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Y. A. El-Kassaby · B. G. Dunsworth · J. Krakowski

Genetic evaluation of alternative silvicultural systems in coastal montane forests: western hemlock and amabilis fir

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Abstract Genetic diversity and mating system were quantified for shelterwood, patch cut and green tree-retention silvicultural systems, and compared to adjacent old-growth. This is a component of a larger study conducted in montane old-growth forests of coastal British Columbia to evaluate the feasibility and ecological consequences of alternative silvicultural systems. The experiment includes replicated treatments representing a range of overstory removal adjacent to old-growth and clearcut areas. Based on 22 electrophoretically assayed loci, the effects of silvicultural systems on genetic parameters of amabilis fir (*Abies amabilis* and western hemlock (*Tsuga heterophylla* were assessed by comparing an average number of alleles per locus, the percent polymorphic loci, and observed and expected heterozygosity between parental populations and naturally regenerated progeny as well as among treatments. Genetic variation in natural regeneration was greater than in parental populations, especially for low-frequency alleles. Silvicultural treatments caused no significant differences in amabilis fir genetic-diversity parameters, while the shelterwood system resulted in lower observed and expected heterozygosity in western hemlock. Nei's genetic distance revealed that all parental populations were extremely similar. The two species had contrasting mating system dynamics with amabilis fir producing higher levels of correlated paternity and inbreeding with wider variation among individual tree outcrossing-rate estimates. Western hemlock had significant levels of correlated paternity only for the green tree and shelterwood treatments demonstrating family structuring inversely related to stand density. Inbreeding in western hemlock was significant but lower than that observed for amabilis fir with a J-shaped distribution for individual tree multilocus outcrossing-rate estimates. The pollination and dispersal mechanisms of the two species represent the most-likely factors causing these differences. Artificial regeneration may be utilized to augment the genetic resources of natural ingress.

Keywords Alternative silvicultural systems · Old growth · Natural regeneration · Mating system · Genetic diversity

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Y. A. El-Kassaby (✉) · J. Krakowski
Department of Forest Sciences,
The University of British Columbia, 3616-2424 Main Mall,
Vancouver, British Columbia V6T 1Z4, Canada
e-mail: yelkassaby@cellfor.com
Tel.: (604)-822-1821, Fax: (604)-822-9102

B. G. Dunsworth
North Island Timberlands, Weyerhaeuser Company Limited,
65 Front St., Nanaimo, British Columbia V9R 5H9, Canada

Introduction

Given the public's growing concern over the importance of biological diversity, foresters must ensure critical stand-attributes remain following silvicultural operations. These include not only physical-attributes, such as stand structure, coarse woody debris and understory species diversity, but also may include the inherent genetic characteristics of a stand (Province of B.C. 1995). Genetic parameters can be utilized as indices of change between pre- and post-harvest stand conditions (Buchert et al. 1997). The genetic composition and diversity of stands can be assessed whether natural or artificial regeneration is employed. When stands are naturally regenerated, direct comparisons can be made between the parents and offspring to evaluate the effects of silvicultural activities on a stand over the long term (Neale and Adams 1985; Morgante et al. 1991; Zheng and Ennos 1997; Rajora 1999; Stoehr 2000).

Clearcutting as a silvicultural system has recently been falling into public disfavour as a multiplicity of simultaneous forest-management objectives are implemented on public land in British Columbia (B.C.). Visual quality objectives, wildlife habitat, watershed values, and recreational and aesthetic values all must be considered prior to harvesting stands (Forest Practices Code of B.C. Act

1996). This has engendered a shift in coastal temperate and mesothermal forestry away from uniform clearcutting and towards a mixture of alternative silvicultural systems. While such studies have been conducted at low elevations (Adams et al. 1998), montane forests, which represent a significant proportion of future timber harvest in British Columbia, have been subject to little research in this area (Kopenaal and Mitchell 1992).

If future stands are genetically depauperate compared to the parent pre-harvest stands, then managers must supplement the genetic resources of the stand by planting seedlings from appropriate areas. Provenance transfer is the overriding concern in reforestation throughout most of B.C., but it is also essential that a comparable level of genetic diversity be maintained in regenerated stands (Clayoquot Scientific Panel 1995). It is currently unknown how silvicultural manipulation would affect the genetics of most-important montane forestry species in the Pacific northwest.

While prior research indicates that density typically has a major effect on mating system and genetic diversity of many key forestry species, the life history and reproductive biology of each species also plays an important role (El-Kassaby 2000). For this reason, generalizations may not be appropriate across species as each follows a unique phenological cycle and responds to different environmental and ecological parameters. The species selected for this study were western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] and amabilis fir [*Abies amabilis* (Dougl. ex Forbes)]. Both are late-seral shade-tolerant monoecious species and can survive for long periods in the understory of a dense canopy. Hemlock produces abundant small, pendant cones throughout the entire crown with tiny, winged seeds (Edwards 1976; Packee 1990), whereas amabilis fir has a strongly periodic cone cycle resulting in mast years of upright cones at the top of the crown which disintegrate upon maturity, releasing somewhat larger, winged seeds (Crawford and Oliver 1990). Inherent variability among individuals is also an important determinant of mating systems and partitioning of genetic diversity within stands (Farris and Mitton 1984; Schoen and Stewart 1986, 1987; El-Kassaby et al. 1987; Denti and Schoen 1988). For this reason, it is important to assess inbreeding (including consanguineous mating) and outcrossing both at the stand and individual tree levels (Aldrich and Hamrick 1998).

The objective of this study was to quantify the effects of different silvicultural systems on genetic diversity and the mating system of western hemlock and amabilis fir for both individual trees and within stands, relative to an old growth control. Based on this information, appropriate recommendations can be made regarding the need for artificial regeneration of these species with respect to genetic diversity, compared to the efficacy of natural regeneration.

Materials and methods

Study site

In conjunction with research, government and industry partners, the MASS (Montane Alternative Silvicultural Systems) research project was established in 1993 to investigate the effects of a variety of silvicultural systems on a host of ecological and operational factors in the montane coastal forest of British Columbia (Beese and Arnott 1999). Located on private land formerly owned by MacMillan Bloedel, now Weyerhaeuser, near Campbell River, B.C., (49°55′N, 125°25′E), situated in the Coastal Western Hemlock Montane Moist Maritime biogeoclimatic variant (CWHmm2) (Green and Klinka 1994), the site has a north aspect, a slope of <20% and ranges in elevation from 740 to 850 m. The pre-harvest old growth forest encompassing the entire study area featured (by the basal area) approximately 45% western hemlock, 25% amabilis fir, 25% western red-cedar (*Thuja plicata*) and 5% Alaska yellow-cedar (*Chamaecyparis nootkatensis*), with dominant trees ranging in age from 200 to 800 years (Arnott and Beese 1997).

Prominent understory species, varying in abundance with soil moisture and nutrient regimes, include *Achlys triphylla, Athyrium filix-femina, Brachythecium* spp., *Cornus canadensis, Dicranum fuscescens, Goodyera oblongifolia, Gymnocarpium dryopteris, Listera caurina, Listeria cordata, Oplopanax horridus, Rhytidiopsis robusta, Rubus spectabilis, Sorbus sitchensis, Veratrum viride* and *Viola glabella* (Beese 1995). Many of these species are indicative of nitrogen-poor soils; humus-form classification and thickness varied from Mors to Mulls with microslope position and mineral soil texture. Mineral soils were generally Loams to Clay Loams overlaying glacial till, sandstone and conglomerate; the most common soil types were (CSSC, Canada) Podzols, Gleysols and Brunisols (USA) Spodosols, Entisols, Alfisols, Inceptisols and Histosols (Beese and Bryant 1999).

Experimental design and treatments

Three replicates of approximately 10 ha each for each alternative silvicultural treatment (PC, patch cut; SW, shelterwood; GT, green tree retention) were randomly installed adjacent to a 69-ha clearcut (CC) treatment and a 20-ha old-growth (OG) control stand from 1992 to 1993 (Fig. 1). Land use and operational constraints precluded the installation of three separate CC and OG blocks of suitable dimensions, so this was mitigated by making the CC and OG treatments much larger to minimize microsite and edge effects. Old-growth buffer stands surround the entire experimental site. The

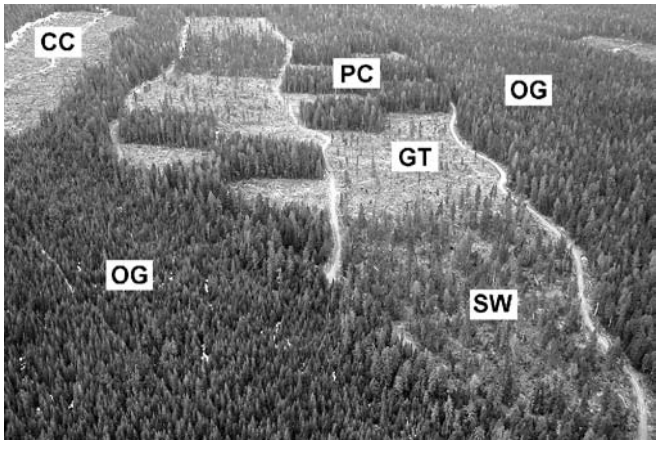

Fig. 1 Aerial view of the study area showing the three replications of the alternative silvicultural systems (PC: patch cut, SW: shelterwood and GT: green tree) as well as old-growth (OG) and clearcut (CC) areas

Table 1 List of electrophoretically assayed enzyme systems and loci used for each analysis

Enzyme	Code	E.C.	Genetic diversity		Mating system	
			Hemlock	Amabilis	Hemlock	Amabilis
Uridine-5′-diphosphoglucose dehydrogenase	UGP	1.1.1.22	1	1	–	–
Shikimate dehydrogenase	SKDH	1.1.1.25	1,2	1,2	–	–
Malate dehydrogenase	MDH	1.1.1.37	1,2,3	1,2	1,2,3,4	1,2,3
Malic enzyme	ME	1.1.1.40	1	1	–	–
Isocitrate dehydrogenase	IDH	1.1.1.42	1	1	1	1
6-Phosphogluconic dehydrogenase	6PGD	1.1.1.44	1	1	2	1
Superoxide dismutase	SOD	1.15.1.1	–	1	–	–
Glutamate dehydrogenase	GDH	1.4.1.2	1	1	–	–
Aspartate amino-transferase	AAT	2.6.1.1	1,2,3	1,2	–	–
Esterase	EST	3.1.1.2	1	1	–	–
Fructose diphosphatase	FDP	3.1.3.11	1	1	–	–
Leucine aminopeptidase	LAP	3.4.11.1	1	1,2	–	–
Aconitase	ACO	4.2.1.3	1	1	–	1
Phosphoglucose isomerase	PGI	5.3.1.9	1,2	1,2	1,2	1
Phosphoglucomutase	PGM	5.4.2.2	1,2	1,2,3	1	1
Glucose-6-phosphate dehydrogenase	G6P	1.1.1.49	–	–	1,2	1

PC removed 50% of the stand basal area (>17.5 cm DBH) in three patches per block of 1.5 to 2 ha; advance regeneration was retained within cutblocks, which were a maximum of two tree lengths (80 m) wide to promote natural regeneration and provide protection for ingress. The GT treatment aimed to leave approximately 25 evenly distributed relatively wind-firm trees per hectare post-harvest to provide both a continual seed source and wildlife habitat, although residual stand damage during operations and windthrow following harvesting reduced the number of leaf trees to a mean of 15 stems per hectare for amabilis fir (Beese 1995). The SW removed 30% of stand basal area across crown classes; clumps of trees and advance regeneration were left following removal of selected trees; leaf trees were selected based on operational feasibility, stand structural attributes and safety, although the final number of mature trees was lower than anticipated due to damage during harvesting and windthrow (Beese 1995). The CC included removal of all mature trees and ingress, and natural regeneration from adjacent areas was allowed to establish (Arnott and Beese 1997; Beese and Arnott 1999).

Sampling

From the two most common species, amabilis fir and western hemlock, dormant vegetative buds and seed cones were collected using a helicopter from up to 30 parent trees in the old-growth reserve (control) and from each of the three replications of the three silvicultural systems (SW, GT retention and PC). All remaining mature trees were sampled in the GT replicates, although there were still fewer than in the other treatments. No sampling was done in the clearcut block: the old-growth parental population was treated as the parents of both the old-growth and clearcut seedling populations, since seedling samples were taken from natural ingress, which must have originated in the clearcut from the surrounding old-growth trees.

Dormant buds from natural regeneration of the two species were also manually collected from the OG, the three alternative silvicultural system treatments as well as the CC. Great care was taken to ensure that the natural regeneration seedlings sampled were from seed crops produced after the start of the experiment Sampling was conducted during 1997 and 1998 for amabilis fir and western hemlock, respectively. Sample sizes for genetic diversity estimates (parent trees and natural regeneration) and for the mating system evaluation from each species and treatment combination are in Tables 2 and 5; sample numbers from mature trees were restricted by the number of leaf trees within each block, and availability and cost of helicopter time. Samples were then placed in plastic bags and stored on ice to prevent protein deterioration, shipped to the laboratory and stored at 2 °C until protein extraction.

Isozyme analyses

Isozyme analyses were conducted on the dormant bud tissues of both parent trees and the natural regeneration for estimating genetic-diversity parameters. Vegetative bud primordia were removed from the bud scales and proteins were extracted using a slightly modified extraction buffer developed by Cheliak and Pitel (1984). Protein electrophoresis was conducted on 11% horizontal starch gels using four gel-electrode buffer systems. The buffer systems used were: (1) histidine citrate pH 7.0 (Fildes and Harris 1966), (2) morpholine citrate pH 6.1 (Clayton and Tretiak 1972), (3) tris citrate: lithium borate pH 8.5 (Ridgeway et al. 1970), and (4) tris citrate pH 7.0 (Siciliano and Shaw 1976).

Twenty two loci were resolved for each species. Those used to assess genetic diversity and mating system parameters for each species are listed in Table 1. Staining methods used followed O'Malley et al. (1980) and Conkle et al. (1982). Additional isozyme analyses were conducted on seeds (germinated diploid embryo and haploid maternal megagametophyte tissue extracted from seeds) for estimating mating-system parameters employing the same methodology mentioned above.

Data analyses

Prior isozyme studies on amabilis fir (Davidson and El-Kassaby 1997) and on the closely related mountain (*Tsuga mertensiana*; Ally et al. 2000) and eastern (*T. canadensis*; Zabinski 1992) hemlock revealed that loci assessed in this study were not linked and were inherited according to Hardy-Weinberg expectations, so all loci were retained for subsequent analyses. Genetic-diversity parameters (average number of alleles per locus, N_a; percent polymorphic loci at the 95% allele frequency and no cutoff levels, PLP_{95} and PLP_{no} respectively; observed and expected heterozygosity, H_o and H_e, respectively) were determined for each combination of tree species, treatment and generation (see Table 2), which were subsequently compared using Nei's (1978) genetic distance (see Tables 4 and 5). These parameters were calculated using the BIOSYS-2 computer program (Swofford et al. 1997). Mating-system parameters [single-locus (t_s) and multilocus (t_m) estimates of outcrossing rates] were estimated using the maximum-

likelihood procedure of Ritland and El-Kassaby (1985) based on exclusion of pollen donors from the gametic pool (Meagher 1986). Outcrossing rates (t) and pollen allelic frequencies (p) were jointly estimated over 100 bootstrapped iterations. The correlated matings, r_p, were estimated following the method of Ritland (1989) after modification to account for the added information obtained from the megagametophytic tissue of conifer seeds (Ritland 1989). The sample sizes (number of trees and seeds per treatment) used in estimating the mating system parameters are listed in Table 6.

Differences among blocks for the alternative silvicultural systems were assessed using Fisher's exact (for 2 × 2 contingency tables) and chi-square tests (for comparisons with more than two factors) of the allele frequency distribution of each locus (Neale 1985). Where differences among blocks were not significant, the blocks were pooled within treatments. This also enabled analyses on larger sample sizes since some blocks within treatments (e.g., GT) had less than 30 mature trees remaining after harvesting, although El-Kassaby and Sziklai (1983) demonstrated that allele frequencies of virtually all frequency distributions can be accurately determined by a sample size of 20 to 30 individuals per population.

The impact on single-tree multilocus outcrossing rates among harvesting methods was tested using one-way analysis of variance at the 5% significance level. The general model for the two-factor GLM ANOVA was:

$$y_{(ij)k} = \mu + T_i + G_j + TG_{ij} + \varepsilon_{(ij)k},$$

where $y_{(ij)k}$ is the value of each genetic parameter for each replication k of the combination of treatment i and generation j, μ is the overall mean, T is the treatment mean, G is the generation mean (parents or offspring), and ε is the residual value associated with that specific combination of factors. All model parameters were fixed.

Statistical differences between genetic-diversity parameters among treatment means and among parent-offspring pairs within each treatment type were quantified using the Student-Newman-Keuls multiple-range test. Differences among treatment by generation combinations were evaluated by a multiple-range test using least-squares means, incorporating the Tukey-Kramer adjustment for sample sizes (SAS Institute 2000) (see Table 4).

Results

Allele frequencies

Amabilis fir

On an individual replicate (block) basis, only one (GT-1) of 11 blocked treatment units had no significant differences between allele frequencies of the parent and offspring populations. The number of significantly different loci per replicate ranged from one (SW-3, GT-3) to four (CC), representing 3 to 18% of the total possible. At the $\alpha = 0.05$ level, the threshold value for statistically significant differences between parent and offspring allele frequencies would be four out of the 22 loci assayed. Eight of the 11 treatment blocks had significantly different allele frequencies. Replications showed similar amounts of variation, but no loci were consistently significant across replicates or treatments. Since the differences were neither large nor systematic, replicates were pooled enabling the direct comparison of treatments (chi-square test, $\alpha = 0.05$). All treatments but GT had some significantly different frequencies between generations; seedlings tended to have more low-frequency alleles. Relative to the OG control, which had significant differences in two of the 22 loci assayed, only the CC treatment had more allele frequency discrepancies between generations (four out of 22, or 18%), although there were no patterns apparent in terms of allelic distribution: some loci had more alleles in the parental population, while others were the converse.

Western hemlock

When individual replicates or blocks were tested, every one had some significant differences among loci when parents and offspring were compared. Only seven of the 11 were statistically significant ($\alpha = 0.05$), where two or more of the 21 loci-assayed differed. As for amabilis fir, no loci were consistently significantly different among cohorts, and replicates of the same treatment tended to vary less than the differences among treatments. The replicates within treatment types were then pooled for subsequent analyses. In SW, PC and GT treatments, seedlings had more rare alleles and lower frequencies of the dominant allele, while for CC the parental population (i.e., the OG treatment) tended to have a lower frequency of the dominant allele. OG showed no consistent patterns, and differed significantly for two of the 21 loci assayed. All treatments but SW had more significant differences between parents and offspring than the OG control: in increasing order of deviation from expectations, treatments were SW (6%), OG (9%), GT (11%), CC (14%), PC (14%).

Genetic diversity

Amabilis fir

All data for these findings are presented in Table 2. Over all treatments combined, 6 and 7 loci out of the 22 surveyed were monomorphic in the natural regeneration (seedlings) and parental (adult) populations, respectively. Overall N_a was 2.1 and 2.2 alleles per locus for the adult and seedling populations, respectively. N_a varied both within and among treatments. OG had the highest value of alleles per locus for parental trees (1.80), and the lowest for the regeneration (1.50), while CC was highest (1.70). PLP_{95} of 27.3 and 31.8% were observed for the seedling and adult populations, respectively. This parameter was highly variable both between generations (adults: 22.7–45.5%; seedlings: 18.2–30.3%) and among and within treatments (PC and GT adults: differences of 18.2%; seedlings: differences of 9.1% in PC, SW and GT). PLP_{no} values for some treatments were double those of PLP_{95}. Differences were most pronounced for the seedling populations (PC: 19.7 vs 50.0; GT: 18.2 vs 43.9) and lowest for the OG parental population (45.5 vs 50.0). Combined H_e estimates of 0.085 and 0.071 were calculated for the adult and seedling populations, respectively. In general, H_e estimates followed the same trend as N_a

Table 2 Sample size (n), average number of alleles per locus (N_a), proportion of polymorphic loci at the 95% and no frequency cutoff limits (PLP_{95} and PLP_{no}), and expected heterozygosity (H_e) for parents and natural regeneration (regen.) for amabilis fir (Ba) and western hemlock (Hw) for all silvicultural treatments [old growth (control) (OG), patch cut (PC), shelterwood (SW), green tree (GT), clearcut (CC) and combined]. Ranges in parentheses

Population	Treatment	n	N_a	PLP_{95}	PLP_{no}	H_o	H_e
Pacific silver fir							
Parents	OG	30	1.80	45.5	50.0	0.079	0.103
	PC	96	1.53 (1.5–1.6)	22.7 (13.6–31.8)	40.9 (36.4–45.5)	0.062 (0.061–0.062)	0.072 (0.062–0.090)
	SW	86	1.63 (1.5–1.8)	28.8 (27.3–31.8)	45.5 (36.4–59.1)	0.069 (0.049–0.082)	0.081 (0.078–0.084)
	GT	55	1.57 (1.4–1.7)	24.3 (18.2–36.4)	39.4 (27.3–50.0)	0.078 (0.060–0.097)	0.084 (0.060–0.103)
	Combined	267	2.10	31.8	42.7	0.069	0.085
Regeneration	OG	33	1.50	18.2	31.8	0.040	0.054
	PC	195	1.63 (1.6–1.7)	19.7 (13.6–22.7)	50.0 (40.9–59.1)	0.059 (0.047–0.064)	0.059 (0.047–0.071)
	SW	149	1.63 (1.5–1.7)	30.3 (27.3–36.4)	50.0 (40.9–54.6)	0.077 (0.068–0.084)	0.085 (0.080–0.090)
	GT	123	1.60 (1.5–1.7)	18.2 (13.6–22.7)	43.9 (36.4–50.0)	0.062 (0.058–0.066)	0.072 (0.069–0.075)
	CC	67	1.70	22.7	50.0	0.059	0.068
	Combined	567	2.20	27.3	46.7	0.061	0.071
Western hemlock							
Parents	OG	30	1.60	38.1	57.1	0.098	0.113
	PC	90	1.57 (1.5–1.7)	36.5 (33.3–38.1)	47.6	0.073 (0.071–0.076)	0.080 (0.028–0.086)
	SW	90	1.75 (1.7–1.8)	41.3 (38.1–47.6)	58.7	0.085 (0.083–0.087)	0.095 (0.089–0.101)
	GT	60	1.57 (1.5–1.6)	42.9 (38.1–47.6)	49.2	0.086 (0.079–0.090)	0.095 (0.087–0.102)
	Combined	270	2.00	42.9	52.4	0.083	0.093
Regeneration	OG	30	1.60	42.9	47.6	0.133	0.115
	PC	202	1.87 (1.7–2.0)	38.1 (33.3–42.9)	65.1 (57.1–71.4)	0.100 (0.085–0.108)	0.110 (0.097–0.123)
	SW	145	1.70 (1.6–1.8)	31.7 (23.8–38.1)	55.6 (47.6–61.9)	0.075 (0.072–0.077)	0.086 (0.083–0.090)
	GT	125	1.80 (1.6–2.0)	42.9 (38.1–47.6)	63.5 (47.6–76.2)	0.103 (0.092–0.115)	0.119 (0.117–0.122)
	CC	65	1.80	33.3	71.4	0.108	0.114
	Combined	567	2.40	33.3	61.0	0.097	0.109

Table 3 Adjusted *p*-values comparing least-squares means of western hemlock genetic parameters between parents and offspring across treatments. Abbreviations are as in Table 1. Values in *italics* are statistically significant ($\alpha = 0.05$)

Parents Offspring	SW-P SW-S	PC-P PC-S	GT-P GT-S	OG-P OG-S	OG-P CC-S
H_e	0.963	*0.008*	*0.032*	1.000	1.000
H_o	0.952	*0.024*	0.348	0.213	0.997
A	1.000	0.141	0.349	1.000	0.973
PLP_{95}	0.466	1.000	1.000	0.999	0.999
PLP_{no}	1.000	0.304	0.535	0.996	0.951

and PLP_{95} showing variation among (adults: 0.072–0.103; seedlings: 0.054–0.090) and within (GT adults: 0.060–0.103; PC seedlings: 0.047–0.071) treatments. H_o was always lower than H_e: parents tended to have a wider difference between the two than seedlings (OG parents: 0.079 vs 0.103), except for PC seedlings which had identical values for H_o and H_e (0.059). The OG control had the largest discrepancy between observed and expected heterozygosity values for both parent and offspring populations.

Estimates of genetic distances (Table 3) between adult populations for the three alternative silvicultural systems (PC, SW and GT) and the old-growth (OG) control were very low, ranging between 0.003 (between PC and GT) and 0.005 (between OG and PC, OG and SW). This was not surprising since the adult populations originated from the same source. It was even more interesting to note the extremely low genetic distance estimates among seedling populations, ranging from 0.0 to 0.004, indicating that the adult population is clearly the source of the seedling population in each treatment. Finally, genetic-distance estimates between adults and seedlings over all treatments were also small, ranging from 0.001 to 0.007.

Significant Type-3 ANOVA differences were found only between all (pooled) parents and seedlings for expected and observed heterozygosity and PLP_{95} (data not shown). No significant effects were found for treatment by generation interactions or among treatments overall. The homogeneous effects of treatments, and lack of difference between treatments and the control (OG) on genetic-diversity parameters, were corroborated by the multiple range tests which failed to detect any significant differences among treatments. Multiple range tests detected significantly higher expected heterozygosity in parents (0.085) than seedlings (0.071), but not for observed heterozygosity. A slightly higher proportion of polymorphic loci (95% criterion) were detected in parents (32%) than in seedlings (27%); PLP_{no} was not significantly different. Treatment differences were never significant. Least-squares means-tests of differences between parents and offspring by treatment showed no significant differences for any parameter in any treatment.

The number of rare alleles (frequency ≤ 0.05) differed somewhat between parents and offspring among treatments: OG had the same number (5), SW had an average of one more rare allele in seedlings, while all other treatments had from one to four more low-frequency alleles in the parental populations (Fig. 2a). There were no systematic differences indicating that either generation

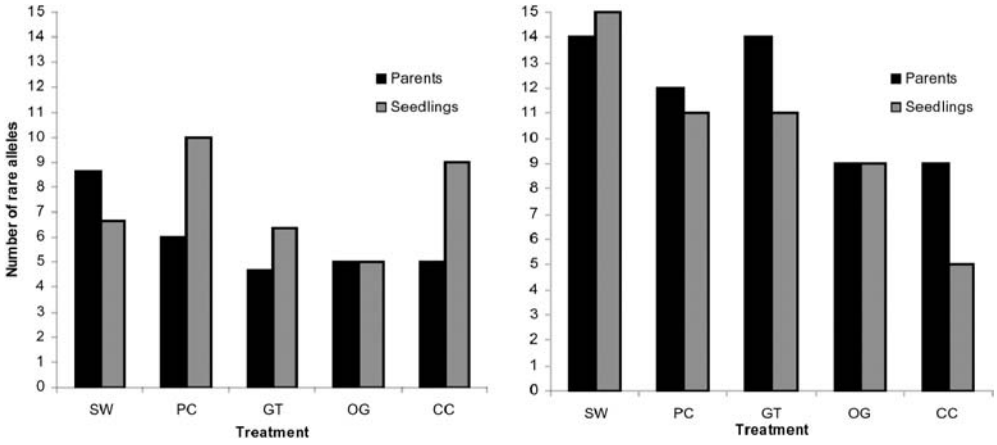

Fig. 2a, b Number of rare alleles before and after harvesting treatments in amabilis fir (**a**, left) and western hemlock (**b**, right). Treatment abbreviations are as in Table 2

had more rare alleles, although CC had the largest number of low-frequency alleles differing between cohorts (9 in seedlings, 5 in parents).

Western hemlock

All data referred to in this section are in Table 2. Over all treatments combined, 2 and 7 loci out of the 21 surveyed were monomorphic in the natural regeneration and parental populations, respectively. The remaining loci were polymorphic producing an overall N_a of 2.0 and 2.4 alleles per locus for the adult and seedling populations, respectively. N_a varied among (adults: 1.6–1.8; seedlings: 1.6–1.9) and within (PC adults: 1.5–1.7; GT seedlings: 1.6–2.0) treatments. A mean PLP_{95} of 33.3 and 42.9% was observed for seedlings and adult populations, respectively. PLP_{95} thus varied considerably between generations as well as among (adults: 36.5–42.9%; seedlings: 31.7–42.9%) and within (adults: 9.5% difference; SW seedlings: 14.3% difference) treatments. PLP_{no} was an average of 9.5% higher than PLP_{95} in parents, but a striking 27.7% higher in seedlings. CC had the largest difference (38.1%). OG had the smallest difference among seedling populations (4.7%) while GT had the smallest difference among the parental populations (6.3%). Combined H_e estimates of 0.093 and 0.109 were observed for the adult and seedling populations, respectively. Expected heterozygosity estimates varied among (adults: 0.080–0.113; seedlings: 0.086–0.119) and within (PC adults: 0.028–0.086; PC seedlings: 0.110–0.123) treatments. H_o was always less than H_e, and tended to vary less. PC had the lowest H_o of the parental populations (0.073), while SW had the lowest estimate among the seedling populations (0.075). Except for SW (parents: 0.083–0.087; seedlings: 0.072–0.077), all seedling populations had higher values than their respective populations.

Similar to amabilis fir, estimates of genetic distances (Table 4) between adult populations for the three alternative silvicultural systems (PC, SW and GT) and the (OG) control were very low, ranging between 0.0 (between SW and GT) and 0.004 (between OG and PC). Similar results occurred among the seedling populations (range: 0.0 to 0.006). Over all treatments, low genetic distances were estimated between the adult and seedling populations, ranging from 0.002 to 0.008.

ANOVA revealed statistically significant differences between combined parents and offspring for both observed and expected heterozygosity, as well as the number of alleles per locus (Table 5). The interaction between treatment and generation was significant for both heterozygosity parameters, preventing the quantification of differences between the main effects (both of which were significant for H_o and H_e). The percentage of polymorphic loci (at the 95% or no criteria) showed no significant differences.

Differences among individual treatments were reflected in Student-Newman-Keuls multiple range tests, adjusted for sample size. Parental populations were less diverse than seedling populations, except for PLP_{95}, which disregards rare alleles; there was no significant difference between means. H_e was significantly lower in shelterwood (0.090) than in old-growth (0.114), clearcut (0.114) and green-tree (0.107) treatments. Observed heterozygosity was significantly higher in old growth (0.116) than in all other treatments but the clearcut (0.103); shelterwood H_o was significantly lower (0.081). No other values among treatments were significantly divergent. Least-squares means were used for parent-offspring comparisons: the only statistically significant differences were for H_e in the PC and GT treatments, and for H_o in the patch cut (Table 5).

A census of the number of rare alleles (frequency ≤ 0.05) was conducted for each generation by treatment combination. Mean values showed the same number of rare alleles present in the old-growth control (i.e., no logging) (Fig. 2b). There were no consistent patterns in the shelterwood treatment; parents had an average of two more rare alleles than the offspring. In all other treatments, the regeneration had from 1.5 (GT) to 5 (CC) more rare alleles (Fig. 2b).

Table 4 Estimates of mean genetic distances (unbiased, Nei 1978) among amabilis fir within and among parents and offspring by treatment. Abbreviations as in Table 1

Item	PC	SW	GT	
A. Parents				
OG	0.005 (0.001–0.007)	0.005 (0.004–0.006)	0.004 (0.001–0.009)	
PC	–	0.004 (0.000–0.013)	0.003 (0.001–0.007)	
SW		–	0.004 (0.001–0.014)	
	PC	SW	GT	CC
B. Regeneration				
OG	0.001 (0.001–0.001)	0.004 (0.001–0.007)	0.002 (0.001–0.004)	0.001 (0.001–0.001)
PC	–	0.004 (0.001–0.008)	0.002 (0.001–0.004)	0.001 (0.001–0.001)
SW		–	0.002 (0.001–0.004)	0.002 (0.001–0.004)
GT			–	0.001 (0.001–0.002)
	OG-P	PC-P	SW-P	GT-P
C. Parents (P) – Regeneration (R)				
OG-R	0.007	0.002 (0.001–0.003)	0.006 (0.001–0.012)	0.004 (0.001–0.006)
PC-R	0.006 (0.004–0.007)	0.002 (0.001–0.005)	0.005 (0.001–0.014)	0.003 (0.001–0.008)
SW-R	0.004 (0.003–0.005)	0.003 (0.001–0.006)	0.003 (0.001–0.007)	0.003 (0.001–0.008)
GT-R	0.004 (0.003–0.006)	0.002 (0.001–0.003)	0.003 (0.001–0.008)	0.002 (0.001–0.004)
CC-R	0.004	0.001 (0.001–0.002)	0.004 (0.001–0.009)	0.003 (0.001–0.004)

Table 5 Estimates of mean genetic distances (unbiased, Nei 1978) among western hemlock within and among parents and offspring by treatment. Abbreviations as in Table 1

Item	PC	SW	GT	
A. Parents				
OG	0.004 (0.003–0.006)	0.002 (0.001–0.003)	0.001 (0.001–0.001)	
PC	–	0.002 (0.001–0.009)	0.003 (0.001–0.011)	
SW		–	0.000 (0.001–0.001)	
	PC	SW	GT	CC
B. Regeneration				
OG	0.003 (0.002–0.004)	0.005 (0.004–0.006)	0.006 (0.003–0.009)	0.001 (0.001–0.001)
PC	–	0.002 (0.001–0.003)	0.002 (0.001–0.004)	0.002 (0.002–0.003)
SW		–	0.002 (0.001–0.003)	0.004 (0.002–0.005)
GT			–	0.004 (0.002–0.006)
	OG-P	PC-P	SW-P	GT-P
C. Parents (P) – Regeneration (R)				
OG-R	0.005	0.006 (0.005–0.009)	0.008 (0.006–0.010)	0.008 (0.006–0.009)
PC-R	0.004 (0.003–0.006)	0.005 (0.002–0.009)	0.005 (0.003–0.007)	0.004 (0.003–0.007)
SW-R	0.004 (0.003–0.005)	0.004 (0.001–0.009)	0.002 (0.001–0.005)	0.002 (0.001–0.005)
GT-R	0.004 (0.002–0.006)	0.007 (0.004–0.014)	0.005 (0.003–0.008)	0.004 (0.001–0.007)
CC-R	0.006	0.007 (0.006–0.008)	0.007 (0.006–0.010)	0.007 (0.007–0.007)

Mating system

Amabilis fir

Data for this section are found in Table 6A. Average single-locus outcrossing rate (t_s) estimates ranged between 0.847 (GT) and 0.882 (OG); multilocus estimates (t_m) ranged from 0.796 (SW) to 0.837 (PC). All outcrossing estimates (t_s and t_m) were significantly different from $t = 1.0$ (t-test, $\alpha = 0.05$). In addition, the four treatment t_m estimates were smaller than their average t_s counterparts indicating the presence of mating among relatives in all treatments (Clegg 1980; Furnier and Adams 1986; Lewis et al. 2000). Single-tree outcrossing rates varied among trees within treatments (Fig. 3). With the exception of the OG that produced skewed distributions (more trees with higher t estimates), individual tree estimates in the three remaining treatments produced fairly even frequency distributions, with trees yielding a wide range of outcrossing rates (Fig. 3).

The correlation of outcrossed paternity (r_p) varied between 0.105 (GT) and 0.165 (OG) (Table 6A). In general, higher estimates of correlated matings were obtained for the OG than from the other three silvicultural treatments (range: 0.105–0.140). The high rate of correlation of male paternity observed for OG (0.165) indicates that 17% of the progeny are full-sibs as opposed to 11 to 14% from the other three silvicultural treatments (Ritland 1989). All p-values comparing least-squares means of t_m and r_p values among treatments, adjusted for sample size, were not statistically significant (data not shown): silvicultural treatments had no statistically detectable impact on multilocus outcrossing rate or the correlation of paternity in amabilis fir.

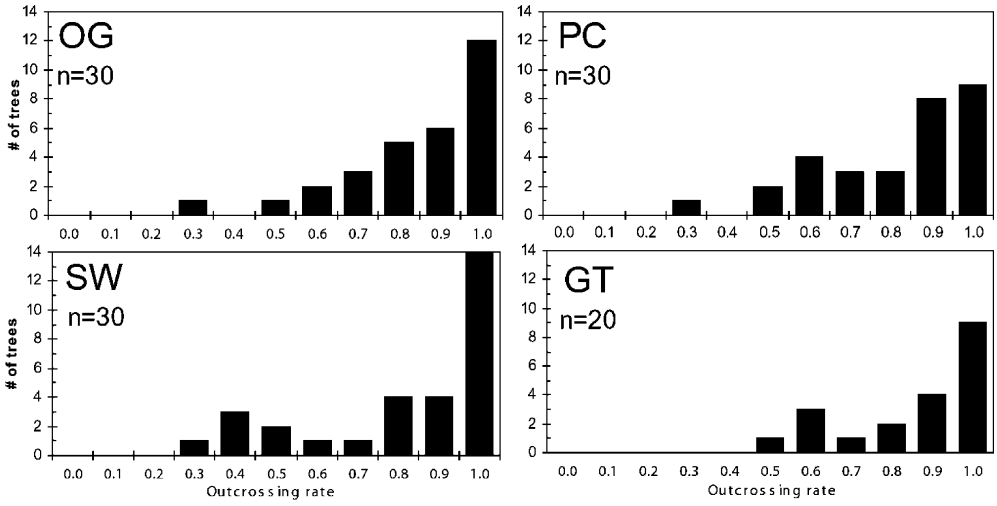

Fig. 3 Frequency distribution of amabilis fir's single-tree multilocus outcrossing rate by silvicultural system Treatment abbreviations are as in Table 2

Table 6 Population estimates of single-locus (t_s), multilocus (t_m), and correlated paternity (r_p) by species by treatment. Abbreviations as in Table 1, standard deviations in parentheses

Estimate	OG	GT	SW	PC
A. Pacific silver fir				
t_s[a]	0.882 (0.041)	0.847 (0.037)	0.852 (0.057)	0.867 (0.037)
t_m	0.801 (0.044)	0.798 (0.039)	0.796 (0.050)	0.837 (0.038)
r_p	0.165 (0.072)	0.105 (0.033)	0.140 (0.250)	0.123 (0.035)
N[b]	26	19	18	37
n[c]	980	662	625	1,452
B. Western hemlock				
t_s[a]	0.903 (0.017)	0.951 (0.027)	0.948 (0.018)	0.880 (0.021)
t_m	0.946 (0.015)	0.922 (0.030)	0.921 (0.022)	0.925 (0.019)
r_p	–0.062 (0.031)	0.043 (0.146)	0.098 (0.030)	–0.053 (0.026)
N[b]	30	20	30	30
n[c]	1,200	800	1,200	1,200

[a] Single-locus minimum variance mean
[b] # of trees sampled
[c] # of seed censused

Western hemlock

Data referred to here are in Table 6B. Mean t_s estimates ranged from 0.880 (PC) to 0.951 (GT) while t_m estimates ranged between 0.921 (SW) and 0.946 (OG). Similar to amabilis fir, all estimates, single- and multi-locus, were significantly different from $t = 1.0$ (t-test, $\alpha = 0.05$). The relationship between the four treatments, t_s and t_m estimates were different from those of amabilis fir. Two treatments (OG and PC) had higher t_m than their t_s counterpart estimates, suggesting that there was no mating among relatives. Their resultant negative estimates of correlated matings (r_p) support the observed relationship between t_s and t_m estimates and indicate that the only inbreeding detected was the product of selfing. The two remaining treatments, GT and SW, produced a trend similar to that observed in amabilis fir, confirming successful mating among relatives. The GT and SW treatments yielded positive r_p estimates (4–10%), however these estimates were substantially lower than those observed for amabilis fir. Single tree outcrossing rates varied among trees within treatments (Fig. 4). Without exception, all treatments produced skewed distributions indicating that the majority of the trees had high outcrossing-rate estimates (Fig. 4). It is noteworthy to mention that no single tree outcrossing-rate estimate was below 0.3. This was not the case for amabilis fir where estimates of 0.1 were observed in all four treatments (Figs. 3 and 4).

All p-values comparing least-squares means of t_m values among treatments, adjusted for sample size, were not statistically significant (data not shown). Thus, silvicultural treatments did not have a statistically significant effect on estimates of the multilocus outcrossing rate in western hemlock. The only significant difference among correlation of paternity (r_p) was that of SW which, in turn, was significantly higher than OG and PC. The confidence interval around the GT mean, most likely due to the smaller sample size, precluded any assertions about the effect of treatment on this parameter.

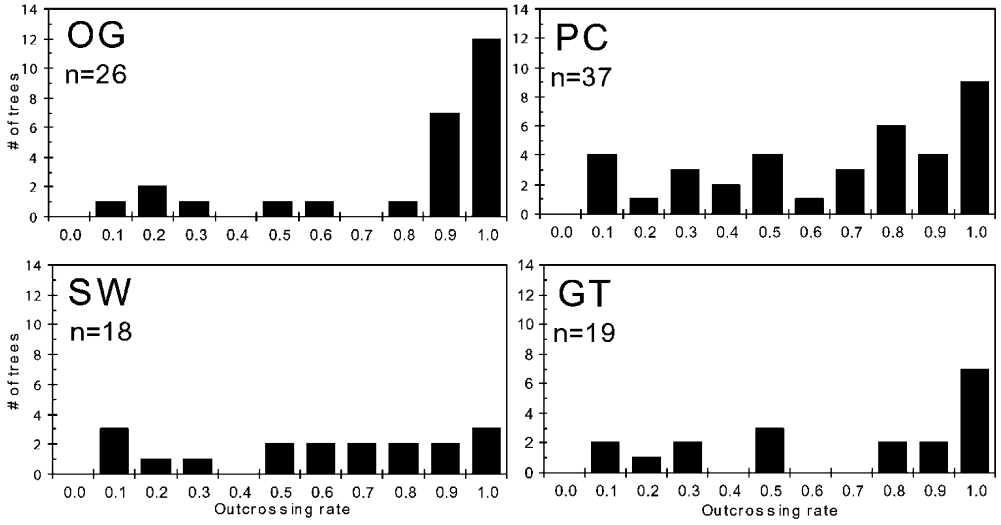

Fig. 4 Frequency distribution of western hemlock's single-tree multilocus outcrossing rate by silvicultural system Treatment abbreviations are as in Table 2

Discussion

Genetic diversity

Both species had similar numbers of alleles per polymorphic locus (*Na*) across treatments and generations, although western hemlock had higher variability among the regeneration, and regeneration pooled across treatments had higher *Na* than the adults (Tables 2 and 5). Rajora (1999) also found that seedlings had similar to slightly higher allelic diversity than parents in harvested *Picea glauca* populations. The slightly higher variability and mean value for western hemlock regeneration could result from age-dependent selection against individuals homozygous for low-frequency, possibly deleterious alleles found throughout the family Pinaceae (of which both species are members), whereby older cohorts have fewer unique, low-frequency alleles and higher heterozygosity than young stands (Mitton and others 1977, 1997; Rajora 1999). This trend was also supported by the direct allele frequency comparisons within replicates of each treatment, where seedlings had more low-frequency alleles as well as lower frequencies of the common allele than parents. These patterns were apparent across nearly all replicates and both species.

Contrary to effects on *Pinus strobus* (Buchert et al. 1997), where allelic diversity and richness were significantly decreased after 75% canopy removal, treatment effects in the present study were not evident for any genetic-diveristy parameter in amabilis fir, although some differences were evident based on allele frequency comparisons between parents and regeneration. Some qualitative differences were apparent, although they were not statistically significant: all harvesting methods resulted in a non-significant decrease in both PLP_{95} and PLP_{no} relative to the control (OG). Conversely, natural regeneration from all harvesting methods had significantly higher percentages of polymorphic loci (PLP_{95}) than the control, although only the SW was substantially higher. When all alleles were taken into account (PLP_{no}) all harvesting methods resulted in a non-significant increase of polymorphic loci relative to the regeneration in the unharvested control. All harvesting methods had similar qualitative but non-significant effects. The gene pool was always more varied in the regeneration, except in the control stand, although the differences were not statistically significant. The most pronounced effects were found in the heterozygosity indices: both observed and expected overall were significantly higher in parental populations when treatments were pooled. No individual treatment effects were apparent, most likely due to the variation within treatments, especially among parental populations. This was similar to Neale's (1985) finding in *Pseudotsuga menziesii* that a shelterwood system comprising 30% overstory removal did not affect genetic diversity or allele frequency distributions between parents and offspring.

Western hemlock showed more complex relationships between treatments and genetic-diversity parameters, indicated by the significant interaction terms among generations and treatments in the ANOVA. This may indicate that leaf-tree density can affect the population structure of western hemlock. Seedlings had higher genetic diversity than parental populations and more low-frequency alleles. Generational cohorts did not differ significantly when only common alleles were considered (PLP_{95}). This was similar to effects found for *P. glauca*, where old-growth stands had the highest heterozygosity (Rajora 1999). Expected heterozygosity was only significantly reduced in the shelterwood treatment, but observed heterozygosity was significantly lower than the control in all silvicultural treatments with the somewhat surprising exception of the clearcut. The SW treatment, which had the highest H_e (based on allele-frequency calculations) of all alternative silvicultural systems, had the lowest H_o, which reflects actual individual-tree diversity. Adams et al. (1998) did not find any significant effects caused by a shelterwood system on coastal *P. menziesii*, although only dominant canopy trees were

removed, whereas in this experiment a representative cross-section of crown classes was retained.

The higher expected, relative to observed, heterozygosity found in both species most likely reflects the effects of inbreeding (Wright 1951; Knowles et al. 1987; Rajora et al. 1998). The decrease in low-frequency alleles and homozygosity with increasing age also implies age-dependent selection against homozygotes, which may harbour higher frequencies of deleterious rare alleles (Neale 1985; Hosius et al. 2000). Genetic distances based on allele frequencies indicate that there are few differences either among treatments or between generations, or any combination of the two, for both species (Adams et al. 1998).

Amabilis fir had a similar number of rare alleles in parents and offspring, but the common alleles differed significantly. The converse was true for hemlock, based on the SNK multiple-range tests. This substantiates Berg and Hamricks' (1997) assertion that all alleles should be censused without confidence limits, since rare alleles (frequency ≤ 0.05) by definition are excluded. These alleles are the ones which, by virtue of their low frequency, will likely to be eliminated by drift through harvesting practices (Adams et al. 1998). Although isozymes are widely recognized as neutral to nearly neutral markers, some have been linked to genes which directly impact fitness (Bush and Smouse 1992) or are indicative of effects caused in overall genomic diversity (Buchert et al. 1997). The evidence for this would be manifested in varying survival regimes of different genotypes, particularly those homozygous for deleterious, low-frequency alleles (Adams et al. 1998). While most rare alleles are most likely neutral to mildly deleterious, they also comprise much of the pool from which future adaptation to changing environments may develop. The numbers of rare alleles before and after harvesting also support this. The higher numbers of low-frequency alleles at isozyme loci in the seedling populations are most likely mildly deleterious alleles not found in the adult population due to selection (Farris and Mitton 1984). This phenomenon has been documented in many conifers, where the higher heterozygosity and higher numbers of low-frequency alleles in early life stages may be linked to traits favourable to seedling establishment or pathogen resistance – traits advantageous to seeds and seedlings which may have a cost in terms of growth for mature trees (Bush et al. 1987).

Mating system

The differences among correlated paternity between species and among treatments can be primarily explained in terms of the pollination biology of each species. In the case of hemlock, where pollen competition can occur, density may also affect the mating system. Amabilis fir has a slightly different reproductive mechanism and pollen grains all have equal opportunity to fertilize the ovule, regardless of proximity and relatedness.

Amabilis fir in this area has overlapping pollination and ovule receptivity periods of approximately 1 week, which would increase the potential for consanguineous mating and selfing (Owens and Molder 1977b). Generally, an average of two pollen grains may land on each receptive ovuliferous scale, with pollen-tube germination and fertilization taking 5 to 6 weeks. Female cones are located at the top of the crown, which generally has a narrow habit: there is therefore little spatial stratification during fertilization with respect to female parents (Crawford and Oliver 1990). Ovuliferous scales near the bottom and top of each cone are infertile, and amabilis fir generally has fairly low seed-set efficiency under wind-pollinated conditions (approximately 18–25%) (Owens and Molder 1977a). This low rate of success has been attributed partially to seed predation by *Megastigmus* sp., and partially to abortion of selfed seed (Owens and Molder 1977b). The relatively large seeds of this species, although winged, tend to fall close to the maternal parent. This, combined with the high shade-tolerance of the species, would lead to family structure within stands (Davidson and El-Kassaby 1997).

Western hemlock cones, although very small compared to amabilis fir cones, are abundantly distributed throughout the crown of mature trees (Packee 1990). During pollination, as up to 100 spiny pollen grains land on the bracts of each receptive cone scale; a projection grows out of the bract, anchoring the pollen, as the cone scales grow over the pollen, trapping it within the cone scale. Pollen tubes subsequently grow towards the micropyles, with an average of one to six pollen tubes per scale, although ten have been found (Colangeli and Owens 1988, 1989, 1990). Multiple pollen tubes afford the opportunity for competition among paternal genotypes, and selection among potential pollen donors at the point of fertilization. Generally, pollen grains landing closest to the cone axis are more successful (Colangeli and Owens 1989). Selfed pollen can thus be screened out at high pollen densities. Similar to amabilis fir, this species can occupy a climax role in ecological succession and is highly shade-tolerant, although this species features frequent asexual reproduction by layering (Packee 1990).

For both single- and multi-locus outcrossing rate estimates based on individual trees, there were no significant differences among treatments for amabilis fir. Although there was clearly some inbreeding occurring as all outcrossing estimates differed from complete outcrossing, it was due to mating among relatives and not selfing. This was also the case for the old-growth control, suggesting that the silvicultural system did not significantly impact the degree or nature of inbreeding in this species. Neale and Adams (1985) found no significant impacts on *P. menziesii* in the Pacific northwest following shelterwood treatments, and similarly density was found to be independent of outcrossing rates in *Pinus jeffreyi* (Furnier and Adams 1986) and *Picea abies* (Morgante et al. 1991).

Hemlock showed a small, but significant amount of inbreeding. For this species, only the PC treatment, where

large contiguous blocks of the original stand are left following harvesting, approximated the outcrossing-rate distribution of the control stand, whereas the other treatments led to a slight but significant increase in inbreeding. The far-higher value of the correlation of paternity (r_p) after the SW treatment compared to the OG and PC treatments was an interesting effect, again suggesting a slight effect of density on mating system. *Pinus caribaea* (Zheng and Ennos 1997) also reflected an effect of density on outcrossing rates. This was also found for *Larix laricina* (Knowles et al. 1987). The small openings with disturbed seedbed caused by the PC option may provide enough space for pollen from the surrounding parent trees to compete with each other, and to exclude pollen from close relatives, and also enable seed to drift farther as more wind turbulence at higher velocities can develop in larger openings, contributing to a more heterogeneous mixture of genotypes in the advance regeneration. This trend is also substantiated by the genetic-diversity parameters after the GT and CC silvicultural treatments, which provide even larger openings. The dense conditions remaining for the OG and SW treatments may prevent comprehensive pollen mixing and restrict seed-fall distances so that seedlings tend to be more related to mature trees nearby.

Management implications

Selecting the best silvicultural options for reforestation must take the genetic composition of the future stand into account. Using the undisturbed, parental stand as a benchmark (Buchert et al. 1997; Rajora et al. 1998), it is possible to determine which of the four silvicultural treatments may best suit regeneration of single or mixed species stands of amabilis fir and western hemlock. Leaving natural ingress to provide the future mature forest will lead to a mixture of hemlock and fir, with hemlock predominating under the current conditions. The degree of soil disturbance will further influence the species composition of the stand, as a mineral soil seedbed would provide ideal establishment conditions for faster-growing early seral species, such as Douglas-fir (*P. menziesii*) or competing herbaceous species (Green and Klinka 1994; Beese and Bryant 1999).

While there were no significant statistical differences among treatments in terms of either mating-system effects or genetic diversity for amabilis fir, there may be some biological effects inherent in allele-frequency differences. These effects are undoubtedly difficult to test for, although maintaining the highest possible diversity of alleles and more heterozygous individuals acts as an insurance policy over the long life span of these organisms, and ensures their evolutionary potential. Since differences in all genetic parameters among treatment effects were small, amabilis fir may be harvested with any of the four silvicultural systems. While clearcutting is the most economically efficient in the short term (Beese 1995), negative public attitudes towards this silvicultural system may prevent it in many coastal forests. This species would be able to regenerate naturally in higher densities in smaller openings, such as those left by the patch cut or shelterwood options (Crawford and Oliver 1990). The green-tree retention system is most likely not cost-effective when compared with the current and projected market value of amabilis fir, whose wood is susceptible to fungal decay and highly resinous (Crawford and Oliver 1990). Any silvicultural system used would benefit from planting seedlings from within the same seed zone, in order to minimize the opportunities for mating among relatives, or to employ supplemental mass pollination (SMP) in orchards supplying reforestation seed for these species (El-Kassaby and Ritland 1986). The likelihood that related trees would be responsible for an even higher proportion of the gene pool in the future would depend on the silvicultural system and rationale for selecting leave trees. Phenological synchronicity among relatives would increase both selfing and consanguineous mating (El-Kassaby et al. 1988; Erikson and Adams 1989), so selecting the shelterwood or patchcut options to minimize correlated paternity and planting additional seedlings would provide a sufficiently diverse stand in the future, as would the traditional clearcut treatment, supplemented by planted seedlings.

Although the control stand had slight but significant inbreeding, as did all other treatments, the nature of the inbreeding tended to change with leaf tree density. As density decreased, selfing increased, evidenced by the higher correlation of paternity. In order to ensure that potentially detrimental effects of selfing, exacerbated by low leaf-tree density (Farris and Mitton 1984; Erickson and Adams 1989; Zheng and Ennos 1997), do not occur throughout the rotation, it may be desirable to supplement genetic potential of hemlock stands by planting seedlings from within the seed-zone transfer guidelines, which would harbour a reservoir of genetic diversity (El-Kassaby 2000). Since similar levels of inbreeding (both multi-locus and single-locus) were observed in the control and all harvested treatments, the slight inbreeding found in this study may not have a very detrimental impact on hemlock: the mixed mating system observed in so many conifers may simply be an adaptation to survive bottlenecks or other extreme events experienced by long-lived woody perennials. Observed and expected heterozygosity was higher when silvicultural systems with larger openings were used. These options also showed lower correlated paternity values. Although current legislation caps clearcut size at 40 hectares on publicly owned lands in coastal British Columbia, in order to promote outcrossing and retain genetic diversity levels extant in old-growth stands, the patch-cut option appears optimal. In order to capitalize on advance regeneration without excessive operating costs, the clearcut option may also be suitable; the patch-cut system may also serve, provided that a slight increase in selfing in advance regeneration is acceptable when mitigated with planted seedlings. Caveats mentioned regarding clearcutting amabilis fir also apply to western hemlock.

Conclusion

For both amabilis fir and western hemlock, natural regeneration had more low-frequency alleles than parents, and lower frequencies of the dominant allele. No statistically significant effects on genetic-diversity parameters or mating system were detected in amabilis fir across a variety of silvicultural treatments (clearcut, patchcut, shelterwood, and green-tree retention) compared to the old-growth control. Lower leaf-tree density following silvicultural treatment appeared to slightly reduce the correlation of paternity and lower selfing rates in western hemlock. A shelterwood system, comprised of 30% basal area removal, resulted in the largest reduction of heterozygosity compared to the old-growth control for hemlock. Clearcutting or patch cutting (50% merchantable basal area removal) resulted in stands with the highest heterozygosity, and lowest correlation of paternity. These differing responses can be accounted for by the effects of stand density on mating system, combined with each species' unique pollination mechanism and selfing tolerance. Supplementing natural regeneration with planted seedlings from the appropriate seed zone would introduce a further reservoir of genetic diversity into stands to ameliorate any detrimental effects on the gene pool from harvesting.

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