R: CCTTAGAGTGTCAGCCATAG	58	$(CAT)_6$	189-210	2	
PST04L04	F: GATTCAAATTCAAACGCGCAC R: CTCGTCTAGCACCAGCTTGC	58	$(TTA)_6$	243-270	14	

Table 4. Average measured values in interior and Sitka spruce sample plots

	Interior Spruce			**Sitka Spruce**		
	Church (CH)	Woodlot (WL)	PGTIS (PG)	Grafton (GR)	Camp 4 (CF)	DM1400 (DM)
Stand Age	5 yrs	13 yrs	23 yrs	8 yrs	13 yrs	23yrs
Total Leaders	15	18	19	17	17	14
Tree Height (cm)	117.1	375.3	432.5	306.5	479	842.6
Tree DBH	N/A	6.0	7.3	11.7	11.5	13.3
Leader Length (cm)	35.2	47.4	53.7	61.2	66.6	71.8
Leader Diameter (cm)						
Top	4.7	8.1	8.2	7.3	10.8	12.0
Middle	6.8	10.9	11.7	9.9	14.0	16.0
Base	10.1	14.6	16.3	14.1	17.8	20.0
Feeding Punctures	18.6	19.3	16.4	22.9	33.8	21.9
Oviposition Plugs	69.3	81.2	76.4	127.5	188.5	103.3
Weevils Present	9	27.8	11.5	28.7	38.2	71.4

Table 5. Sibships inferred from the parentage analysis program Colony (Wang 2004)

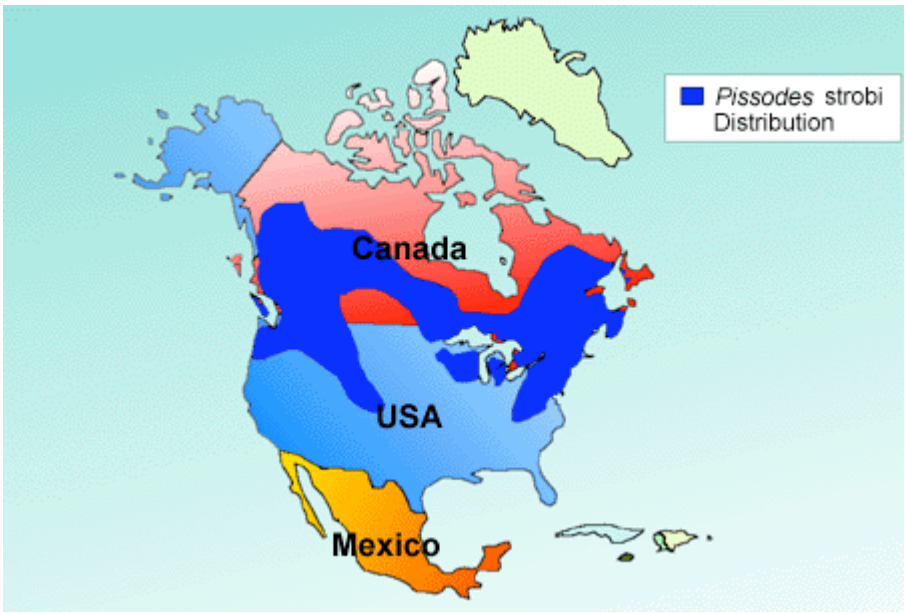
Site	Total Genotypes	Shared parents	Unique Parents	Total Full Sib Families
CH	50	15	35	13
WL	62	21	41	10
PG	48	16	32	13
GR	47	20	27	13
CF	48	28	20	13
DM	45	27	18	7

Table 6. F_{is}, F_{st}, F_{it} and Nm for *P. strobi* in each plot calculated using Genepop (Raymond and Rousset 1995)

Site	Fis	Fst	Fit	Nm
Church Road	0.078	0.174	0.238	0.567
Woodlot	0.247	0.094	0.318	1.773
PGTIS	0.126	0.168	0.273	1.154
Grafton Road	0.037	0.116	0.149	0.945
Camp 4	0.211	0.094	0.285	2.030
DM1400	0.208	0.091	0.280	1.843

Table 7. Number of alleles in each locus for interior spruce and Sitka spruce plots

Locus	Interior spruce number of observed alleles	Sitka spruce number of observed alleles
We2-7.2	15	7
We2-19	19	15
We 3-14	33	24
We3-16	24	21
We3-18	15	10
PST02D01	6	5
PST02J22	3	3
PST02J24	2	6
PST02K14	5	3
PST02L13	5	7
PST02P01	10	6
PST04C09	5	4
PST04E11	11	12
PST04I09	2	1
PST04L04	10	12



Source: http://cfs.nrcan.gc.ca/subsite/weevil/about-apropos

Figure 1. Distribution of *Pissodes strobi* in North America (Alfaro 1994)

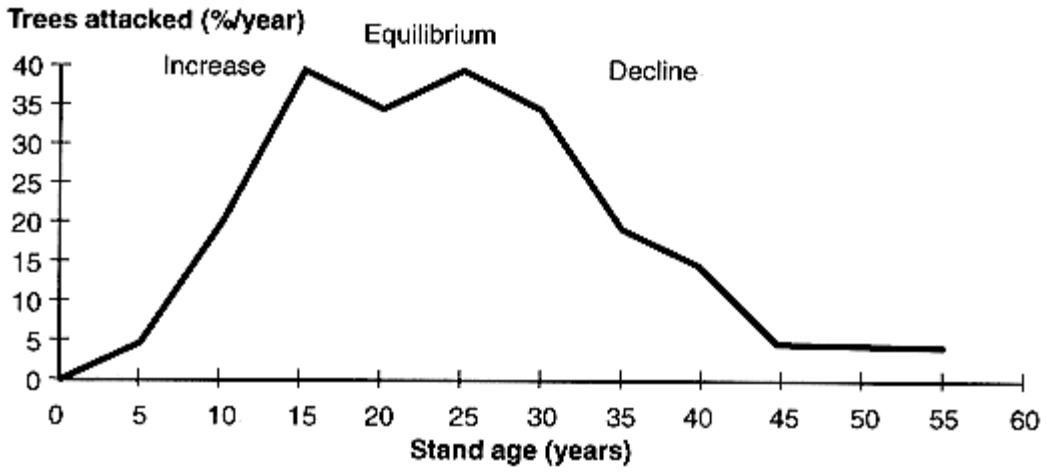


Figure 2. Attack dynamics of *Pissodes strobi* in open grown Sitka spruce stand in British Columbia (Alfaro 1994)

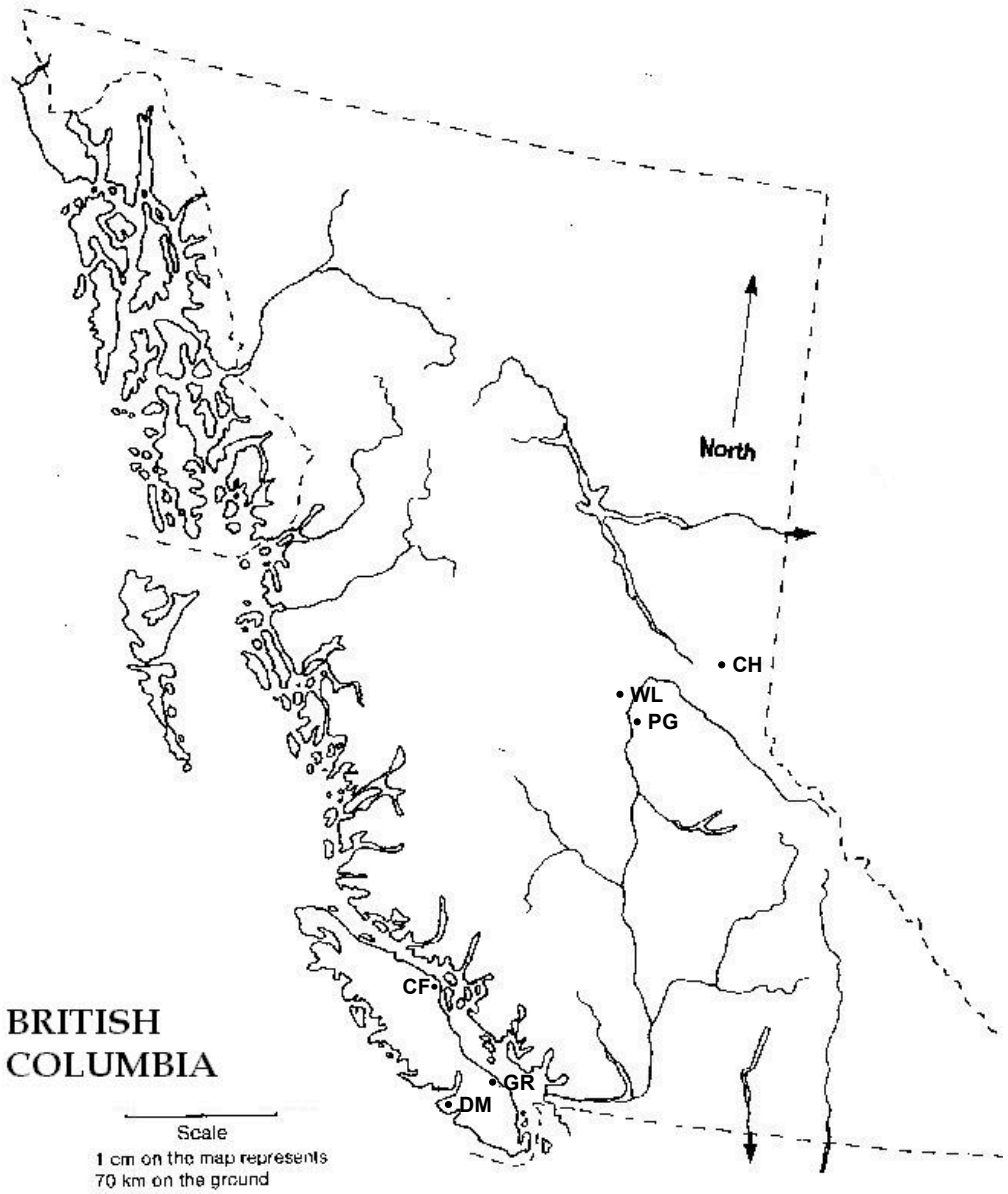


Figure 3. Map of British Columbia Canada showing the sampling locations for the interior spruce and Sitka spruce plots. Church Road (CH) 5 yrs, Woodlot (WL) 13 yrs, PGTIS (PG) 23 yrs, Grafton Road (GR) 8 yrs, Camp 4 (CF) 13 yrs, DM1400 (DM) 23yrs (see Table 4).

Figure 4. Example of trees sampled from interior spruce plots: a) Church Road (CH) 5 yrs, b) Woodlot (WL) 13 yrs, c) PGTIS (PG) 23 yrs and Sitka spruce plots: d) Grafton Road (GR) 8 yrs, e) Camp 4 (CF) 13 yrs, f) DM1400 (DM) 23 yrs.

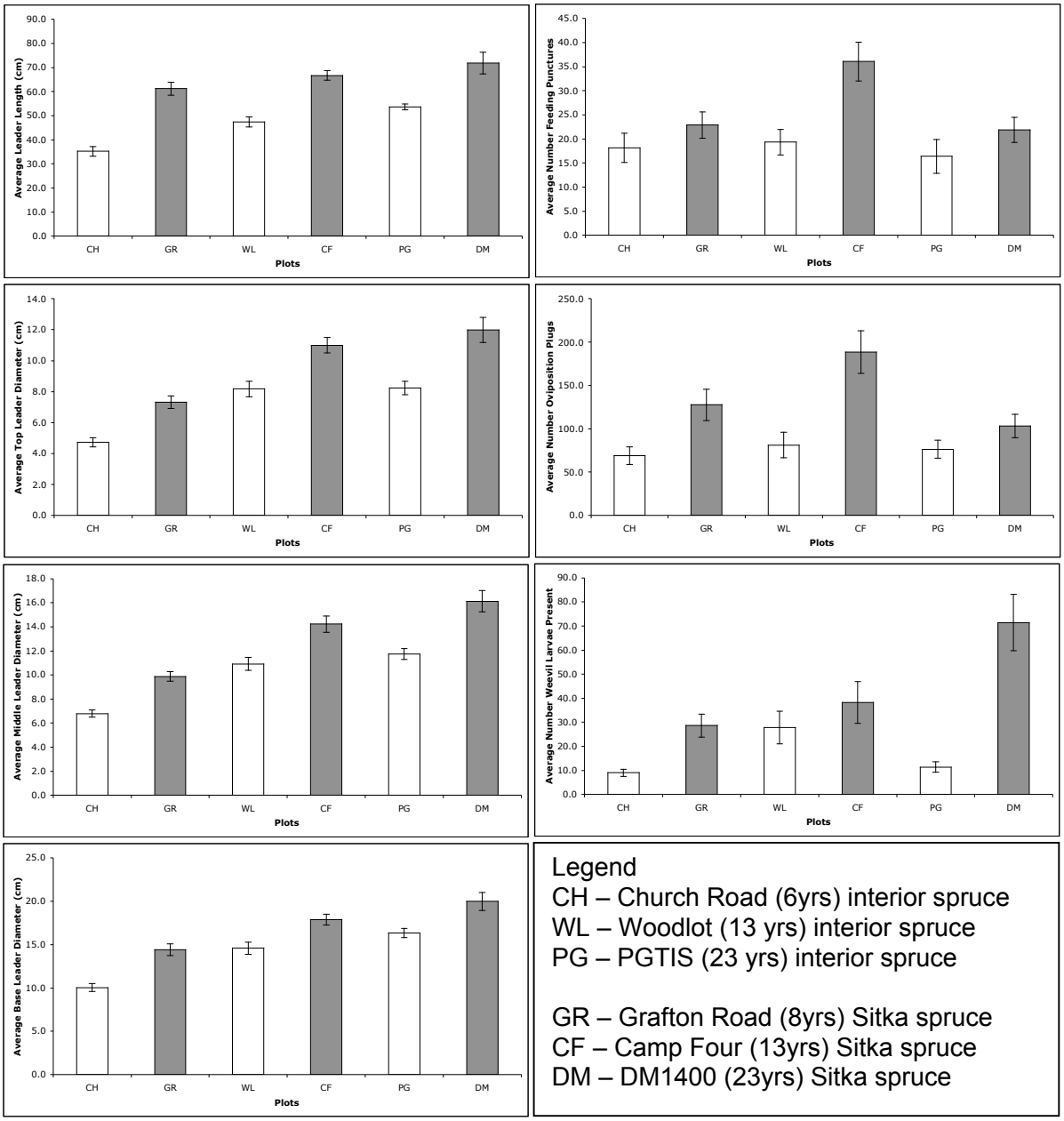


Figure 5. Graphical representation of average values collected from all plots. White bars represent interior spruce plots, grey bars represent Sitka spruce plots.

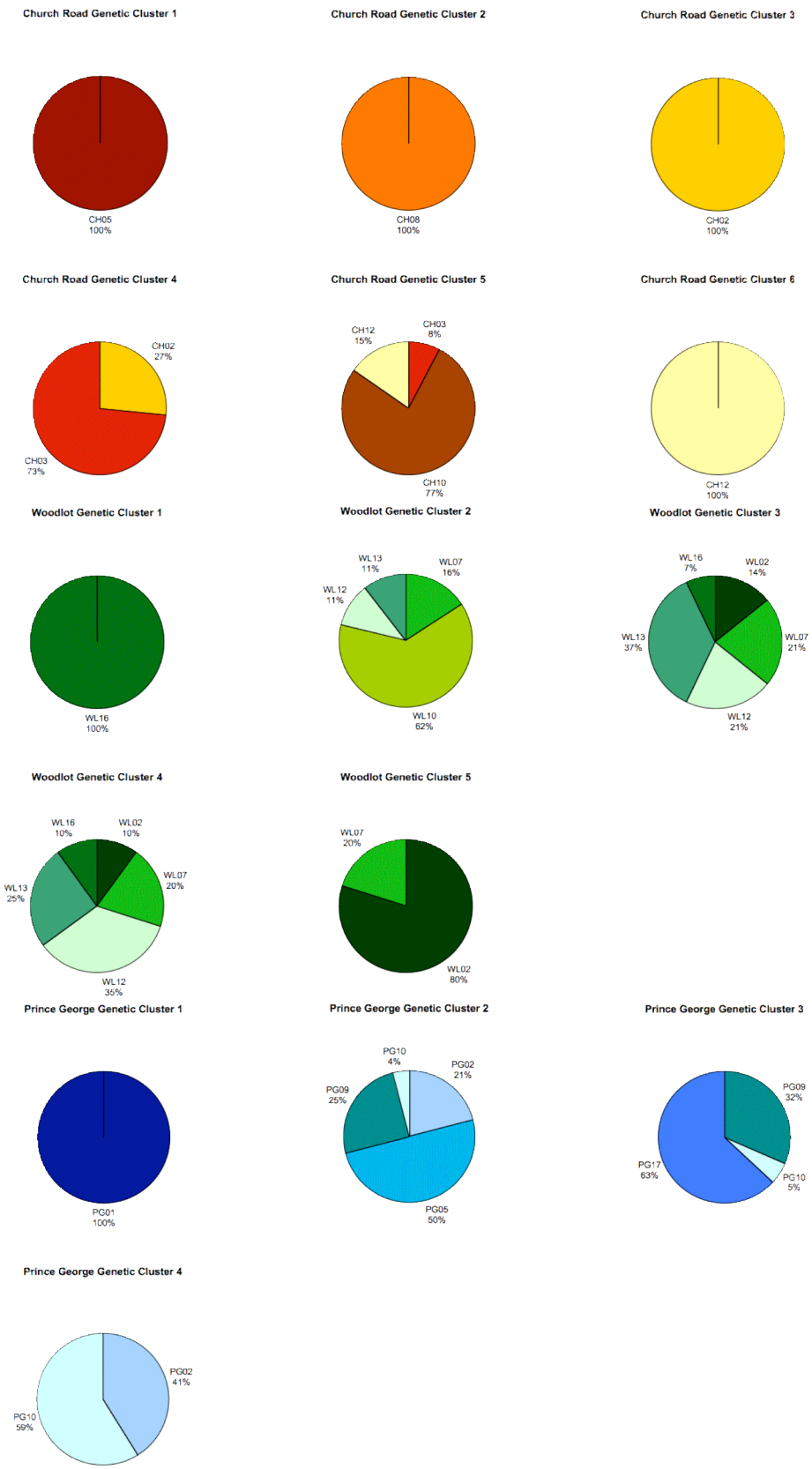


Figure 6. Structure population allocation in interior spruce plots. a) Church Road (CH), b) Woodlot (WL), and c) Prince George (PG)

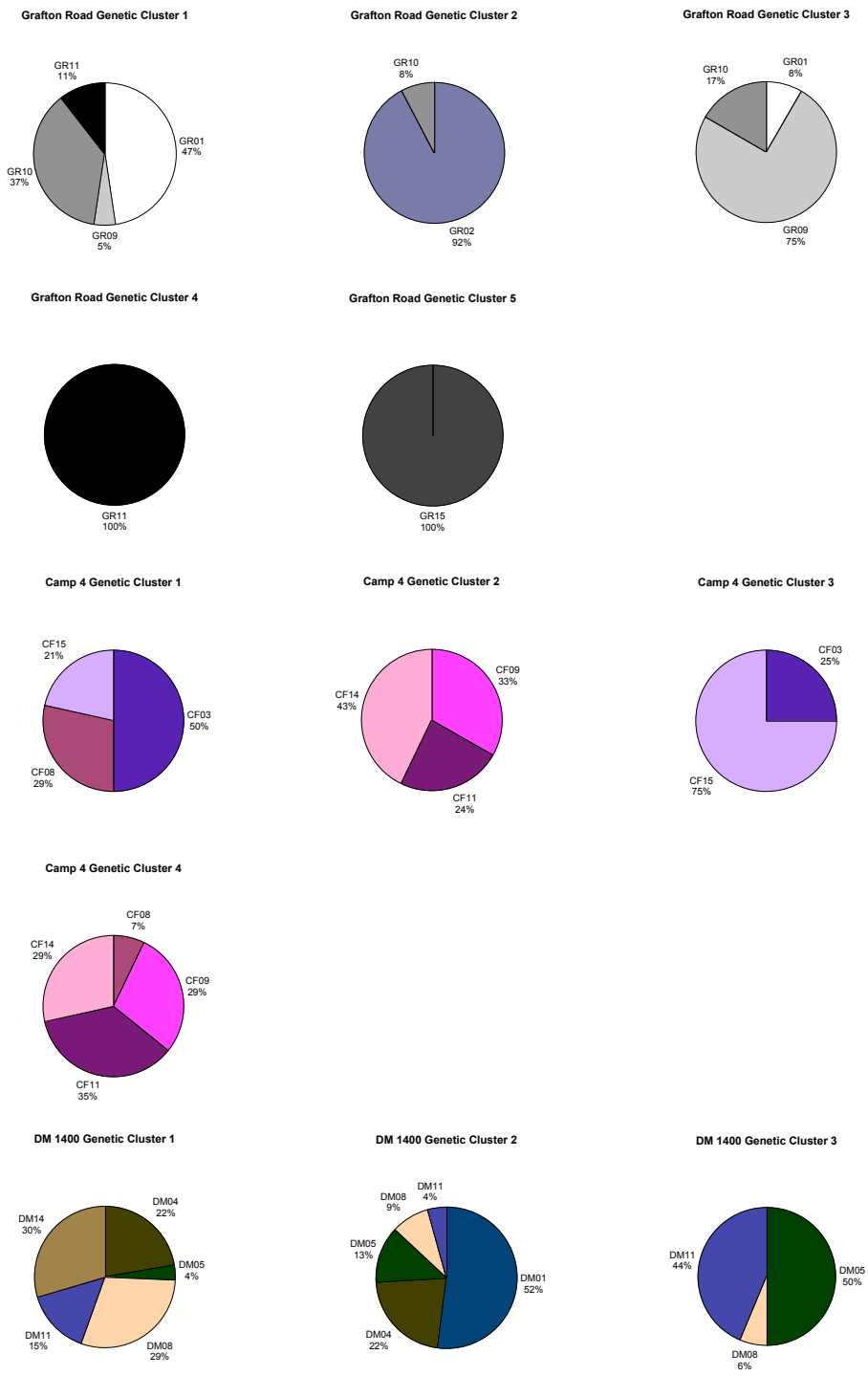


Figure 7. Structure population allocation in Sitka spruce plots. a) Grafton Road, b) Camp 4, and c) DM 1400

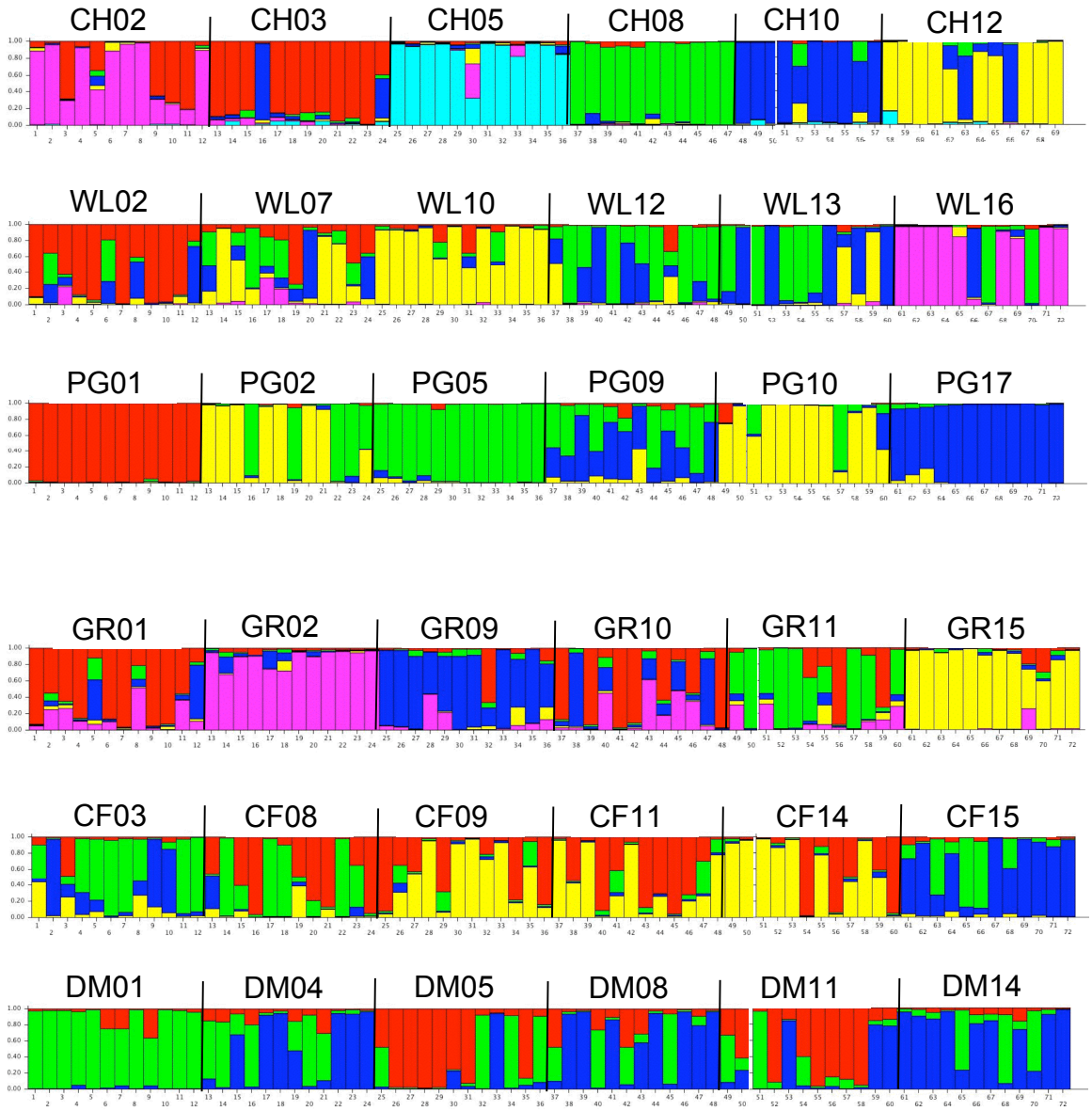


Figure 8. Output from Structure (Pritchard et al. 2000) showing genetic clustering associated with each tree in all six sample plots. Black bars delineate individual trees and each coloured bar represents individual larvae analysed from each tree

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Appendices

Appendix 1 Layout of Sampled Trees

Layout of trees sampled in each plot. A) Church Road, B) Woodlot and C) PGTIS

Layout of trees sampled in each plot. D) Grafton Road, E) Camp 4 and F) DM1400

Appendix 2 Total Numbers of Weevils Sampled Per Tree

Total number of weevils sampled and collected from both interior spruce and Sitka spruce plots

Site	Sampled	Total	Percent Sampled		Site	Sampled	Total	Percent Sampled
CH02	12	12	100		GR01	12	65	18
CH03	12	18	67		GR02	12	43	28
CH05	12	12	100		GR09	12	37	32
CH08	11	11	100		GR10	12	37	32
CH10	10	10	100		GR11	12	42	29
CH12	12	23	52		GR15	12	37	32
WL02	12	85	14		C403	12	68	18
WL07	12	72	17		C408	12	121	10
WL10	12	57	21		C409	12	33	36
WL12	12	45	27		C411	12	55	22
WL13	12	82	15		C414	12	84	14
WL16	12	24	50		C415	12	99	12
PG01	12	29	41		DM01	12	54	22
PG02	12	13	92		DM04	12	140	9
PG05	12	18	67		DM05	12	63	19
PG09	12	33	36		DM08	12	65	18
PG10	12	26	46		DM11	12	125	10
PG17	12	13	92		DM14	12	142	8

Appendix 3 SNPs in *Pissodes strobi*

Mining a *Pissodes strobi* [Peck] expressed sequence tag (EST) library for single nucleotide polymorphisms (SNiPs)

Introduction

A Single nucleotide polymorphism (SNP) is an alteration in a DNA sequence at a single base position. SNPs can be located in coding and no coding regions. Changes in non-coding regions generally have no effect on the phenotype. SNPs in coding regions are classified into two groupings, synonymous and non-synonymous. A Synonymous SNP is when a base change occurs in the coding region and does not alter the phenotype (no amino acid change). Non-synonymous SNPs occur when the base change alters the amino acid sequence, which may alter the function of the protein. In general non-synonymous substitutions are more desirable as greater information can be gained i.e. association mapping. However synonymous SNPs and non-coding SNPs are also a valuable tool for genetic studies (i.e. population analysis). SNPs are considered bi-allelic markers as they usually consist of only two alleles. Substitution rates are very low for single base pair substitutions (10^{-8} changes/ nucleotide/ generation) making the probability of similar changes in other individuals or populations rare (Vignal et al. 2002, Allendorf and Luikart 2007).

Pissodes strobi is an insect pest of spruce trees in British Columbia. Effective control of the beetle has not been achieved through any currently available method or combination of methods. The discovery of trees that are resistant to this beetle has renewed interest in this system. In order to effectively utilise these new resistant trees the genetics of the beetle should also be further investigated to understand the adaptation potential of the beetle populations. A preliminary EST database was established for *Pissodes strobi*, as to date no SNP work has included this beetle. Although the library is still under construction we utilized the current sequences to preliminary mine the data base for single nucleotide polymorphisms.

Materials and Methods

An EST library was established at the University of British Columbia Treenomix Lab using cDNA from both *Pissodes strobi* adults and larvae. Twelve larvae were collected from one tree in a Sitka spruce plantation (Grafton Road) on Vancouver Island. A normalised full length enriched directionally cloned library was constructed using total RNA pooled from all larvae. Adult weevils were collected from two British Columbia Forest Service plantations in Campbell River on Vancouver Island. Total RNA was extracted from eleven females and 9 males, and pooled together. Larval and adult DNA was combined and the Creater SMART cDNA Library Construction Kit (Clontech, Mountain View, CA, USS) was used for cDNA construction. CAP3 was used for initial EST alignments (Huang and Madan 1999). EST sequences were mined at UBC, by the Treenomix lab using Polyphred (Nickerson et al. 1997). A total of 12 288 sequences were discovered to construct the first EST library for *Pissodes strobi*. These sequences were filtered for quality and 11 090 (90%) were of high enough quality to mine for the presence of SNPs.

The library was aligned using cap3 and this alignment was then placed in Polyphred for further analysis (Nickerson et al. 1997). All sequences containing 3 or more reads were aligned with Polyphred for SNP discovery. The trace files were placed in a separate folder and a Polyphred script was applied to identify SNPs and create useable output files. These files were checked for the number of sequences and anything less than three was put aside and all results were collated, and a blast search was performed on each usable contig. Each contig and blast result was verified by hand to ensure the program did not included questionable SNPs. All SNPs had to have quality values of no less than 25 and the five flanking bases on either side were no less than 18. All SNPs were then classified based on substitution (transition vs. transversion), type of SNP (synonymous vs. non-synonymous) and location of the SNP. Manual inspection of all sequences was performed using CodonCode Aligner (CodonCode Corp., Dedham, MA, USA) to verify the SNP.

Results

SNP summary of identification

A total of 12 288 sequences were discovered before filtering for quality. After sequences were filtered, 11 090 high quality sequences remained for analysis. The 11 090 sequences were composed of 1554 singletons and 9536 reads which were constructed using CAP3 (Huang and Madan 1999) into 3402 total contigs. The total assembly consisted of 1554 singletons, 2405 contigs with 2 reads, 754 contigs with 3-5 reads, 211 contigs with 6-10 reads and 32 contigs with 11-10 reads. The average length of reads for the total 11 090 sequences was 737bp.

The EST sequences were further filtered to remove contigs with 2 or less reads, a total of 997 contigs remained to mine for SNPs. Of the 997 contigs 109 were found to contain 202 SNPs. Among the 109 contigs, 53 (49%) contained one SNP, 27 (25%) contained 2 SNPs, 22 (20%) contained 3 SNPs, 6 (6%) contained 4 SNPs, and 1 (1%) contained 5 SNPs (Figure 1). The average length of these contigs was 1124.7 nucleotides with a minimum length of 608 nucleotides and a maximum length of 2109 nucleotides. The longest contig was constructed with 5 reads while the shortest had 6 reads. The most reads in one contig was 15 and this contig had only 2 SNPs.

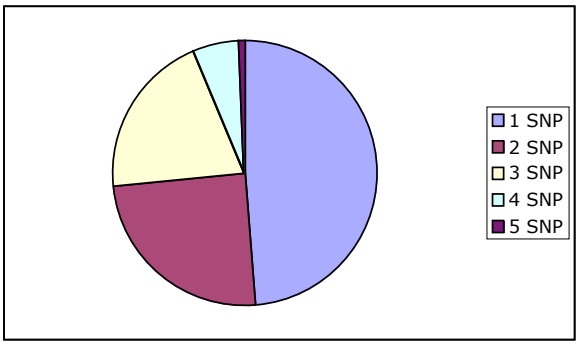

Figure A3.1. Percent of contigs containing one to five SNPs per contig

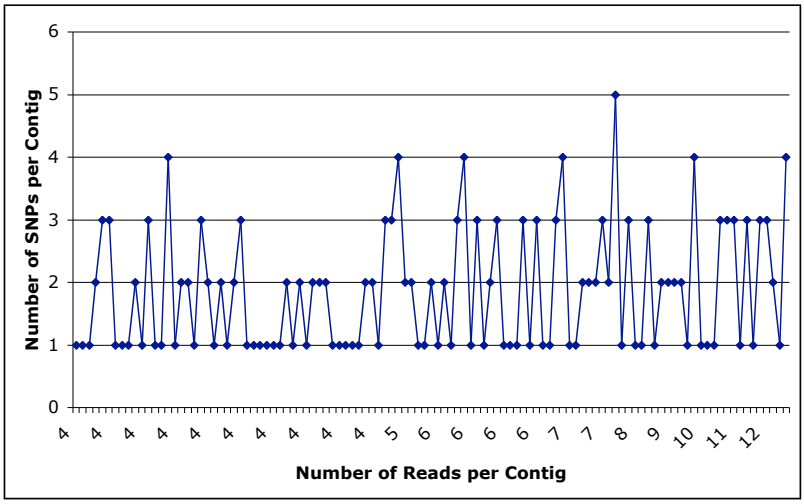

Figure A3.2. Number of SNPs per Contig. One point represents one contig

SNP density ranged from 0.45 to 4.6 per 1000 nucleotides with an average density of 1.8 SNPs per Kb. The densest contig (4.6 SNP/Kb) contained five SNPs in 1082 nucleotides consisting of 8 reads. The next three densest contigs had 4 SNPs each and consisted of 5, 6 and 11 reads respectively. The least dense contig (0.45SNP/Kb) contained one SNP in 1197 nucleotides and consisted of 4 reads.

SNP characteristic

All of the SNPs were further classified based on nucleotide substitution type: transitions (pyrimidine↔pyrimidine or purine↔purine) or transversions (pyrimidine↔purine). Of the 202 SNPs identified 118 (58%) were transitions, which were further broken into groups, 58 (25%) were classified as T/C and 60 (30%) were classified as G/A. The 202 SNPs also contained, 84 (42%) transversion of which 36 (18%), 17 (8.4%), 17(8.4%) and 14 (6.9%) were classified as T/G, C/A, T/A and G/C respectively. The highest frequency of changes occurred in the T/C transition while the lowest frequency occurred in the G/C transversion. The ratio of transitions to transversions was 1.4:1.

Table A3.1. Frequency of substitution type in all SNPs and in only coding SNPs

Substitution Type	Total in all 202 SNPs	Percentage	Total in 113 Coding SNPs	Percentage
T/C	58	25	31	27
G/A	60	30	35	31
T/G	36	18	24	21
C/A	17	8	9	8
T/A	17	8	8	7
G/C	14	7	6	5

Synonymous vs. non-synonymous

SNPs that were found to be in coding regions were further classified according to amino acid sequence changes. Synonymous substitutions occur when there is no change in the amino acid whereas non-synonymous substitutions have base changes that lead to changes in amino acids. From the 202 SNPs, 113 were located in coding sequences, the remaining 87 were not considered for this analysis as they were considered non-coding. There were 54 (47%) synonymous substitutions and 59 (52%) non-synonymous substitutions recorded.

Of the 113 coding SNPs 34 (30%) had a first codon change, 37 (33%) had a second codon change and 42 (37%) had a third codon change. Codon changes were further broken down between synonymous and non-synonymous substitutions. The synonymous substitutions occurred in the first codon 21 times and the third codon 33 times. The non-synonymous substitutions occurred in the first codon 15 times, in the second codon 37 times and finally in the third codon 9 times. Further information on synonymous and non-synonymous SNPs is found in table 2.

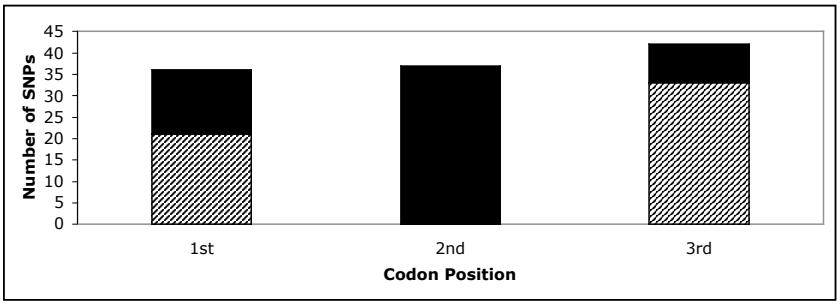

Figure A3.3. The number of SNPs involving the first, second and third codon. Solid bars represent synonymous substitutions, hatched bars represent non-synonymous substitutions.

Table A3.2. Information on current synonymous and non-synonymous SNPs found in *Pissodes strobi* EST sequences

Contig Name	SNP Position	Annotation	Codon #1	Codon #2	Amino acid change
W_Contig1053-contig2	633	PREDICTED: similar to glyceraldehyde 3-phosphate dehydrogenase [Tribolium castaneum]	AGT	TGT	T/T
W_Contig1064-contig2	681	PREDICTED: similar to Sparc [Tribolium castaneum]	GAT	TAT	D/Y
W_Contig1064-contig2	769	PREDICTED: similar to Sparc [Tribolium castaneum]	GAG	GAA	E/E
W_Contig1113-contig1	705	PREDICTED: similar to proclotting enzyme [Tribolium castaneum]	CTC	CCC	L/P
W_Contig112-contig5	344	PREDICTED: similar to CG10542 CG10542-PA [Tribolium castaneum]	GTA	GCA	V/A
W_Contig1182-contig1	619	PREDICTED: similar to CG14949 CG14949-PA [Tribolium castaneum]	GGA	GTA	G/V
W_Contig1197-contig1	518	trypsin-like serine proteinase [Anthonomus grandis]	CAG	CGG	Q/R
W_Contig1197-contig1	846	trypsin-like serine proteinase [Anthonomus grandis]	ACA	TCA	C/*
W_Contig1223-contig1	408	PREDICTED: similar to CG12811 CG12811-PA [Tribolium castaneum]	CGG	TGG	P/P
W_Contig1223-contig1	515	PREDICTED: similar to CG12811 CG12811-PA [Tribolium castaneum]	AAT	ACT	N/T
W_Contig1231-contig1	574	PREDICTED: hypothetical protein [Tribolium castaneum]	CTA	CTG	L/L
W_Contig1236-contig1	319	PREDICTED: similar to signal peptidase 18 kDa subunit [Tribolium castaneum]	CAG	CGG	Q/R
W_Contig1236-contig1	571	PREDICTED: similar to signal peptidase 18 kDa subunit [Tribolium castaneum]	AGA	AAA	R/K
W_Contig1236-contig1	640	PREDICTED: similar to signal peptidase 18 kDa subunit [Tribolium castaneum]	AAC	AGC	N/S
W_Contig1312-contig2	451	PREDICTED: similar to cop9 complex subunit 7a [Tribolium castaneum]	GAA	GAG	E/E
W_Contig1367-contig3	405	PREDICTED: similar to acyl-protein thioesterase 1,2 [Tribolium castaneum]	TTG	TTA	L/L
W_Contig1367-contig3	738	PREDICTED: similar to acyl-protein thioesterase 1,2 [Tribolium castaneum]	TCG	TCC	S/S
W_Contig1389-contig1	278	PREDICTED: similar to heat shock protein 1 [Tribolium castaneum]	AGG	ATG	R/M
W_Contig1389-contig1	398	PREDICTED: similar to heat shock protein 1 [Tribolium castaneum]	CTA	CGA	L/R
W_Contig14-contig5	440	PREDICTED: similar to ribosomal protein S3e [Tribolium castaneum]	GGC	GGT	G/G
W_Contig14-contig5	695	PREDICTED: similar to ribosomal protein S3e [Tribolium castaneum]	AAA	AAG	K/K
W_Contig140-contig3	625	PREDICTED: similar to p27BBP/eIF6-like [Tribolium castaneum]	TTG	TTT	L/F
W_Contig1404-contig1	453	PREDICTED: similar to mRNA cap-binding protein eIF4E [Tribolium castaneum]	TAC	TTC	Y/F
W_Contig1404-contig1	480	PREDICTED: similar to mRNA cap-binding protein eIF4E [Tribolium castaneum]	AAC	AGC	N/S
W_Contig1404-contig1	576	PREDICTED: similar to mRNA cap-binding protein eIF4E [Tribolium castaneum]	TTT	TAT	F/Y
W_Contig143-contig2	481	Endoglucanase; Endo-1,4-beta-glucanase	CTG	CTT	L/L
W_Contig144-contig1	738	PREDICTED: similar to smad nuclear-interacting protein 1 [Tribolium castaneum]	GAA	GTA	E/V
W_Contig1507-contig1	614	PREDICTED: hypothetical protein [Tribolium castaneum]	GCC	GGC	A/G
W_Contig1543-contig1	251	PREDICTED: hypothetical protein [Tribolium castaneum]	GCG	GCT	A/A
W_Contig160-contig1	356	trypsin precursor [Diaprepes abbreviata]	AGC	ATC	S/I
W_Contig160-contig1	413	trypsin precursor [Diaprepes abbreviata]	TGG	TGT	W/C
W_Contig1700-contig2	589	PREDICTED: similar to mitochondrial ribosomal protein S2 CG2937-PA [Tribolium castaneum]	TAG	TAC	*/Y
W_Contig1716-contig1	429	castaneum]	GTC	GCC	V/A
W_Contig1845-contig1	629	PREDICTED: similar to adaptin [Tribolium castaneum]	AGA	AGG	R/R
W_Contig189-contig1	364	ribosomal protein L7e [Biphyllus lunatus]	CTA	TTA	L/L
W_Contig189-contig1	442	ribosomal protein L7e [Biphyllus lunatus]	CGT	TGT	R/C
W_Contig189-contig1	628	ribosomal protein L7e [Biphyllus lunatus]	CGA	GGA	R/G
W_Contig189-contig1	825	ribosomal protein L7e [Biphyllus lunatus]	TCT	CCT	S/P
W_Contig1923-contig1	559	PREDICTED: hypothetical protein isoform 1 [Tribolium castaneum]	CCT	CTT	P/L
W_Contig1923-contig1	577	PREDICTED: hypothetical protein isoform 1 [Tribolium castaneum]	AGG	AGT	R/S
W_Contig1923-contig1	603	PREDICTED: hypothetical protein isoform 1 [Tribolium castaneum]	TCT	TAT	S/Y
W_Contig1927-contig1	575	castaneum]	GAG	CAG	L/L
W_Contig2005-contig1	324	cytochrome c oxidase subunit III [Priasilpha obscura]	GAA	AAA	F/F
W_Contig2005-contig1	331	cytochrome c oxidase subunit III [Priasilpha obscura]	TAT	TAC	Y/Y
W_Contig2032-contig1	434	castaneum]	CCT	TCT	R/R
W_Contig213-contig1	352	PREDICTED: similar to AGAP011620-PA [Tribolium castaneum]	CTG	CTT	L/L
W_Contig213-contig1	417	PREDICTED: similar to AGAP011620-PA [Tribolium castaneum]	CCG	CTG	P/L
W_Contig2150-contig1	608	PREDICTED: similar to putative alcohol dehydrogenase [Tribolium castaneum]	AAG	ATG	K/M
W_Contig2197-contig1	456	PREDICTED: hypothetical protein [Tribolium castaneum]	GGA	GGG	G/G
W_Contig2238-contig1	593	PREDICTED: similar to AGAP010237-PA [Tribolium castaneum]	CTA	TTA	*/*
W_Contig2238-contig1	704	PREDICTED: similar to AGAP010237-PA [Tribolium castaneum]	TTA	ATA	*/Y
W_Contig2307-contig2	396	PREDICTED: similar to CG7630 CG7630-PA [Tribolium castaneum]	GGC	GGA	G/G
W_Contig2438-contig1	535	PREDICTED: similar to Mitochondrial tumor suppressor 1 homolog (Angiotensin-II type 2 receptor-interacting protein) (AT2 receptor-binding protein) (Coiled-coiled tumor suppressor gene 1 protein) [Tribolium castaneum]	TTG	GTG	Q/H
W_Contig2438-contig1	674	PREDICTED: similar to Mitochondrial tumor suppressor 1 homolog (Angiotensin-II type 2 receptor-interacting protein) (AT2 receptor-binding protein) (Coiled-coiled tumor suppressor gene 1 protein) [Tribolium castaneum]	ATT	ATC	I/I
W_Contig2585-contig2	247	cathepsin L protease inhibitor 1 [Diaprepes abbreviatus]	GTG	GTA	V/V
W_Contig2585-contig2	374	cathepsin L protease inhibitor 1 [Diaprepes abbreviatus]	GCG	GAG	A/E
W_Contig2736-contig1	372	ribosomal protein L10e [Scarabaeus laticollis]	TGT	GGT	T/T
W_Contig2737-contig1	559	PREDICTED: similar to proteasome alpha 4 subunit [Tribolium castaneum]	TTG	TTT	L/F
W_Contig2798-contig4	742	PREDICTED: similar to mitochondrial import receptor subunit tom20 [Tribolium castaneum]	ACC	ATC	T/I
W_Contig2965-contig1	668	PREDICTED: hypothetical protein [Tribolium castaneum]	TAG	TAT	*/Y
W_Contig2974-contig2	210	PREDICTED: similar to CG32633 CG32633-PA, partial [Tribolium castaneum]	CCT	ACT	R/S
W_Contig302-contig2	608	pectin methylesterase [Sitophilus oryzae]	GGG	GGA	G/G
W_Contig308-contig1	317	small heat shock protein 21 [Gastrophysa atrocyanea]	CTC	CTT	L/L
W_Contig3092-contig1	606	PREDICTED: similar to AGAP010515-PA [Tribolium castaneum]	GTT	GGT	V/G
W_Contig3148-contig1	676	PREDICTED: similar to mediator complex [Tribolium castaneum]	TAG	CAG	L/L
W_Contig3322-contig1	478	PREDICTED: similar to AGAP008106-PA [Tribolium castaneum]	GTG	GTA	V/V
W_Contig3322-contig1	505	PREDICTED: similar to AGAP008106-PA [Tribolium castaneum]	GTA	GTG	V/V
W_Contig362-contig5	474	PREDICTED: similar to CG5454 CG5454-PA [Tribolium castaneum]	AAG	AGG	K/R
W_Contig364-contig2	1140	PREDICTED: similar to CG3244 CG3244-PA isoform 2 [Tribolium castaneum]	CCC	CTC	P/L
W_Contig375-contig1	381	PREDICTED: similar to chaperonin [Tribolium castaneum]	AGA	GGA	S/S
W_Contig385-contig1	248	PREDICTED: similar to ribosomal protein L13e [Tribolium castaneum]	CAA	TAA	L/L
W_Contig385-contig1	263	PREDICTED: similar to ribosomal protein L13e [Tribolium castaneum]	AGT	GGT	T/T
W_Contig385-contig1	381	PREDICTED: similar to ribosomal protein L13e [Tribolium castaneum]	TGG	TGA	W/*
W_Contig385-contig1	671	PREDICTED: similar to ribosomal protein L13e [Tribolium castaneum]	GCC	TCC	G/G
W_Contig386-contig2	224	PREDICTED: similar to succinyl-CoA synthetase small subunit, putative isoform 2 [Tribolium castaneum]	CGG	CTG	R/L
W_Contig402-contig2	527	PREDICTED: similar to VAMP-associated protein, putative [Tribolium castaneum]	AGG	AAG	R/K
W_Contig422-contig2	316	PREDICTED: similar to troponin C [Tribolium castaneum]	CTC	CTT	L/L
W_Contig422-contig3	463	PREDICTED: similar to troponin C [Tribolium castaneum]	CAA	TAA	L/L
W_Contig436-contig1	671	castaneum]	ACA	GCA	C/C
W_Contig440-contig1	197	PREDICTED: similar to ribosomal protein L18Ae [Tribolium castaneum]	AGT	AGG	S/R
W_Contig454-contig3	357	PREDICTED: similar to troponin C type IIb [Tribolium castaneum]	AGA	AAA	R/K
W_Contig454-contig3	399	PREDICTED: similar to troponin C type IIb [Tribolium castaneum]	TCG	TTG	S/L
W_Contig51-contig2	479	PREDICTED: similar to CG32633 CG32633-PA, partial [Tribolium castaneum]	AAT	AAG	N/K
W_Contig512-contig2	727	PREDICTED: hypothetical protein [Tribolium castaneum]	ACA	ACG	T/T
W_Contig512-contig3	706	PREDICTED: hypothetical protein [Tribolium castaneum]	TCA	CCA	S/P
W_Contig518-contig2	623	PREDICTED: similar to thaumatin-like protein [Tribolium castaneum]	CCC	CAC	P/H
W_Contig542-contig5	242	PREDICTED: similar to vesicle amine transport protein [Tribolium castaneum]	GAG	GAA	E/E
W_Contig570-contig1	279	PREDICTED: hypothetical protein isoform 2 [Tribolium castaneum]	CCG	CGG	P/R
W_Contig570-contig1	303	PREDICTED: hypothetical protein isoform 2 [Tribolium castaneum]	TGT	TTT	C/F
W_Contig589-contig1	563	PREDICTED: similar to Cuticular protein 49Ah CG8515-PA [Tribolium castaneum]	AAC	AAT	N/N
W_Contig589-contig1	623	PREDICTED: similar to Cuticular protein 49Ah CG8515-PA [Tribolium castaneum]	GCT	GCC	A/A
W_Contig614-contig1	632	PREDICTED: similar to GA15448-PA [Tribolium castaneum]	CAT	TAT	M/I
W_Contig622-contig2	426	PREDICTED: similar to CG2765 CG2765-PA [Tribolium castaneum]	TAC	GAC	Y/D
W_Contig622-contig2	615	PREDICTED: similar to CG2765 CG2765-PA [Tribolium castaneum]	CGA	TGA	R/*
W_Contig623-contig1	530	PREDICTED: similar to anterior fat body protein [Tribolium castaneum]	GTA	GCA	V/A
W_Contig625-contig1	632	PREDICTED: similar to eIF2 alpha subunit [Tribolium castaneum]	GCT	ACT	S/S
W_Contig642-contig3	569	PREDICTED: similar to predicted protein [Tribolium castaneum]	CCG	CCA	P/P
W_Contig676-contig1	569	trypsin precursor [Diaprepes abbreviata]	TCG	ACG	R/R
W_Contig792-contig1	854	PREDICTED: similar to AGAP012394-PA [Tribolium castaneum]	CGA	GGA	S/S
W_Contig792-contig1	965	PREDICTED: similar to AGAP012394-PA [Tribolium castaneum]	CAT	TAT	M/I
W_Contig802-contig2	669	PREDICTED: hypothetical protein [Tribolium castaneum]	ACC	CCC	G/G
W_Contig803-contig2	648	endopolygalacturonase 1 [Chrysomela tremulae]	GGA	GAA	G/E
W_Contig808-contig1	613	PREDICTED: hypothetical protein [Tribolium castaneum]	TGG	GGG	P/P
W_Contig830-contig3	310	PREDICTED: similar to Transmembrane protein 50B (HCV p7-trans-regulated protein 3) [Tribolium castaneum]	TTC	TTT	F/F
W_Contig830-contig3	388	PREDICTED: similar to Transmembrane protein 50B (HCV p7-trans-regulated protein 3) [Tribolium castaneum]	GAT	GAC	D/D
W_Contig830-contig3	574	PREDICTED: similar to Transmembrane protein 50B (HCV p7-trans-regulated protein 3) [Tribolium castaneum]	TTC	TTT	F/F
W_Contig864-contig1	285	PREDICTED: similar to CG7872 CG7872-PA [Tribolium castaneum]	AAG	AAA	K/K
W_Contig864-contig1	381	PREDICTED: similar to CG7872 CG7872-PA [Tribolium castaneum]	TCA	TCG	S/S
W_Contig864-contig1	454	PREDICTED: similar to CG7872 CG7872-PA [Tribolium castaneum]	CGG	CAG	R/Q
W_Contig864-contig1	548	PREDICTED: similar to CG7872 CG7872-PA [Tribolium castaneum]	GTC	ATC	D/D
W_Contig959-contig2	447	PREDICTED: similar to ribosomal protein S6 [Tribolium castaneum]	ACA	ACG	T/T
W_Contig959-contig2	562	PREDICTED: similar to ribosomal protein S6 [Tribolium castaneum]	TAG	TGG	*/W
W_Contig981-contig1	572	PREDICTED: similar to ecdysteroid regulated 16 kDa [Tribolium castaneum]	GTA	GTC	V/V

Discussion

Single nucleotide polymorphisms are becoming the marker of choice especially amongst mammals. It is clear that entomological use of SNPs is lagging behind as a search of the NCBI SNP data base reveals only; *Drosophila*, *Apis mellifera*, *Bombyx mori*, Aedes and Anopheles species. Using SNPs in the *Pissodes strobi* system should open up new avenues for researcher to investigate interactions between this forest pest and its' host tree species.

We identified a total of 202 SNPs with 113 of these in coding regions and an average density of 1.8 per 1000 base pair. The ratio of transitions to transversions was 1.4:1 regardless of whether the ratio is from all the SNPs, coding only or non-coding only. This ratio varies from those found in Drosophila, Anopheles, and humans which all had a 2:1 transition to transversion ratio (Wondji et al. 2007, Vignal et al. 2002, Brookes 1999, Moriyama and Powell 1996). When comparing to Drosophila some caution is warranted as Berger et al. 2001 reported a ratio of 1.08:1 using different Drosophila strains than other studies. However, *Pissodes strobi* show a lower ratio than mammals and could be considered less conservative when compared to mammals. Since this is a preliminary analysis and the entire EST genome has not been sequenced this ratio may change as more SNPs are discovered and individuals are sequenced. There appears to be no strong relationship between the number of reads and the number of SNPs, more reads does not necessarily mean more SNPs (Figure 2).

Nucleotide diversity of *Pissodes strobi* can be estimated from the sequences that we have currently obtained. In the 109 contigs that contained 202 SNPs approximately 120214 bp were derived to have an estimated nucleotide diversity of 1.7×10^{-3}. *Tribolium castaneum* is the closest insect to *Pissodes strobi* to be completely sequenced (~160 Mb) and could be used to roughly estimate the number in *P. strobi*. Once the entire genome size of *P. strobi* is discovered, estimates of SNP density in this insect can be calculated within and between populations.

The SNP data in this study is a preliminary step in the further investigation into *Pissodes strobi* and its interaction with spruce in British Columbia. Further SNP discovery and analysis may hold the key to understanding interactions of this forest pest and its host species.

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Appendix 4 Regression and Correlation Values

	Linear Regression		Pearson Product Correlation	P-value
	Multiple R2 value	Adjusted R2 value		
Church Road (CH)				
Weevils Present vs Leader Length	0.136	0.071	0.369	0.175
Weevils Present vs Leader Top Diameter	0.212	0.084	-0.460	0.084
Weevils Present vs Leader Middle Diameter	0.006	-0.076	0.019	0.945
Weevils Present vs Leader Base Diameter	0.194	-0.056	0.139	0.621
Weevils Present vs Feeding Punctures	0.3216	0.269	0.567	0.027
Weevils Present vs Oviposition Plugs	0.2872	0.232	0.536	0.039
Woodlot (WL)				
Weevils Present vs Leader Length	0.014	-0.048	0.119	0.639
Weevils Present vs Leader Top Diameter	0.128	0.073	0.357	0.145
Weevils Present vs Leader Middle Diameter	0.531	0.501	0.728	0.000
Weevils Present vs Leader Base Diameter	0.604	0.579	0.777	0.000
Weevils Present vs Feeding Punctures	0.064	0.006	0.253	0.311
Weevils Present vs Oviposition Plugs	0.121	0.066	0.348	0.157
PGTIS (PG)				
Weevils Present vs Leader Length	0.061	0.005	-0.246	0.309
Weevils Present vs Leader Top Diameter	0.024	-0.033	0.156	0.524
Weevils Present vs Leader Middle Diameter	0.002	-0.057	-0.041	0.868
Weevils Present vs Leader Base Diameter	0.002	-0.056	0.048	0.848
Weevils Present vs Feeding Punctures	0.003	-0.055	-0.059	0.809
Weevils Present vs Oviposition Plugs	0.002	-0.057	-0.042	0.865
Grafton Road (GR)				
Weevils Present vs Leader Length	0.097	0.037	0.312	0.223

	Linear Regression		Pearson Product Correlation	P-value
	Multiple R2 value	Adjusted R2 value		
Weevils Present vs Leader Top Diameter	0.194	0.140	0.440	0.077
Weevils Present vs Leader Middle Diameter	0.164	0.108	0.405	0.107
Weevils Present vs Leader Base Diameter	0.129	0.071	0.359	0.156
Weevils Present vs Feeding Punctures	0.108	0.048	0.328	0.198
Weevils Present vs Oviposition Plugs	0.148	0.091	0.408	0.127
Camp 4 (CF)				
Weevils Present vs Leader Length	0.226	0.175	0.476	0.053
Weevils Present vs Leader Top Diameter	0.057	-0.006	0.238	0.358
Weevils Present vs Leader Middle Diameter	0.090	0.029	0.300	0.242
Weevils Present vs Leader Base Diameter	0.089	0.029	0.299	0.244
Weevils Present vs Feeding Punctures	0.132	0.074	0.363	0.152
Weevils Present vs Oviposition Plugs	0.128	0.070	0.358	0.158
DM1400 (DM)				
Weevils Present vs Leader Length	0.010	-0.719	0.103	0.727
Weevils Present vs Leader Top Diameter	0.005	-0.078	-0.072	0.807
Weevils Present vs Leader Middle Diameter	0.053	-0.026	0.230	0.428
Weevils Present vs Leader Base Diameter	0.004	-0.079	0.061	0.834
Weevils Present vs Feeding Punctures	0.189	0.121	0.435	0.120
Weevils Present vs Oviposition Plugs	0.257	0.195	0.507	0.061