Population genetics of *Gaultheria shallon* in British Columbia and the implications for management using biocontrol

J.E. Wilkin, S.F. Shamoun, C. Ritland, K. Ritland, and Y.A. El-Kassaby

Abstract: *Gaultheria shallon* Pursh. (salal), an ericaceous shrub native to the Pacific Northwest, often out-competes regenerating conifer species in managed forests. A naturally occurring fungus, *Valdensinia heterodoxa* Peyronel, is being considered as a potential biocontrol agent for salal. Knowledge of the genetic diversity and population structure of salal will help assess the effectiveness and the potential risks of using a biocontrol agent in natural populations. Salal samples were collected from five populations, four on Vancouver Island and one on coastal mainland British Columbia. DNA fingerprints were obtained based on 230 amplified fragment length polymorphisms (AFLPs), of which 99.1% were variable. While salal has been reported to be a polyploid, it is likely that over time it is moving toward a diploid state. Based on a comparison of allele frequencies with known diploids, the AFLP loci used in this study appear to follow a diploid pattern; however, the levels of variation reported in this study may be an underestimation depending on the ploidy of salal. An intensively sampled population on Vancouver Island (Shawnigan Lake) showed isolation by distance and low kinship correlations, indicative of more sexual reproduction than expected for a predominantly clonal population. Our findings suggest that salal may be clonal at a very local scale (less than 5 m), and that with high levels of diversity within populations and little differentiation among populations, developing an effective biocontrol for salal may be challenging.

Key words: population structure, genetic diversity, AFLP, biocontrol, *Gaultheria shallon*.

Résumé : La *Gaultheria shallon* Pursh. (salal), une éricacée arbustive indigène de la côte nord-ouest du Pacifique, compétitionne fréquemment avec des espèces de conifères dans les forêts aménagées. Le *Valdensinia heterodoxa* Peyronel, un champignon venant naturellement, constitue un agent de lutte biologique potentiel contre le salal. Une connaissance de la diversité génétique et de la structure des populations du salal aiderait à évaluer l'efficacité et les risques potentiels liés à l'utilisation d'un agent de lutte biologique dans les populations naturelles. Les auteurs ont récolté des échantillons de salal dans cinq populations, quatre sur l'île de Vancouver, et une en région côtière continentale, en Colombie-Britannique. Ils ont obtenu des empreintes ADN basées sur 230 polymorphismes de longueurs d'amplifications (AFLPs), dont 99,1 % sont variables. Bien qu'on ait rapporté de la polyploïdie chez le salal, il semble, qu'avec le temps, il se déplace vers l'état diploïde. À partir d'une comparaison des fréquences des allèles avec des diploïdes connus, les lieux AFLP utilisés dans cette étude semblent suivre un patron diploïde; cependant, les niveaux de variation rapportés dans cette étude pourraient être sous-estimés, selon la ploïdie du salal. Une population intensément échantillonnée sur l'île de Vancouver (Lac Shawnigan) montre une isolation par la distance et de faibles corrélations de parentalité, ce qui indique une plus forte reproduction sexuelle qu'attendue, pour des populations à prédominance clonale. Les résultats suggèrent que le salal peut être clonal à une échelle très locale (moins de 5 m), et que de hauts niveaux de diversité dans les populations et une faible différenciation entre les populations, pourraient rendre difficile le développement d'un agent de lutte biologique efficace contre le salal.

Mots clés : structure des populations, diversité génétique, AFLP, lutte biologique, *Gaultheria shallon.*

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Introduction

Gaultheria shallon Pursh. (salal) is a perennial evergreen ericaceous shrub native to the Pacific Northwest, occurring from California to the panhandle of Alaska (Fraser et al. 1993). Salal ranges from 0.2 to 5 m in height with thick, ovate, leathery leaves (Pojar and Mackinnon 1994). From March through July, salal produces many urn-shaped flowers that are pollinated by bees, flies, and hummingbirds (Pojar 1974). The purple fruits often remain attached to the

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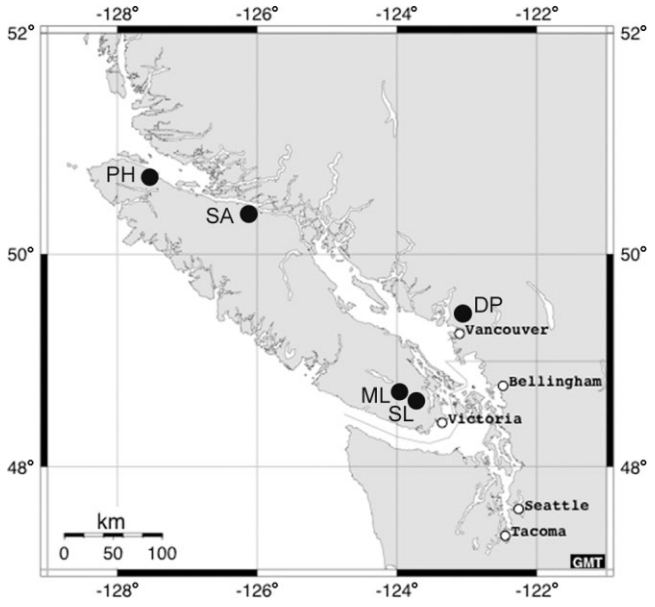
plant for several months and contain many seeds, which are dispersed by birds and mammals (Haeussler et al. 1990, Fraser et al. 1993). Overall, seedling establishment and survival is poor (Huffman et al. 1994), and salal relies predominantly on vegetative growth through an extensive root system and ericoid mycorrhizal associations (Xiao and Berch 1993). Salal has been reported as octoploid ($2n = 88$), derived from two duplication events as evidenced by multivalent chromosome associations during meiosis (Callan 1941). More recently, chromosome preparations from salal in British Columbia showed salal to be diploid ($n = 44$) with no observed multivalent chromosome associations (Pojar 1974).

Salal stems are connected via underground mats of rhizomes, which are thought to create a clonal population structure (Bunnell 1990). Following Cook (1983), clonal fragments include all stems connected by rhizomes. Ramets are stems capable of independent survival and genets include all genetically identical clonal fragments and ramets, which combined, make up a clone. Huffman et al. (1994) excavated clonal fragments and found that as clones age they become physically fragmented. The largest clonal fragment excavated contained 218 m of total rhizome length and 292 ramets, and covered an area of 29 m^2. Clonal expansion was greatest under open canopies (Huffman et al. 1994).

As a predominant understory species, nearly continuous at mid to low elevations, salal has many important roles in coastal British Columbia forests (Fraser et al. 1993; Messier et al. 1990). Salal provides food for many birds, mammals, and microorganisms (Fraser et al. 1993). It is traditionally important to the aboriginal people of coastal British Columbia (Pojar and MacKinnon 1994) and is now commonly used in floral arrangements, which has generated demand for healthy, disease-free plants. In contrast, salal has become a management problem in forested areas where it competes with regenerating conifers (Messier 1992). There has been much research on methods to control salal in these areas (Prescott 1999); however, none of the traditional control methods, such as herbicide treatment, burning, manual brushing, or other means of scarification, have provided an economical and reliable solution (D'Anjou 1990; Tirmenstein 1990).

Biological control may provide an alternative management strategy for salal. To investigate this, a potential biocontrol agent, *Valdensinia heterodoxa* Peyronel (Sclerotiniaceae), has been selected (Shamoun et al. 2000). *Valdensinia heterodoxa* is an ascomycete fungus and a natural pathogen of salal such that the inundative approach to biocontrol can be used, where large amounts of an indigenous pathogen are applied to the target plant to create disease and suppress the host (Wall et al. 1992). The effectiveness of the biocontrol will depend in part on the diversity and structure of both the host and pathogen natural populations. This includes the amount of homogeneity among individuals within a population and the rates of gene flow, mutation, and sexual reproduction in natural populations of both the host and pathogen (McDonald and McDermott 1993; Hintz et al. 2001). This study on salal and a complementary one on *V. heterodoxa* (Wilkin 2004) are the first population genetic investigations into these species and will assess the amount and distribution of genetic diversity in natural populations in British Columbia, which will assist in the evaluation and development an effective biocontrol system.

Fig. 1. Map of Vancouver Island and mainland British Columbia, showing the locations of the five *Gaultheria shallon* (salal) populations sampled in this study.



Materials and methods

Site location

Salal was collected from forested areas in Shawnigan Lake (SL), Mesachie Lake (ML), Sayward (SA), and Port Hardy (PH) on Vancouver Island, and Deek's Peak (DP) on the west coast of mainland British Columbia (Fig. 1). Samples collected from one site were considered to represent one population.

Sample collection

In ML, SA, PH, and DP, five salal stems were selected from each of 10 *V. heterodoxa* infection centres (patches). Up to three infected and uninfected salal leaves (no visible lesions or scars) were collected from each stem; the uninfected leaves were used for this study, while the infected leaves were used to assess the genetic diversity of *V. heterodoxa* (Wilkin 2004). One sample of healthy salal was also collected between infected patches. Overall, sampling of 10 patches, with five stems sampled per patch, plus an additional 10 samples between patches, resulted in a sample size of 60 individuals from each population. The SL population was sampled more intensively to study population structure in greater detail. A 2500 m^2 area sectioned into 5 m × 5 m grids was established in a continuous salal patch. One to three leaves were collected from a single stem at each intersection point for a total of 121 individuals. Discs of leaf tissue (0.33 cm^2) were cut, dried in silica gel, and stored at 4 °C until DNA isolation.

DNA isolation

For DNA isolation, approximately 100 mg of dried leaf tissue was ground with liquid nitrogen and small mortars

and pestles. The isolation protocol followed Weising et al. (1995) with the following modifications: CTAB buffer (2.5% cetyltrimethylammonium bromide, 1% PVP-40, 1.4 mol/L NaCl, 0.02 mol/L EDTA, 0.1 mol/L Tris-HCl, and 0.5% β-mercaptoethanol added just before use) was preheated to 65 °C and 800 mL was added to each sample, which was then vortexed and incubated for 90 min at 65 °C, with gently mixing every 15 min; after chloroform extraction, layers were separated by centrifuging at room temperature for 5 min at 10 000 rpm (Eppendorf Centrifuge 5417R, Hamburg, Germany); following DNA precipitation with isopropanol, the pellet was washed with 1 mL of wash buffer (10 mmol/L ammonium acetate and 70% ethanol) and centrifuged at 14 000 rpm for 5 min at 4 °C; and final pellets were resuspended in 25 µL Tris-EDTA buffer (10 mmol/L Tris-HCL, pH 8.0, and 1 mmol/L EDTA). DNA was isolated from two replicates of each individual sample and then combined for a final volume of 50 µL Tris-EDTA. Concentration and quality of DNA was tested with 1.2% agarose gel electrophoresis in 1× Tris-borate-EDTA and also using a spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Amersham, Piscataway, New Jersey, USA). DNA was diluted to 100 ng/µL for the amplified fragment length polymorphisms (AFLP) reactions.

AFLP analysis

AFLP primer pairs used on *Rhododendron* (Escaravage et al. 1998; Pornon et al. 2000) were screened, and additional new pairs were tested. Four primer pairs that produced clear banding patterns and polymorphisms were selected. To ensure reproducibility, the procedure was independently repeated on a subset of six samples. The AFLP protocol was from the Genetic Data Centre at the University of British Columbia (C. Ritland, personal communication) and used 500 ng DNA in each reaction. A pre-amplification was done using *Eco*RI + A and *Mse*I + C primers. For the final selective amplification, one +3/+4 and three +3/+3 primer pairs were used, where the first sequence refers to the *Eco*RI primer and the second refers to the *Mse*I primer: ACC/CCTT, ACC/CGT, AGG/CTC, and ACA/CGT.

Scoring of AFLP gels

The LI-COR 4200 was used to electrophorese polymerase chain reaction generated fragments (LI-COR Inc., Lincoln, Nebraska, USA). Gels were scored using SAGA-MX for AFLP bands (LI-COR Inc.). When scoring the AFLP fingerprints, it was assumed that comigrating bands on a gel were identical in sequence and that repeatable loci represented stable fragments. Because of the dominant nature of AFLPs, bands were scored as present or absent. Individuals showing no band at a locus were considered homozygous recessive absent (null), and those with a band were assumed to have at least one copy of the amplifying allele.

Data analysis

Scorable bands ranged from 64 to 660 bp. In total, 230 loci were scored across all populations, and all loci were used in the analyses. Individuals with missing data were not included in the analyses because they biased pairwise comparisons.

Salal has 88 chromosomes, which suggests octoploidy. However, this has not been confirmed. Because of this unconfirmed ploidy, wherever possible, indices of band sharing were used that do not require knowledge of ploidy. When necessary for certain analyses, salal was assumed to be diploid. This assumption can be justified by comparing the distribution of the null allele frequency (q) when calculated as diploid (square root of the null frequency) and as octoploid (eighth root of the null frequency, Fig. 2) to the expected distribution, which, from Zhivotovsky (1999) is expected to be flat under a neutral model. To further support the assumption that salal is behaving as a diploid, the distribution of q was compared with two known diploids, *Pinus contorta* Dougl. ex. Loud. (lodgepole pine) and *Arbutus menziesii* Pursh (arbutus). The null allele frequencies for both the lodgepole pine (C. Liewlaksaneeyanawin, unpublished data) and arbutus (Beland 2004) data sets were calculated from AFLP loci as the square root of the null band frequency and compared with that of salal to see if salal followed the same distribution as confirmed diploids (Fig. 2). Also, if salal is in the process of diploidization, it is likely that varying levels of ploidy exist in these populations. To test this, the inheritance of each locus would have to be tested individually, which would be extremely laborious. Instead, by showing that the distribution of allele frequencies follows the same pattern as other known diploids, it suggests that at least for these loci, salal is behaving predominantly as a diploid (Fig. 2). As well, wide ranges in band intensity were not readily observed to account for salal behaving as an octoploid, nor is scoring AFLP bands for intensity reliable enough to indicate copy number or ploidy (Nybom 2004).

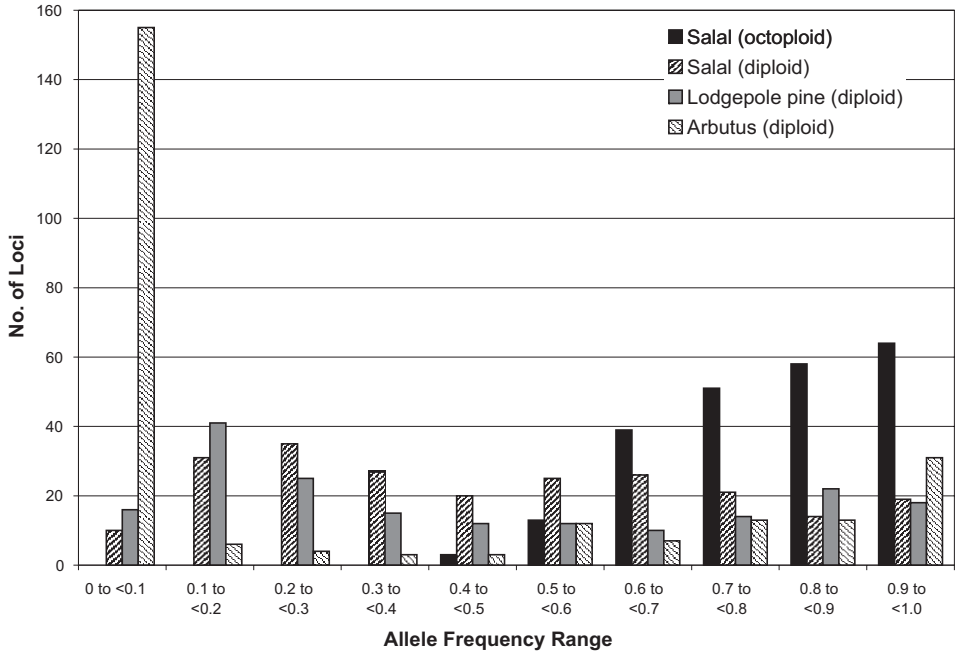
Differentiation among populations was determined using principal component analysis in SAS (Version 8.0, SAS Institute Inc., Cary, North Carolina) and ANOVA procedures in SPAGeDi (Version 1.1, Hardy and Vekemans 2002) to calculate the level of variance partitioned among populations (global F_{ST}) and pairwise genetic distances (F_{ST}) between populations. The F_{ST} values calculated in SPAGeDi use a nested ANOVA procedure from Weir and Cockerham (1984) and are based on allele identity. The pairwise F_{ST} values used to create the bootstrapped unweighted pair group with arithmetic averages (UPGMA) dendrogram were based on the method of Reynolds et al. (1983) invoked in PHYLIP Version 3.57c (Felsenstein 1995) using diploid null allele frequencies.

To gain some insight into the level of similarity among individuals within populations and to study fine-scale population structure in the Shawnigan Lake (SL) population, SPAGeDi, which does not assume Hardy–Weinberg equilibrium (Hardy 2003), was used. Since the inbreeding coefficient must be known, the analysis was run using a range of coefficients. Hardy's kinship estimator for dominant data (Hardy 2003) was used to test whether genetic relatedness between pairs of individuals was correlated with spatial distance.

Results

The four primer pairs resulted in 230 scorable loci, of which only two were monomorphic for the dominant allele. Some individuals had missing data, reducing total sample

Fig. 2. Comparison of *Gaultheria shallon* (salal) null allele frequency distributions to those of two known diploids, lodgepole pine (*Pinus contorta*) and arbutus (*Arbutus menziesii*). The comparison shows that the distribution of diploid frequencies is more flat than that of octoploid frequencies and that the null allele frequency of salal follows the same pattern as two known diploids, lodgepole pine and arbutus.



size from 361 to 273 individuals (Table 1). Over all populations, 99.1% of the loci showed variation (Table 1), but the average percentage of polymorphic loci (within populations) was 89.7%; SL had the lowest diversity (87.0%) while SA and DP had the highest (92.2%; Table 1).

Over all populations, the average frequency of the null fragment was 0.30 and the average frequency of q was 0.48 assuming diploid and 0.80 assuming octoploid (based on 228 polymorphic loci). Figure 2 shows that for salal, diploid gene frequencies have a flat and more even distribution than octoploid frequencies, which are skewed above 0.5. As well, the distribution of the diploid frequencies matches those of other known diploids. For the SL population, the average gene diversity of the null allele (q_{avg}) was 0.40 (diploid) and 0.69 (octoploid), which was the lowest of the five populations. In comparison, DP had the highest q_{avg} with values of 0.58 (diploid) and 0.80 (octoploid). No two individuals had identical fingerprints.

Kinship coefficients between pairs of individuals within each population ranged from –0.348 to 0.811 and from the average kinship coefficients, suggest that most individuals are no more related to each other than to the rest of the population (Table 2). However, some individuals did show high levels of similarity to each other. In particular, DP had the highest average kinship coefficient (0.147) and the most individuals with kinship coefficients greater than 0.5, although this population also had the fewest number of pairs (Table 2).

Differentiation among populations

Principal component analysis indicated that many loci contribute to the variation observed in these populations, as 72 principle components had eigenvalues greater than one and explained 80% of the variation. The plot of the first two principle components showed that some of the variation is due to differences among populations as seen by the clustering of individuals from the same population, particularly for SL and ML, and the separation of the medians of each group (Fig. 3). This was also supported by the global F_{ST}, for which 9.27% of the variation was partitioned among the populations while the majority of variation was within populations ($P < 0.0001$).

The matrix of pairwise F_{ST} values based on allele identity (Table 3) suggests that SA and DP are the least differentiated populations ($F_{ST} = 0.0319$), although they also had the smallest sample sizes (Table 1). SL and DP had the highest F_{ST} (0.1603, Table 3). A UPGMA dendrogram of pairwise F_{ST} values based on diploid gene frequencies (Fig. 4) shows that SL and ML formed one cluster while PH, SA, and DP formed a second cluster, in which SA and DP were the most similar.

A Mantel test that evaluated the association of pairwise F_{ST} values with pairwise spatial distances was not significant, although this could reflect the low power of the test with only five populations. Qualitative comparisons of the pairwise F_{ST} values and the bootstrap values of the UPGMA dendrogram suggest there may be some isolation by distance (Table 3, Fig. 4). For example, SL is most genetically similar to ML, which is the closest population geographically, and most genetically differentiated from DP (on the coastal mainland), which is the population most isolated from SL (located in the interior of Vancouver Island). It also suggests that populations are separated latitudinally, as SA is more differentiated from PH than it is from DP.

Table 1. Sample size, number of monomorphic loci, and % polymorphic loci in each population of *Gaultheria shallon* based on 230 loci.

Population*	Sample size†	No. of monomorphic loci		% polymorphic loci
		No. with dominant alleles‡	No. with recessive alleles§	
SL	111	2	28	87.0
ML	56	4	19	90.0
SA	36	5	13	92.1
PH	39	7	22	87.4
DP	31	11	7	92.1
Total	273	2	0	99.1

*SL, Shawnigan Lake; ML, Mesachie lake; SA, Sayward; PH, Port Hardy; DP, Deek's Peak.
†Individuals with missing data were excluded.
‡Dominant refers to presence of a band.
§Recessive refers to absence of a band.

Table 2. Summary of pairwise kinship coefficients among individuals within *Gaultheria shallon* (salal) populations.

Population*	No. of pairs†	Mean kinship coefficient	Variance	Minimum kinship coefficient	Maximum kinship coefficient	% pairs with kinship > 0.5
SL	6105	–0.002	0.017	–0.348	0.714	0.52
ML	1540	0.063	0.021	–0.294	0.578	0.58
PH	741	0.065	0.025	–0.278	0.684	0.94
SA	630	0.071	0.020	–0.287	0.742	0.63
DP	465	0.147	0.036	–0.209	0.811	7.31

*SL, Shawnigan Lake; ML, Mesachie lake; SA, Sayward; PH, Port Hardy; DP, Deek's Peak.
†Number of pairs = $n(n–1)/2$, where n is the population size.

Fig. 3. Clustering of populations of *Gaultheria shallon* in a plot of principle components (Prin) from principal component analysis. The median of each population is indicated by the large symbol corresponding to each group. SL, Shawnigan Lake; ML, Mesachie lake; SA, Sayward; PH, Port Hardy; DP, Deek's Peak.

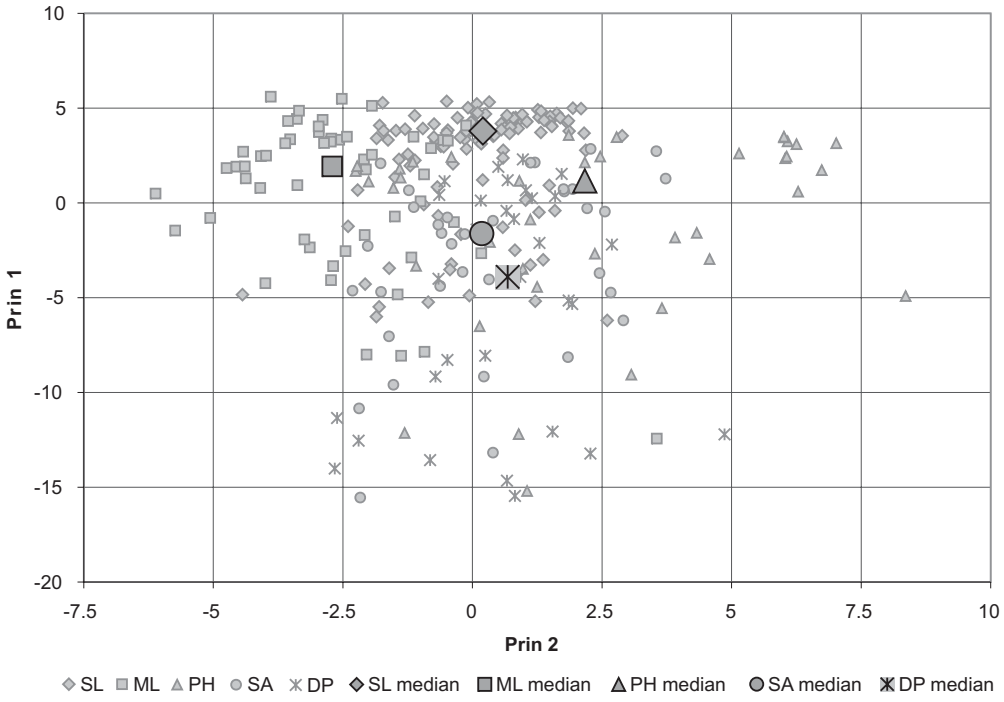
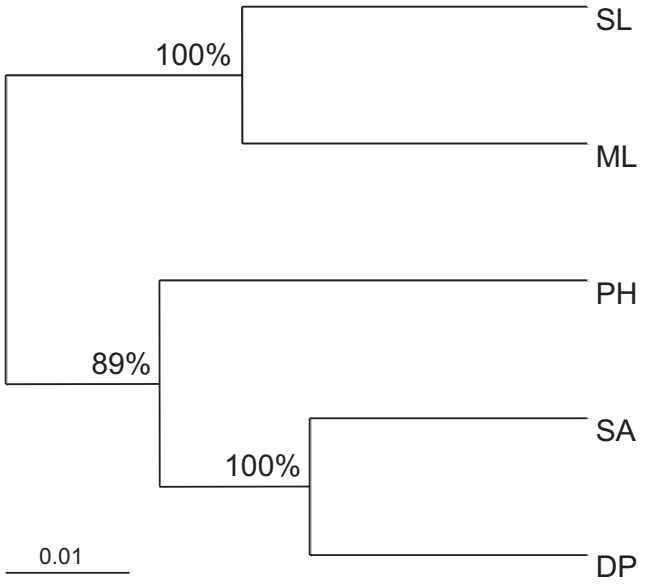


Table 3. Matrix of population pairwise genetic distances among *Gaultheria shallon* (salal) populations calculated as F_{ST} values from a modified ANOVA procedure.

Population*	SL	ML	SA	PH
ML	0.0732			
SA	0.0892	0.0851		
PH	0.1043	0.0874	0.0572	
DP	0.1603	0.1282	0.0764	0.0319

*SL, Shawnigan Lake; ML, Mesachie lake; SA, Sayward; PH, Port Hardy; DP, Deek's Peak.

Fig. 4. UPGMA dendrogram of *Gaultheria shallon* (salal) populations based on Reynold's (1983) pairwise F_{ST} genetic distances. The percent support of 100 bootstrapped replicates is indicated at each branch point. SL, Shawnigan Lake; ML, Mesachie lake; SA, Sayward; PH, Port Hardy; DP, Deek's Peak.



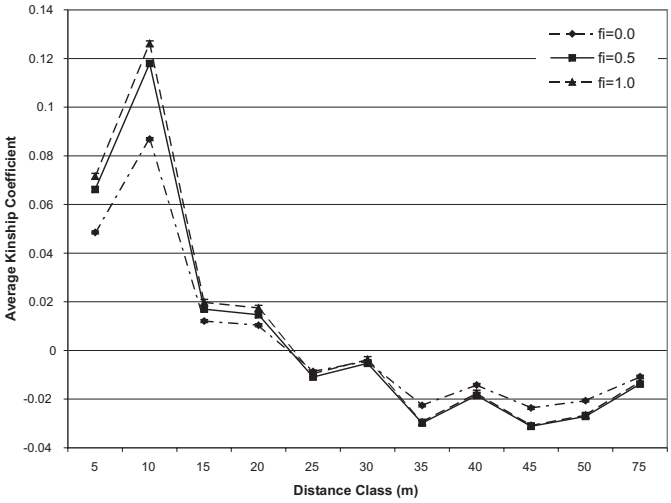
Fine-scale structure within the Shawnigan Lake population

SL was not as genetically diverse as the other populations (Table 1). As spatial distance increased, genetic similarity decreased (Fig. 5). Kinship coefficients increased between the 5-m and 10-m spatial distance classes. When pairwise kinship coefficients were partitioned into groups, we found that most individuals were no more related within groups than to the whole population (Fig. 6).

Discussion

The levels of genetic diversity and polymorphism found in this study are similar to those found in studies of other Ericaceous species. Using intersimple sequence repeat (ISSR) markers on *Gaultheria fragrantissima* Wall, Deshpande et al. (2001) detected 83.7% polymorphism, and using AFLPs, Escaravage et al. (1998) found *Rhododendron ferrugineum* L. to be 88% polymorphic. Albert et al. (2003) found levels of genetic diversity in three clonal *Vaccinium* species to be equivalent to those in nonclonal species. While

Fig. 5. Relationship between average kinship coefficients (genetic similarity) and spatial distance classes for *Gaultheria shallon* (salal) individuals from Shawnigan Lake at a range of inbreeding coefficients (f_i). Error bars are average jackknifed standard errors based on measures of actual variance over all loci for each inbreeding coefficient (Ritland 1996).



the likely variable ploidy in salal may have resulted in higher polymorphism than would be observed in strictly diploid Ericaceous species, these other studies indicate that genetic diversity in the Ericaceae is very high, which is consistent with the results obtained in this study.

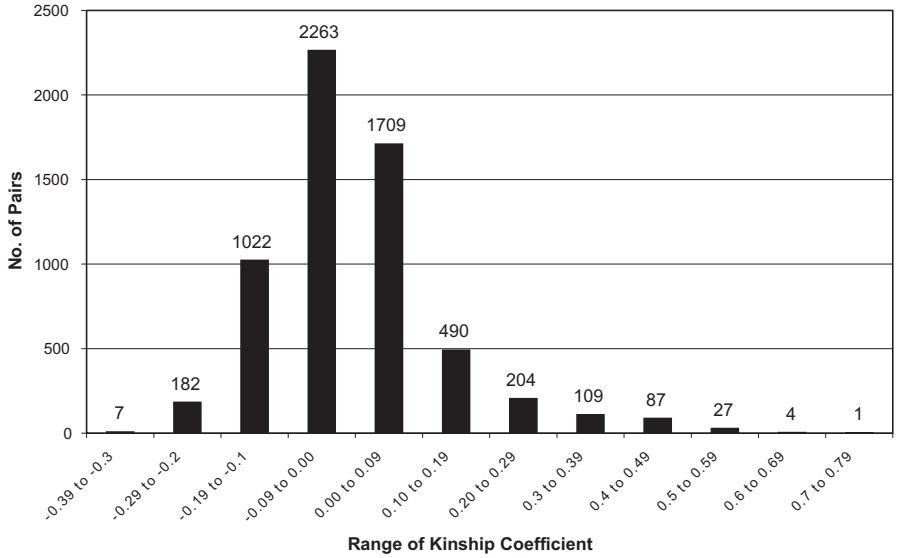
Differentiation among populations

Principal component analysis and analysis of molecular variance showed little population differentiation among the salal populations in this study. The overall F_{ST} of 0.0927 is comparable to the level of isozyme population differentiation observed for other long-lived woody perennial species (0.084, Hamrick et al. 1992), although lower than that observed for RAPD markers (0.25, Nybom 2004). A study of *Leucopogon obtectus* Benth. found that 10.3% of the observed AFLP variation was partitioned among populations (Zawko et al. 2001). Low population differentiation in salal could be explained by high gene flow among populations, particularly since relatively few migrants per generation are necessary to reduce F_{ST}. High migration rates are consistent with the wildlife-mediated pollen and seed dispersal in salal.

Based on pairwise F_{ST} values, SA and DP appear to be the most similar populations (lowest F_{ST}). From the dendrogram (Fig. 4), there is support for isolation by distance as demonstrated by SL and ML, which had low F_{ST} and were geographically close to each other. However, isolation by distance was not supported by the Mantel test. Also in contrast is SA, which was closest geographically to PH but most genetically similar to DP on the mainland. This relationship could result from prevailing winds causing mainland populations to be pollinated by island populations or from a founder effect, with SA founded by genetically different individuals than PH, perhaps from mainland populations as evidenced by its high similarity to the DP populations.

Low population differentiation was also observed in a study on phenotypic variation among salal provenances

Fig. 6. Frequency distribution of 6105 pairwise kinship coefficients between individuals of *Gaultheria shallon* (salal) from Shawnigan Lake (n = 111). The number of pairs within each kinship coefficient class is noted above each bar.



(Dorworth et al. 2001). The authors proposed that genetically different individuals established geographically close populations or that birds disperse seeds over wide geographic distances. High gene flow, as suggested by Dorworth et al. (2001), could explain the low levels of differentiation observed. Alternatively, the sampling scale may not have captured clonal patterns in the natural populations. As well, the populations used for this study represented only the center of the species' range; such central populations often have high diversity and low differentiation (Gapare 2005).

Fine-scale structure in Shawnigan Lake

A significant decrease in genetic similarity was observed as spatial distance increased (Fig. 5), as was found in *Rhododendron ferrugineum* populations with AFLP markers (Escaravage et al. 1998). Salal was predicted to have a predominantly clonal population structure based on its life history; however, the level of genetic similarity between individuals in this study does not support this hypothesis. For example, no two individuals had identical fingerprints and only a few individuals likely differed by somatic mutations alone (e.g., in disconnected ramets of a clone). As well, high levels of diversity were observed compared with the moderate levels of diversity expected in a clonal population (Ellstrand and Roose 1987). Individuals 10 m apart had higher kinship coefficients than those 5 m apart (Fig. 5), which could be explained by local competition among clones or possibly by a growth form characterized by widely spaced, intermingling ramets. This may also reflect the pattern of seed dispersal within the population, although depending on canopy cover, salal is thought to rely predominantly on vegetative reproduction (Xiao and Berch 1993) and seedling establishment is often poor (Huffman et al. 1994). This suggests that 10 m may indicate the limit to clone size or the size at which the dominant reproduction mode switches from clonal to asexual.

The observed genetic differentiation among individuals (Fig. 6) suggests quite high levels of random mating. This was unexpected because in clonal populations many individuals would have high kinship correlations (i.e., >0.95). Significant levels of relatedness in salal are likely found at smaller, more local spatial scales, such as within the 5-m sampling grid. This is supported by Huffman et al. (1994) who found that the largest salal clone in their study covered 29 m^2. As well, with the ericaceous shrub *Elliottia racemosa*, Godt and Hamrick (1999) found that while populations were highly clonal, the distribution was not related to spatial distance within the population. They proposed that ramets separating and dying off made room for the growth of other clones, leading to widely dispersed stems of the same clone. However, if this is true with salal, one should find many individuals with high genetic similarities within populations regardless of spatial distance, which was not observed in this study. These results suggest that for further population-level studies on salal, samples should be collected more than 10 m apart to obtain different individuals. Clonal population structure was not readily observed in this study and finer-scale sampling may be necessary to further clarify clonal dynamics and mutation rates.

Implications for biocontrol

Relevant to the development of a biocontrol agent to control salal is the population genetics of salal. The levels of genetic diversity and population structure in the host plant can help predict the suitability of biocontrol as a control method and also indicate the potential effects of introducing large amounts of the biocontrol agent into the environment. One major concern is that continued application of a virulent pathogen will select for resistant plant strains. For example, successive application of the biocontrol agent is expected to cause epidemics throughout the targeted area and induce population bottlenecks resulting in a residual population of resistant plants. Depending on the amount of gene flow among populations, these resistant plants may increase in frequency making the population more difficult to control, necessitating the development of a more virulent biocontrol (Slatkin 1987; McDonald and McDermott 1993). As well,

the stress from the biocontrol epidemic may induce the host plant to shift from predominantly vegetative growth to sexual reproduction, resulting in more recombination and thereby increasing the probability of developing new resistance phenotypes in the plant on which selection could act (Barrett 1982).

An ideal situation would be to have differentiated populations with very low gene flow, which would effectively restrict the biocontrol strain to the target area, potentially making it easier to control and monitor. However, this study has shown salal to have very high genetic diversity, suggestive of high gene flow and low population differentiation. With highly diverse populations, it may be difficult to find one biocontrol strain effective over a whole population. As a result, a cocktail of strains may be necessary for the biocontrol to be effective. High diversity and polyploidy in salal may also increase its ability to adapt to the biocontrol strain, particularly if the additional gene copies resulting from increased ploidy level are a source of variation (Soltis and Soltis 2000). In this case, further studies should be done to determine the current ploidy of salal in natural populations and the level of divergence among the genome copies. While the populations in this study showed low differentiation, a range-wide study would be interesting to see if population differentiation occurs at a larger scale, enabling the controlled use of a biocontrol agent over an entire region.

This is the first study to use molecular markers in natural populations of salal in coastal British Columbia. It revealed that salal has very high genetic diversity and low population structure, suggesting that precautions should be taken in management strategies of salal. As well, this study found evidence for more sexual reproduction in these populations than predicted and that clonal reproduction may be limited to small, localized areas.

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