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

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

by

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EXECUTIVE SUMMARY

Black spruce (*Piceamariana* [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 are: 1) to evaluate the genetic diversity and the level of inbreeding of black spruce populations regenerated by layering after harvest; and 2) to compare these parameters with those of populations regenerated naturally by seed 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 next phase will determine whether consanguineous mating is more likely in stands regenerated by layering, by estimating the comparing mating system parameters. These results will permit to validate at the genetic biodiversity level the current management regime of black spruce forests, ensuring that natural genetic resources are well preserved for the future.

RÉSUMÉ EXÉCUTIF

L'épinette poire (*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 lemploi 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 sont d'évaluer la diversité génétique et le niveau d'endogamie de peuplements d'épinette noire régénéres 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. La prochaine étape permettra de déterminer si le taux de croisements consanguins est plus élevé au sein des peuplements régénérés à partir de marcottes, en estimant et en comparant les paramètres du système d'accouplement. Ces résultats permettront de valider au niveau de la biodiversité génétique le régime actuel d'aménagement des pessières noires, assurant une conservation à long terme des ressources génétiques naturelles.

ACKNOWLEDGEMENTS

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 help with field sampling. As well, we wish to thank Drs. Bob Rutledge 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 (equipment and basic 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, involved 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

INTRODUCTION

Black spruce (*Picea mariana* [Mill.] B.S.P.) has the capacity to regenerate naturally be either of two means. When seedbed conditions are favorable, such as following fire or when mineral soil is exposed by windthrow, regeneration may occur through seedling recruitment. In older stands, the accumulation of organic matter prevents the establishment of black spruce seedlings and regeneration occurs primarily by layering. Historically, silvicultural practices in Quebec have taken advanced of this latter reproductive strategy of black spruce; replacement of harvested stands by advanced clonal regeneration has been encouraged. However, concerns have emerged regarding the possible genetic consequences of management practices that promote the establishment of stands through asexual means. Does reproduction by layering lead to a reduction of genetic diversity? Perhaps inbreeding is enhanced by clonal structuring that may be caused by layering. An increase in inbreeding could lead to a reduction in vigour of future seedling populations. We are addressing these concerns by investing Québec populations of black spruce that differ in establishment history. Using codominant DNA markers, we are examining levels of genetic variation and mating system parameters in clonal black spruce populations that have replaced harvested stands and we are comparing these to stands that have regenerated naturally from seeds following fire.

DATA ANALYSIS AND RESULTS

Development of Molecular Markers for Black Spruce

To assess the genetic diversity of black spruce stands, we had to develop a totally new generation of codominant molecular markers allowing the unambiguous scoring of heterozygous trees from dominant homozygous trees. This is because biochemical codominant markers such as isozymes are notoriously difficult to score from phenol and tannin-rich vegetative tissues such as needles collected on mature conifer trees. DNA markers were based on the polymerase chain reaction (PCR), a process by which a large quantity of a specific segment of DNA can be amplified from a small amount of an organism's total DNA. The DNA segment that is amplified is flanked by two short, known DNA sequences called PCR primers. We designed PCR primers corresponding to each of 50 black spruce genes that were arbitrarily selected from a cDNA library (a collection of cloned, expressed DNAs provided generously by Dr. Bob Rutledge, research scientist at the Canadian Forest Service).

Thirty-nine pairs of PCR primers were successful in preliminary amplification trials and were used to screen a range-wide panel of black spruce trees for PCR products of differing sizes that could be easily separated using agarose gel electrophoresis and made visible by staining with ethidium bromide. Note that heterozygous genotypes often present additional nonallelic products (see arrow in adjacent figure), the result of heteroduplex DNA formation. The range-

wide panel was generously provided by Dr. Jean Beaulieu, research scientist at the Canadian Forest Service.

A total of 12 markers revealed exclusively codominant fragment-size variation in the range-wide panel of black spruce trees. These markers are convenient for use in population studies because they are technically simple to use and they can be assessed unambiguously in diploid tissues such as needles. These results have been published (Perry and Bousquet 1998, appendix II). Also and as an indirect fall out from this project, we have also demonstrated the usefulness of these markers in other conifer species (Perry and Bousquet 1998b, appendix III). It should be noted that grants from NSERC of Canada and FCAR of Québec were also necessary to develop fully these markers and make them available for analysis of field samples.

Estimating the Genetic Diversity of Mature Populations

Genetic variation at 12 codominant marker loci was examined in three stands that had regenerated vegetatively from layering following harvesting (CV1, CV3, PGJ) and in four stands that had regenerated from seeds following fire (FV1, FV2, FV3, NS). Stands were aged 60-75 years. Five of the seven stands were located in the CAAFs of Produits Forestiers Alliance (CV1, CV3, FV1, FV2, FV3) in addition to two geographical control stands, one locate din Parc Des Grands Jardins (from layering after clear-cut, PGJ) and the other one the North Shore Region of the St-Lawrence River (from seeds after fire, NS), both in Québec. Stand origin was validated using historical records, the presence of stumps as well as verifying the reproductive origin of mature trees, whether from pre-established layering following clear-cut or from seeds following fire. This was done in collaboration with Dr. René Doucet, ecophysiologist for the Ministère des Ressources naturelles du Quebec, by checking on a number of trees the ring accumulation pattern with trees established from layering, which usually show a heterogenous and asymmetrical growth pattern in the early years. DNA was extracted from needles of 30 trees per stand and genetic fingerprints were obtained for each of these trees for 12 marker loci. Sampled trees were spaced at least 20 m apart.

Briefly, no trends in either the amount of variation (as measured by heterozygosity or the number of alleles per locus) or the level of inbreeding (as measured by the fixation index) were apparent between the two population types at this scale of sampling. Thus, the genetic biodiversity detected in black spruce stands resulting from the current harvesting regime practiced in Québec does not appear to depart significantly from that of black spruce stands from fire origin, at least after one round of harvesting and at the scale of study (0.5 hectare). These results will be published, together with those from the third part of the project (see below), in a paper to be submitted during next Winter. Again here, complementary funding from NSERC of Canada and FCAR of Québec should be acknowledged.

Table 1. Mean genetic parameters values estimated in mature populations of black spruce

Origin	Population	Mean nb of alleles/locus[1]	Heterozygosity[2]	Inbreeding level[3]
Harvest	CV1	2.8	27	-3
	CV3	2.9	26	2
	PGJ	2.6	24	3
Fire	FV1	2.7	30	5
	FV2	2.8	28	0
	FV3	2.9	24	4
	NS	2.7	23	6

[1] Based on a sample of 12 independent genetic loci (STS markers).
[2] Unbiased expected heterozygosity.
[3] In units of fixation index F_{IS}. Positive values indicate increased relatedness relative to random assortment (Hardy-Weinberg) expectations.

Inbreeding levels in seed populations

We are currently investigating the mating system in four of the seven stands previously analyzed at the mature population level: two of fire origin and two clonal. The underlying hypothesis is that clonal micro-substructing promoted by clear-cut and layering stand origin might promote consanguineous mating as compared to stands from seeds and fire origin. By examining genetic markers in embryos extracted from seeds of mature trees, we will determine whether the level of inbreeding in the filial generation is affected by the mature stand's origin.

Codominant DNA markers will be assayed in 2 embryos per tree, 15 trees per stand (a total of 1440 embryos). Single-locus and multilocus outcrossing rates will be estimated from these data using a maximum-likelihood approach. These results together with the results from the previous section will be submitted to publication during the next Winter.

Until now, our efforts under this sub-project have focused on collecting and extracting from cones the required seeds from the different stands and developing innovative methodologies that will facilitate the processing of the large numbers of embryos to be fingerprinted genetically. We have adapted during the last Winter a simple and rapid DNA preparation protocol and we have determined combinations of markers that are amenable to being amplified together in the same PCR (multiplex PCR). To reduce costs, we have settled for the 8 most polymorphic loci, thus those giving us as much genetic information as possible. We have multiplexed most of these, that is, we now amplify more than one genetic marker at a time in order to save in reagents and time. With respect to the genetic markers previously developed (Perry and Bousquet 1998a), we have developed the following successful combinations of

markers: Sb098-Sb21-Sb70, Sb62-Sb29, Sb24-Sb06. We still amplify Sb01 alone. Thus, instead of 8 PCR reactions per embryo, the multiplex approach requires only 4 PCR reactions per embryo.

Following this, we have completed the DNA extractions and have initiated the DNA fingerprint analysis for each of the 1440 embryos. Two stands (CV3 and FV1) have been completed for 5 polymorphic marker loci, for a total 3600 single-locus genotypes obtained. Obtaining the rest of the fingerprints should be completed by the end of September 1999. The numerical analysis should be completed late in the Fall of 1999 or early next Winter. Again here, complementary funding from NSERC of Canad and FCAR of Québec should be acknowledged.

MANAGEMENT IMPLICATIONS

Besides its fundamental benefits for improving our understanding of natural genetic variation in commercially important Canadian conifer species and providing a new generation of molecular markers for the international plant and forest genetics communities (Perry and Bousquet 1998a,b), this project is, to our knowledge, the only one in the Network assessing the possible impacts of current harvesting practices on the genetic biodiversity of an important component of our Canadian boreal forests. As such, the results will provide guidelines to the Québec Ministry of Natural Resources and other provincial agencies in order to enforce or modify current forest policies regarding the management regime of black spruce stands. Thus, these guidelines will help enforcing the first CSA criterion for certification of forest management practices, which is to preserve biodiversity at all levels, including natural genetic resources. Companies such as Produits Forestiers Alliance, our industrial partner in this project, is keenly interested in enforcing these guidelines and accessing sound data to achieve this goal.

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Perry, D.J. and J. Bousquet. 1998b. 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. Appendix III.

OTHER PAPERS OR PRESENTATIONS DERIVED FROM THIS PROJECT

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. Appendix I.

Perry, D.J. and 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.

Perry, D.J. and J. Bousquet. 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).

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

APPENDICES (Hard copy only)

Appendix 1: 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).

Appendix 2: 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.

Appendix 3: 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.