PROJECT REPORT 2000-28

FINAL PROJECT REPORT

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Genetic diversity of black spruce populations regenerated after fire or after harvest with pre-established regeneration protection

Jean Bousquet and Daniel J. Perry

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ISBN 1-55261-088-8

Genetic diversity of black spruce populations regenerated after fire or after harvesting with pre-established regeneration protection

by

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August 2000

Project Title: Genetic diversity of black spruce populations regenerated after fire or after harvesting with pre-established regeneration protection

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EXECUTIVE SUMMARY

Black spruce (*Picea mariana* [Mill.] B.S.P.) regenerates under two different regimes 1) by seed, given adequate seed bed conditions, e.g. post-fire conditions; and 2) by layering, e.g. populations where harvest with protection of pre-established natural regeneration is practiced and where accumulation of organic matter prevents seed germination and seedling establishment. Although the growth of black spruce stands from layering is comparable to that of stands from seeds, questions have been raised about the long-term effects of management regimes favouring layering and clonal structures. The objectives of this project were: 1) to evaluate the genetic diversity and levels of inbreeding of black spruce populations regenerated by layering after harvest; and 2) to compare these parameters with those of populations regenerated naturally by seeds after fire. A set of 12 polymorphic codominant molecular markers have been developed and applied to evaluate the genetic diversity in seven mature populations of black spruce from both types of origin. At the scale of analysis, the results show that the genetic diversity of black spruce populations is essentially the same whether stand origin is from fire or from clear-cut with protection of pre-established regeneration. Inbreeding levels were also essentially the same. The estimation of mating system parameters in the filial generation (seeds) for both types of stands essentially showed similar outcrossing rates. Hence, there was no tendancy in stands regenerated from layering for diminished genetic diversity, functional population structuring or decreased outcrossing rates. Thus, within the limits of the present study, the practice of harvesting black spruce stands with protection of pre-established natural regeneration from layering does not affect the genetic diversity of black spruce, at least after one round of such a management regime. Long-term genetic effects after several rounds of such a management regime could not be ruled out, as no experimental designs are currently available to monitor such possible long-term effects.

Titre de Projet: Diversité génétique des peuplements d'épinette noire régénérés à la suite de feux ou après coupe avec protection de la régénération préétablie

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Autre Chercheur: Daniel J. Perry (Ph.D. Univ. Minnesota), chercheur postdoctoral

Collaboratrice principale: Alison D. Munson, professeure, CRBF, Université Laval

RESUME EXECUTIF

L'épinette noire (*Picea mariana* [Mill.] B.S.P.) se régénère suivant deux stratégies; par les semences, lorsque le lit de germination est propice comme après un feu, et par marcottage, par exemple dans les peuplements où est pratiquée la coupe avec protection de la régénération pré-établie, et où l'accumulation de matière organique prévient la germination des semences et l'établissement des semis. Bien que la croissance des peuplements issus de marcottes semble comparable à celle des peuplements issus de semis, des préoccupations ont été formulées quant à la diversité des peuplements issus de la reproduction asexuée et des effets à long terme de l'emploi de stratégies d'aménagement favorisant le marcottage et la structuration clonale. Une augmentation des niveaux d'endogamie, de consanguinité et une diminution des niveaux de diversité génétique pourraient prendre place si de tels systèmes d'aménagement sont maintenus sur plusieurs générations. Les objectifs du projet étaient d'évaluer la diversité génétique et le niveau d'endogamie de peuplements d'épinette noire régénérés par marcottes après coupe et de comparer ces paramètres à ceux des peuplements naturels régénérés à partir de semences après feu. Un ensemble de 12 marqueurs moléculaires co-dominants polymorphes a été mis au point et utilisé afin d'évaluer la diversité génétique de sept populations matures d'épinette noire représentatives des deux types d'origine. À l'échelle de l'étude, les résultats démontrent que la diversité génétique des populations d'épinette noire est la même, que l'origine soit un feu ou une coupe avec protection de la régénération pré-établie. Les niveaux de consanguinité étaient également similaires. De plus, l'estimation des taux d'inter-fertilisation et d'autres paramètres du système d'accouplement à partir de la génération filiale (semences) n'a pas permis de déceler de différences significatives entre les deux types d'origine. Ces résultats démontrent que, dans les limites de la présente étude, le régime de coupe avec protection de la régénération issue de marcottes n'entraîne pas d'effets décelables sur la diversité génétique de l'épinette noire après une ronde d'application d'un tel système d'aménagement. Des effets à long terme après plusieurs rondes d'application d'un tel système d'aménagement ne peuvent être exclus, mais il n'existe pas actuellement de dispositifs permettant de valider ou non la pratique à très long terme.

ACKNOWLEDGMENTS

We wish to thank Dr. René Doucet, scientist at the Quebec Ministry of Natural Resources, for assistance with the selection of study sites and precious help with the recognition of stand origin. We also thank Dr. Ken Smith Mr. Francois Larochelle, respectively postdoctoral fellow with Dr. Alison Munson and research assistant at C.R.B.F., who helped with field sampling. As well, we wish to thank Drs. Bob Rultedge and Jean Beaulieu, scientists at the Canadian Forest Service, for providing a black spruce cDNA bank and a range-wide panel of black spruce trees, respectively. We also acknowledge Produits Forestiers Alliance for access to study sites. This project was also supported by grants from the Natural Sciences and Engineering Council of Canada (equipments and research grants) and from Fonds FCAR du Québec (team grant).

PROJECT PROFILE

Legacy 1: Understanding disturbance

Sub-legacy: Regeneration under harvesting and natural

Starting Date: November 1996

Completion Date: March 2000

Amounts Awarded:
- 1996-97: 12 500$
- 1997-98: 25 000$
- 1998-99: 20 000$
- 1999-00: 20 000$

Sites of Study

- Black spruce stand sites of Alison Munson's group in Québec, involving Produits Forestiers Alliance
- Two additional sites on Québec North Shore and Parc des Grands Jardins (geographical controls)
- Laboratories: Genetics lab. of the Forest Biology Research Centre (CRBF), Laval University

PAPERS OR PRESENTATIONS DERIVED FROM THIS PROJECT

Perry, D.J. and J. Bousquet. 2000. Genetic diversity and mating system of post-fire and post-harvest black spruce: An investigation using codominant sequence-tagged site (STS) markers. Canadian Journal of Forest Research (in revision).

Bousquet, J., D.J. Perry and M. Perron. 2000. L'écologie génétique de l'épinette noire. Dép. des Sciences biologiques et GREF, UQAM, Montréal, January (invité).

Perry, D.J. and J. Bousquet. 1999. Genetic diversity and mating system of black spruce under fire and harvest regimes: an investigation using codominant DNA markers. Abstract *In* Proceedings of the SFM Network Annual Conference, Edmonton, February.

Perry, D.J. and J. Bousquet. 1998. Sequence-tagged site (STS) markers of arbitrary genes: development, characterization and analysis of linkage in black spruce. Genetics 149: 1089-1098.

Perry, D.J. and J. Bousquet. 1998. Sequence-tagged-site (STS) markers of arbitrary genes: the utility of black spruce-derived STS primers in other conifers. Theoretical and Applied Genetics 97: 735-743.

Perry, D.J. et J. Bousquet. 1998. Codominant sequence-tagged-site polymorphisms: convenient PCR-based markers for population studies. Joint Meeting North American Forest Biology Workshop Western Forest Genetics Association, Victoria, BC, June.

Perry, D.J. and J. Bousquet. 1997. PCR-based codominant markers of expressed black spruce genes. IUFRO Joint Meeting Somatic Cell Genetics and Molecular Genetics of Trees, Québec, August.

Perry, D.J. and J. Bousquet. 1997. Codominant DNA markers in black spruce. 26th Biannuel Meeting of the Canadian Tree Improvement Association, Québec, August.

Bousquet, J. and D.J. Perry. 1997. Marqueurs co-dominants d'ADNc (ESTs) par PCR chez les épinettes. Laboratory of Plant Systematics and Evolution, Univ. of Paris (Orsay), France, October (invited).

Bousquet, J. and D.J. Perry. 1997. ESTs markers in black spruce. Dept. of Plant Physiology and Forest Genetics, Umea, Sweden, December (invited).

Genetic Diversity and Mating System of Post-Fire and Post-Harvest Black Spruce: An Investigation Using Codominant Sequence-Tagged-Site (STS) Markers

(Submitted to CJFR)

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ABSTRACT

cDNA-based sequence-tagged-site markers were used to examine the genetic composition of three mature, layer-origin populations of black spruce, (*Picea mariana* (Mill.) B.S.P.) the result of logging operations in the first half of the twentieth century, and compare them with four mature, seedling-origin populations that regenerated naturally following fire. The amount of STS-marker variation revealed in these populations was very similar to that previously observed in a range-wide panel of black spruce trees. There was little differentiation among populations and no significant differences in heterozygosities, numbers of alleles or fixation indices were evident between layer-origin and fire-origin stands. Likewise, when mating system parameters were estimated in one population of each of these two types, no significant differences were found; outcrossing was essentially complete with no evidence of mating among relatives. The estimated correlation of paternity within progeny arrays was about 17 and 13 percent in the fire-origin and layer-origin stands, respectively, but again the observed difference was not statistically significant. At least at the current scale of sampling, silvicultural practices that result in stand replacement by layer-origin advance regeneration appear not to have had negative impact upon the genetic diversity or level of inbreeding in second-growth black spruce stands.

ACKNOWLEDGEMENTS

This research was supported by grants to J.B. from the NCE Sustainable Forest Management Network, NSERC of Canada and FCAR of Quebec. A.D. Munson and M. Coyea (CRBF) provided much assistance in stand selection. R. Doucet (Ministère des Ressources naturelles du Québec) assisted in the field verification of stand origins. S. Daigneault and S. Légaré (Canadian Forest Service), and F. Larochelle and M. Perron (CRBF) provided much assistance with sample collection. D. Fournier, I. Gamache and P. Perry helped in the laboratory.

INTRODUCTION

Black spruce (*Picea mariana* (Mill.) B.S.P.) is a major component of the boreal forest of North America. It has a transcontinental distribution, with a range that extends from west coast of Alaska, to Newfoundland in the east (Viereck and Johnston, 1990). It grows on a variety of sites and often forms pure stands, particularly on sites having organic soils. Owing to favorable fibre properties and the vastness of the resource, black spruce has also assumed an important economic role. For instance, in Quebec, a major participant in the Canadian forest industry, more than half of the annual pulpwood harvest is black spruce (Doucet 1990).

Black spruce has traditionally been harvested in Quebec by clearcutting and stand replacement has been allowed to occur by the release of advance regeneration. This was the natural progression of hand-felling and horse-skidding operations in the first half of the twentieth century, but with mechanization of harvesting, special efforts have become necessary to protect the survival of advance growth. A forest protection strategy in Quebec now requires "*coupe avec protection de la régénération et des sols*" (CPRS; *i.e.*, harvest with protection of advance regeneration and soils) or so-called "*careful logging*" for most of the black spruce stand types of the boreal forest (Doucet 1992).

In the eastern boreal region, a large majority of black spruce advance regeneration consists of layers (Richardson 1981, Doucet 1988, Paquin *et al*. 1999), which occur when living branches become embedded in moist organic matter on the forest floor and develop roots (Stanek 1961). For this reason, stands that regenerate following careful logging will differ from those that become established after stand destruction by fire, the primary natural disturbance agent in the boreal forest (Rowe and Scotter 1973). Fire generally eliminates advance regeneration and stand replacement then occurs through seedling recruitment. Most natural black spruce stands in Quebec are of seed origin, established after wildfires (Cogbill 1985).

Fire-origin stands are typically even-aged, while careful logging produces second-growth stands that have an uneven-aged structure (Morin and Gagnon 1992). The tallest advance regeneration at the time of release generally maintains dominance in second-growth stands (Paquin and Doucet 1992b, Pothier *et al*. 1995). Following an initial period of acclimation, released layers typically perform as well as naturally established seedlings in terms of height growth, total height and physiological characteristics (Paquin *et al*. 1999). In general, growth and volume production of layer-origin second-growth stands has been found to be comparable to that described in yield tables for natural black spruce stands (Doucet 1990, Morin and Gagnon 1991, 1992, Paquin and Doucet 1992a).

Although considerable research has focused on the comparison of the demographics, growth and physiology of layer- and seed-origin black spruce, the

possibility that current silvicultural practices may have negative genetic consequences has not been investigated. In the current report, we examine the genetic composition of mature, layer-origin populations of black spruce, the result of logging operations in the first half of the twentieth century, and compare them with mature, seedling-origin populations that regenerated naturally following fire. We also estimate mating system parameters for each of these two population types. Our genetic surveys were carried out using a set of sequence-tagged-site (STS) markers that reveal codominant polymorphisms of arbitrarily chosen genes in black spruce (Perry and Bousquet 1998). This represents the first application of these molecular markers to a population genetics study.

MATERIALS AND METHODS

Survey of mature populations

Thirty to thirty-two trees, spaced at least 20 m apart, were sampled from each of seven black spruce populations in Quebec, Canada (Fig. 1) that regenerated naturally following fire (FV1, FV2, FV3 and NS) or from advance regeneration following clearcut logging operations (CV1, CV3 and PGJ). Stands that were previously harvested were identified by the presence of remnant cut stumps and a synchronous growth release in cross-sections of standing trees. In contrast, cross-sections of trees from post-fire stands displayed rapid and relatively even growth through the period from establishment to canopy closure, as is expected of seed-origin trees. The seven stands were of similar ages, with the number of years since seedling establishment or understory release estimated to range from about 50 to 90.

DNA was isolated from about 75 mg of needles from each tree following Bousquet *et al*. (1990), with an additional chloroform:isoamyl alcohol (24:1) extraction. We examined a set of 12 STS markers (*Sb01*, *Sb06*, *Sb07*, *Sb08*, *Sb11*, *Sb21*, *Sb24*, *Sb29*, *Sb31*, *Sb62*, *Sb70* and *Sb72*). These markers were previously found to reveal strictly codominant polymorphisms in black spruce using standard agarose gel electrophoresis, without additional manipulation of amplification products (Perry and Bousquet 1998). Primer sequences, as well as polymerase chain reaction (PCR) and electrophoretic conditions, are described in Perry and Bousquet (1998).

Most statistical analysis were carried out using the computer program GDA Version 1.0 (d15), written by P. Lewis (Department of Ecology and Evolutionary Biology, University of Connecticut) and D. Zaykin (Department of Statistics, North Carolina State University) and distributed from the GDA home page at http://alleyn.eeb.uconn.edu/gda/. Descriptive statistics included the average number of alleles per locus (*A*), average expected heterozygosity per locus (*h*) (unbiased estimate; Nei, 1978), average observed (direct count) heterozygosity per locus (*H*) and the fixation index (*f*). Deviation of genotype frequencies from Hardy-Weinberg expectations was assessed for individual loci

within populations via exact tests, with 3200 generated samples**.** The average effective number of alleles per locus (A_e) was calculated as the mean over loci of $1/\Sigma p_i^2$, where p_i is the frequency of the *i*th allele at the locus (Crow and Kimura 1970). Pairwise unbiased genetic distances (*D*) were estimated following Nei (1978). Differences in measures of diversity and *f* between layer- and seed-origin stands were assessed using *t* tests (Montgomery 1991).

F-statistics, *f*, *F*, and *q* (Weir and Cockerham 1984), which are analogous to Wright's (1951) F_{is}, F_{it} and F_{st}, respectively, were estimated. A hierarchical *F*-statistic analysis was also carried out, with populations defined according to stand origin. This procedure provides two differentiation statistics, q_s and q_p, corresponding to differentiation among subpopulations (stands) in populations (fire or layer) and differentiation between populations, respectively. Confidence intervals (95 percent) of *F*-statistics were determined by bootstrapping over loci to obtain 1000 replicates.

Mating system investigation

Seed cones, all produced in the most recent crop, were collected from 15 trees, spaced at least 20 m apart in each of two populations (CV3 and FV1). Seeds were extracted from dried cones, taking care to maintain the integrity of the single-tree seed lots. Following overnight imbibition of water, ungerminated embryos were excised from seeds under a dissecting microscope. Twenty-four embryos were obtained per single-tree family, giving a total of 360 embryos for each population.

DNA from individual embryos was prepared for PCR using Chelex 100 resin (Bio-Rad Laboratories). Each single embryo was homogenized in 25 µl of a 5 percent Chelex 100 suspension, incubated at 56º C for 1 hr, followed by 5 min incubation in a boiling-water bath. Solids were pelleted by centrifugation (a minimum of 5 min at 13,000 rpm in a microcentrifuge) and 4 µl of supernatent were added directly to each PCR (total reaction volume was 15 µl). Eight polymorphic loci were assayed, of which seven were amplified in 3 sets of multiplexed markers (*Sb08*, *Sb21* and *Sb70*; *Sb62* and *Sb29*; *Sb24* and *Sb06*). *Sb01* was amplified alone. Multiplex reaction conditions were the same as described for amplification of single loci (Perry and Bousquet 1998), except for the inclusion of additional PCR primers. Maternal genotypes were obtained directly using DNA extracted from needles as described above for the survey of mature populations.

Mating system parameters (the multilocus population outcrossing rate, t_m; single-locus population outcrossing rates, t_s; minimum variance average single-locus population outcrossing rate, $\bar{t}_s$; correlation of outcrossed paternity within progeny arrays, r_p; and outcross pollen pool allele frequencies) were estimated using the computer program MLTR, version 1.1. This program, written and distributed by K. Ritland (Department of Forest Sciences, University of British Columbia), is a revision and extension of an earlier program, MLT (Ritland 1990) and includes multilocus formulae for

estimating correlated matings. Confidence intervals (95 percent) of mating system parameters were inferred from the ordered output of 1000 bootstrap replicates.

Estimation of mating system parameters was initially carried out using the complete set of eight loci surveyed in the embryos. However, the observation that both *Sb06* and *Sb29* may be moderately linked to *Sb01* (Perry and Bousquet 1998) raised concerns regarding the potential lack of independence between observations at these loci. Therefore we also repeated the statistical analyses excluding *Sb06* and *Sb29* (we elected to retain *Sb01* because it provided more information).

RESULTS AND DISCUSSION

In terms of observed heterozygosity and observed number of alleles, the level of genetic diversity found in these Quebec populations of black spruce (mean $H = 0.25$, mean $A = 2.8$; Table 1) was almost identical to that previously observed using these same twelve STS markers in a panel of black spruce trees that were selected from across the transcontinental range of the species ($H = 0.26$, $A = 2.8$; Perry and Bousquet 1998). In the present study, previously unobserved alleles were found at two STS marker loci (5 additional alleles at *Sb01* and one at *Sb21*; Appendix 1). Observation of new alleles was not unexpected since the total sample size for the Quebec populations ($N = 212$) was much larger than that of the range-wide panel ($N = 22$). As was the case in the range-wide panel, *Sb01* was again the most variable marker locus, having an average expected heterozygosity of 0.63 and 10 alleles detected in these population samples.

Even though the current study was restricted to a relatively small region, all of the alleles that were found in a range-wide panel (Perry and Bousquet 1998) were also observed in these populations. This apparent ubiquity of alleles is suggestive of extensive gene flow throughout the species range, although it is quite plausible that region-specific, low to medium frequency alleles exist, but were not included in the previous range-wide panel. For the Quebec populations under investigation here, the upper bound of the 95 percent confidence interval of θ was 0.011 (Table 2), indicating that not more than about one percent of the observed genetic variation occurred among populations. Accordingly, the pairwise genetic distances were also very low (mean $D = 0.0018$). Clearly, at least within this region, gene flow among populations appears to have been sufficient to negate essentially all effects of diversifying forces, such as mutation, selection and genetic drift, that may act upon these STS marker loci.

Table 1. Summary of genetic variation revealed by twelve codominant STS markers surveyed in mature black spruce populations in Quebec, Canada. Fire-origin populations were regenerated from seeds, whereas harvest-origin stands consisted primarily of layers. The means over loci of number of alleles (A), effective number of alleles (A_e), expected heterozygosity (h), observed heterozygosity (H) and fixation index (f) are presented. Standard errors are shown in parentheses.

Origin	Population	A	A_e	h	H	f
Fire	FV1	2.67	1.57	0.30	0.28	0.05
		(0.41)	(0.16)	(0.06)	(0.07)	
	FV2	2.75	1.49	0.28	0.28	0.00
		(0.30)	(0.14)	(0.06)	(0.06)	
	FV3	2.92	1.43	0.24	0.23	0.04
		(0.53)	(0.15)	(0.06)	(0.06)	
	NS	2.67	1.39	0.23	0.22	0.06
		(0.41)	(0.12)	(0.06)	(0.06)	
	Mean	2.75	1.46	0.26	0.25	0.04
		(0.06)	(0.04)	(0.02)	(0.02)	(0.01)
Harvest	CV1	2.83	1.59	0.27	0.28	-0.03
		(0.55)	(0.26)	(0.07)	(0.07)	
	CV3	2.92	1.48	0.26	0.25	0.02
		(0.53)	(0.17)	(0.06)	(0.07)	
	PGJ	2.58	1.42	0.24	0.23	0.03
		(0.34)	(0.13)	(0.06)	(0.06)	
	Mean	2.78	1.50	0.25	0.25	0.01
		(0.10)	(0.05)	(0.01)	(0.01)	(0.02)
Grand Mean		2.76	1.48	0.26	0.25	0.02
		(0.05)	(0.03)	(0.01)	(0.01)	(0.01)

Our estimate of interpopulation differentiation among these seven Quebec populations is very similar to most estimates of interpopulation differentiation that were previously obtained for black spruce via allozymes. Boyle and Morgenstern (1987) found F_{st} to be 0.010 for six populations spread over a distance of 52 km in central New Brunswick. Similar estimates were obtained for two proximal populations (about 2 km apart) on upland and lowland sites in Ontario (F_{st} = 0.009; Boyle *et al*. 1990) and for six peatland and upland populations that were sampled over a range of about 120 km in Alberta (F_{st} = 0.010; Wang and MacDonald 1992). In Quebec, Isabel *et al*. (1995) estimated an F_{st} of 0.008 from allozyme frequencies in five black spruce populations that spanned more than 1000 km. It appears that, in general, only about one percent of

variation at allozyme or STS marker loci in black spruce is interpopulational, with the remainder residing within populations. This is considerably lower than an average interpopulational differentiation reported for gymnosperms (mean G_{st} = 0.073; Hamerick *et al*. 1992). An exception to the above trend is apparent in Newfoundland, where Yeh *et al*. (1986) found F_{st} among 21 populations to be 0.059 (s.e. = 0.010). Perhaps historical and(or) environmental factors have resulted in greater differentiation among Newfoundland black spruce populations in comparison to among those on the mainland.

Table 2. *F*-statistics of twelve codominant STS marker loci surveyed in seven mature black spruce populations in Quebec, Canada. The statistics *f*, *F*, and θ were estimated as described by Wier and Cockerham (1984) and are analogous to Wright's (1951) F_{is}, F_{it} and F_{st}, respectively. Confidence intervals (Upper and lower bounds) are of 95 percent confidence intervals that were determined from 1000 bootstrap replicates.

Locus	*f*	*F*	θ
Sb01	-0.018	-0.006	0.011
Sb06	0.150	0.138	-0.014
Sb07	-0.009	-0.007	0.002
Sb08	-0.027	-0.028	-0.002
Sb11	-0.020	0.013	0.032
Sb21	0.051	0.056	0.005
Sb24	0.224	0.226	0.002
Sb29	-0.061	-0.049	0.011
Sb31	-0.084	-0.075	0.008
Sb62	0.048	0.052	0.004
Sb70	0.128	0.122	-0.006
Sb72	0.000	-0.006	-0.006
Overall	0.024	0.029	0.006
upper bound	0.082	0.085	0.011
lower bound	-0.014	-0.008	0.000*

*the lower bound of the 95 percent confidence interval for mean θ was small, but positive.

We found no evidence that the genetic diversity of previously harvested, layer-origin stands differed from that of seedling-origin stands that regenerated following fire. On average, the two groups did not differ significantly in the number of alleles per locus (either actual and effective) or expected heterozygosity (Table 1). The mean genetic distance between fire- and layer-origin stands (0.0013) was not greater than the mean of the pairwise distances between fire-origin stands (0.0035) and a hierarchical *F*-statistic

analysis, with populations defined by stand origin, resulted in a value of -0.003 for θ_p, which did not differ significantly from zero (the upper and lower limits of the 95 percent confidence interval for θ_p were 0.0012 and -0.0062, respectively). These observations indicate that past silvicultural practices have had no apparent effect upon allele frequency differentiation among populations.

Similarly, when the organization of genetic variation within stands was considered, there was no evidence that fire- and layer-origin stands differed. Fixation indices were generally positive, but small (Table 1), and the means did not differ significantly between stands of different origins. In two populations, there was a significant deficiency of heterozygotes at *Sb24* (FV3, $f = 0.46$, $p = 0.033$; NS, $f = 0.64$, $p = 0.012$), but no other loci within populations deviated significantly from Hardy-Weinberg expectations and the overall fixation index did not differ significantly from zero (Table 2), indicating that these mature populations were not inbred.

Knowles (1985) has made similar observations regarding restocking of black spruce clearcuts by alternative means in Ontario. Using allozymes, she found that plantations, derived either from bulk seed-zone seed collections or seed collections from a clonal seed orchard, had levels of variability similar to natural populations. In general, natural regeneration of harvested stands from seed, or artificial regeneration by planting of seedlings has been found to have little, if any, discernible effect upon molecular marker variation in other boreal or temperate conifers including Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*; Neale 1985, Adams *et al*. 1998), lodgepole pine (*Pinus contorta* var. *latifolia*; Thomas *et al*. 1999) and jack pine (*Pinus banksiana*; Knowles 1985). Natural clonal propogation in conifers is relatively rare, and we are not aware of any other investigation of the genetic consequences of silvicultural systems that are based upon it.

Although the sum of our evidence suggested that stand origin had no apparent affect on the amount of variation or level inbreeding present in the mature black spruce populations considered in the present study, the possibility remained that silvicultural practices may have had an impact upon mating system parameters. For example, we considered it plausible that the clonal structure of layer-origin stands may have increased the occurrence of proximal stems of identical genotypes, which in turn may have allowed greater opportunity for self pollination. The consequences of this, or any other alteration in mating patterns, would be manifested in the seed crop which, in the case of an event such as a catastrophic fire, would be relied upon as the source of subsequent regeneration. Any substantial increase in the amount of inbreeding in this filial generation could be detrimental since black spruce seedlings that arise from selfing typically have poorer survival and growth (Park and Fowler 1984).

Our comparison of the mating systems of FV1, a seedling-origin population, and CV3, a layer-origin population, revealed no reason for concern. Multilocus outcrossing

rates were high in both populations, and did not differ significantly from 100 percent (Table 3). Although single-locus estimates of outcrossing are expected to be biased downward by consanguineous matings other than selfing (Shaw and Allard 1982), the means of the single-locus estimates did not differ significantly from multilocus estimates in either population, suggesting that there had been little, if any, mating among relatives in either population type. There was statistically significant correlation of outcrossed paternity within progeny arrays (r_p) in both populations (Table 3). Our estimates of r_p indicate that the probability of a pair of progeny randomly chosen from the same family being full sibs was about 17 percent in FV1 and about 13 percent in CV3. The difference in r_p observed between populations was not statistically significant. Note that when all of these mating system analyses were repeated using only the reduced set of loci (*Sb06* and *Sb29* excluded) to avoid the inclusion of known linked pairs of loci, essentially identical results were obtained (Table 3).

Previous studies have found a greater degree of self-fertilization in black spruce. Multilocus estimates of outcrossing rates were found to range from 0.891 to 0.976 in six natural black spruce populations in New Brunswick (Boyle and Morgenstern 1986); half of those estimates differed significantly from $t = 1$ ($\alpha = 0.05$). In a clonal seed orchard of black spruce in Ontario, t_m was estimated to be 0.837 (Barret *et al*. 1987). Outcrossing rates in two natural black spruce populations in Alberta appeared to be even lower; analyses of seeds from the most recent cone crops provided multilocus outcrossing rate estimates of 0.626 and 0.616 (Sproule and Dancik 1996). In these Alberta populations, the outcrossing rates were shown to increase with the cone crop age, suggesting pre-germination selection against selfed seeds over time. Our sampling was of seeds from the most recent cone crop, which would have minimized the opportunity for selection to eliminate selfed embryos. Also, unlike in the studies above, our sampling was of ungerminated embryos. Although the reduction in germination of self-pollinated seed may be only slight in black spruce (Park and Fowler 1984), by using ungerminated embryos we eliminated another opportunity for selection to act against selfed embryos prior to census. We expected our use of ungerminated embryos from the most recent cone crop to increase the opportunity to observe products of self-fertilization and therefore we did not anticipate finding such extreme levels of outcrossing in these two black spruce populations.

Although outcrossing appeared to be essentially complete, the presence of significant correlation of paternity in the progeny arrays indicates that mating was not random in these two populations. Rather, the number of paternal parents appears to have been limited. If all neighboring trees have equal probability of being the pollen parent, then the probability of outcrossing twice to the same tree (i.e., r_p) is $1/n$, where n is the number of paternal parents (Ritland 1989), and the effective number of pollen parents can be estimated as $1/r_p$. By substituting our observed values of r_p, the effective numbers of pollen parents are estimated to have been 6.0 and 7.6 in FV1 and CV3, respectively. This suggests that in both the fire-origin and the layer-origin stands, the majority of successful

Table 3. Mating system parameters estimated for two black spruce populations in Quebec, Canada. Population FV1 regenerated from seeds following fire, whereas CV3 was a harvest-origin stand consisting primarily of layers. Single- (t_s) and multilocus (t_m) estimates of the outcrossing rate, minimum variance mean of the single-locus outcrossing rate estimates ($\bar{t}_s$) and the correlation of outcrossed paternity within progeny arrays (r_p) are presented, with upper and lower bounds of confidence intervals (95 percent) indicated in parentheses. Sample size (n) is the mean over loci. Multilocus parameters were estimated based upon a full complement of eight STS marker loci, and also upon a reduced set of loci that excluded *Sb06* and *Sb29*.

		Population	
Locus	Parameter	FV1	CV3
Sb01	t_s	1.063 (0.843, 1.264)	0.977 (0.965, 1.096)
Sb06	t_s	---*	0.922 (0.758, 1.060)
Sb08	t_s	0.977 (0.699, 1.220)	1.005 (0.864, 1.151)
Sb21	t_s	0.913 (0.749, 1.002)	1.158 (0.922, 1.334)
Sb24	t_s	0.752 (0.470, 0.955)	1.098 (0.838, 1.201)
Sb29	t_s	1.054 (0.905, 1.231)	1.090 (0.918, 1.286)
Sb62	t_s	0.926 (0.787, 1.028)	1.030 (0.847, 1.212)
Sb70	t_s	0.965 (0.671, 1.030)	---
all loci	t_m	0.989 (0.940, 1.063)	0.993 (0.969, 1.019)
	$\bar{t}_s$	0.974 (0.910, 1.028)	1.009 (0.957, 1.062)
	$(t_m - \bar{t}_s)$	0.016 (-0.023, 0.070)	-0.017 (-0.061, 0.024)
	r_p	0.167 (0.090, 0.651)	0.132 (0.070, 0.494)
	n	346	344
Sb06 and *Sb29*	t_m	0.994 (0.941, 1.047)	0.995 (0.969, 1.047)
excluded	$\bar{t}_s$	0.968 (0.902, 1.025)	1.008 (0.949, 1.071)
	$(t_m - \bar{t}_s)$	0.026 (-0.016, 0.076)	-0.013 (-0.066, 0.035)
	r_p	0.188 (0.110, 0.748)	0.135 (0.073, 0.427)
	n	343	342

*A dash indicates that there was no variation observed among maternal trees at this locus, precluding estimation of t_s.

pollen may have been shed by a few near neighbours. If this was the case, then it would appear that these near neighbours were not close relatives of the maternal trees since there was no suppression of $\bar{t}_S$ relative to t_m. This inference of little or no family structure in populations FV1 and CV3 is consistent with the results of previous allozyme investigations of spatial-genetic structure. Knowles (1991) found the distribution of genotypes to be almost random in two lowland black spruce populations. Boyle *et al*. (1990) found evidence of very localized clustering of similar genotypes in one black spruce population that had regenerated without disturbance, but no indication of genetically divergent neighbourhoods. Spatial arrangement of genotypes on a second site that had regenerated following fire was more random.

We adopted some innovative methodologies to facilitate the processing, at the DNA level, of a large number of embryos for the mating system portion of this investigation. Multiplex PCR increased the amount of information obtained per reaction (Fig. 2). The use of a Chelex extraction protocol allowed the rapid preparation of crude template extracts from ungerminated embryos. In order to obtain template that was suitable for PCR amplification, we found it necessary to grind the black spruce embryos in the Chelex suspension prior to incubation. However, this action may not be necessary in other species. Black spruce has small seeds in comparison to other spruces; there are about 890 seeds per gram for black spruce, compared to 500 seeds per gram for white spruce (*Picea glauca*) or 160 seeds per gram for Norway spruce (*Picea abies*) (Young and Young 1992). We suspect that grinding of black spruce embryos is necessary to expose sufficient cell surface area to the Chelex suspension and that species having larger embryos may present sufficient surface area without grinding. For example, we have achieved amplification of STS markers from Chelex preparations of intact Norway spruce embryos (Perry and Bousquet, unpublished data). While templates prepared from embryos using Chelex were generally suitable for PCR, we have not been successful in using templates prepared in this manner from megagametophytes. For this reason, we limited our sampling for the mating system investigation to embryos only, rather than assaying megagametophyte-embryo pairs as is more common in conifer mating system studies. However, since two embryos are expected to provide more information for population estimates of *t* than is a single megagametophyte-embryo pair (Ritland and El-Kassaby 1985), on a reaction per reaction basis, restricting the sampling to embryos as we have done should actually be more efficient.

For much of this century, foresters in Quebec have relied heavily upon layers for replacement of black spruce stands following clear-cut logging. Careful logging, which preserves the advanced, layer-origin regeneration, is currently mandated on much of the area harvested. Therefore, it is reassuring that this practice has had no discernible negative impact on the genetic diversity or level of inbreeding in the resulting stands, at least after one round of harvesting and at this scale of sampling. Although future monitoring of more advanced generations would be prudent, our current evidence

suggests that the practice of careful logging should promote the maintenance of productive, well-adapted black spruce forests in Quebec.

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Figure 1. The locations of seven black spruce populations sampled in Quebec, Canada. FV1, FV2, FV3 and NS regenerated naturally from seed following fire, while CV1, CV3 and PGJ were of harvest origin and consisted primarily of layers.

Figure 2. Eight STS-marker loci amplified from Chelex 100 preparations of ungerminated black spruce embryos and used for the estimation of mating system parameters. Seven loci were amplified in three sets of multiplexed reactions (panels b, c, and d) while *Sb01* was amplified alone (panel a). The amplification products shown derive from a single family. Size markers (left-hand lanes) are fragments of a 100-bp ladder (Pharmacia, panel a; GIBCO/BRL, panels b, c and d)

FIGURES AVAILABLE IN HARD COPY ONLY

Appendix 1. Allele frequencies observed at 12 sequence-tagged-site (STS) marker loci in mature trees of seven Quebec populations of black spruce. Sample size (*n*) is the number of trees sampled per population.

		Population (*n*)						
Locus	Allele	FV1 (30)	FV2 (30)	FV3 (30)	NS (30)	CV1 (30)	CV3 (30)	PGJ (32)
Sb01	*1870**	---	---	0.017	---	0.017	---	---
	1900	0.083	0.217	0.150	0.217	0.233	0.150	0.188
	1930	0.567	0.583	0.600	0.650	0.383	0.533	0.641
	1960	0.067	0.033	0.067	0.017	0.067	0.033	0.063
	2010	0.200	0.117	0.067	0.033	0.167	0.100	0.094
	*2040**	---	---	---	---	0.017	0.017	---
	2075	0.033	0.050	0.050	0.017	0.100	0.100	---
	*2110**	0.050	---	---	---	---	---	---
	*2155**	---	---	0.033	0.067	0.017	0.050	0.016
	*2180**	---	---	0.017	---	0.000	0.017	---
Sb06	*539*	0.933	0.933	0.950	0.967	0.950	0.933	0.969
	609	0.067	0.067	0.050	0.033	0.050	0.067	0.031
Sb07	*645*	---	0.017	---	0.017	---	0.033	---
	648	1.000	0.983	1.000	0.983	1.000	0.967	1.000
Sb08	*634*	0.017	0.017	0.017	0.017	0.033	0.017	0.016
	645	0.333	0.417	0.250	0.300	0.367	0.350	0.297
	646	0.583	0.517	0.567	0.583	0.517	0.550	0.672
	653	0.067	0.050	0.167	0.100	0.083	0.083	0.016
Sb11	*691*	0.167	0.150	0.017	---	0.133	0.100	0.094
	695	0.833	0.850	0.983	1.000	0.867	0.900	0.906
Sb21	*471*	0.267	0.183	0.133	0.150	0.217	0.117	0.203
	474†	0.683	0.817	0.867	0.833	0.783	0.867	0.766
	*610**	0.050	---	---	0.017	---	0.017	0.031
Sb24	*738*	0.800	0.800	0.817	0.900	0.900	0.867	0.922
	771	0.200	0.200	0.183	0.100	0.100	0.133	0.078
Sb29	*553*	0.050	0.133	0.067	0.200	0.117	0.117	0.109
	574	0.917	0.750	0.883	0.800	0.850	0.833	0.891
	580	0.033	0.117	0.050	---	0.033	0.050	---
Sb31	*439*	0.117	0.033	0.050	0.117	0.050	0.033	0.109
	449	0.883	0.967	0.950	0.883	0.950	0.967	0.891
Sb62	*681*	0.600	0.750	0.783	0.750	0.733	0.733	0.609
	689	0.200	0.100	0.067	0.033	0.117	0.067	0.125
	691	0.059	0.050	0.033	0.017	0.033	0.050	0.094
	706	0.150	0.100	0.117	0.200	0.117	0.150	0.172
Sb70	*404*	---	0.017	0.017	0.050	0.017	---	---
	410	0.133	0.083	0.117	0.033	0.067	0.083	0.063
	417	0.867	0.900	0.867	0.917	0.917	0.917	0.938
Sb72	*515*	---	0.017	0.017	---	---	---	0.016
	523	1.000	0.983	0.983	1.000	1.000	1.000	0.984

*Allele was not previously observed in a 22-tree range-wide panel of black spruce (Perry and Bousquet 1998).

†Alleles *Sb21-473* and *Sb21-474* were pooled into a single allele class (see Perry and Bousquet 1998).