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Genetic Sustainability of Fragmented Conifer Populations from Stressed Areas in Northern Ontario (Canada): Application of Molecular Markers

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1. Introduction

The Earth's genes, species, and ecosystems are the product of over 3 billion years of evolution and the basis for the survival of our own species. Biological diversity, the measure of the variation in genes, species and ecosystems is valuable because future practical uses and values are unpredictable and our understanding of ecosystems is insufficient to be certain of the impact of removing any component. Genetic diversity is an indicator of ecosystem condition and sustainability. It is a fundamental component of biodiversity and it encompasses all of the genetically determined differences that occur between individuals of a species. The loss of biodiversity is due above all to economic factors, especially the low values given to biodiversity and ecological functions such as watershed protection, nutrient cycling, pollution control, soil formation and photosynthesis. Biodiversity is very much a cross-sectoral issue, and virtually all sectors have an interest in its conservation and the sustainable use of its components. Biological resources are renewable and with proper management can support human needs indefinitely. These resources, and the diversity of the systems which support them, are therefore the essential foundation of sustainable development.

The past two decades have been a time of great change in the management of natural resources in Ontario and around the world. Ontario's forest policy has shifted to a more balanced ecological approach as the forest is now viewed as part of a larger ecosystem (OMNR, 2001). All forest policies and associated management practices in Ontario conform to the Policy Framework for Sustainable Forests (OMNR, 2001). Many Ontario communities especially in the North depend on forests. There are some 60.9 million hectares of forested land in the province, representing approximately 57% of the 106.8

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million hectare provincial land base (including water). Crown forest accounts for approximately 91% of this forested land located mostly in the Northern Ontario region. It represents the boreal and the Great Lake St. Lawrence Forests that are composed mainly of conifer species (Natural resources of Canada, 2003; OMNR, 2001). The Forest Resource Inventory is the primary survey for sustainable forest management. Information generated by forest inventories has contributed greatly to our knowledge of one of Ontario's renewable resources and continues to serve as the basis for major forest resource planning and policy decisions in the OMNR. But this information is not sufficient to ensure the sustainable management of the forest resource. To achieve this goal, information on genetic diversity of tree populations is essential.

In addition, forest management practices must change to keep pace with climatically induced changes in forest ecosystems. The sustainability, biodiversity, health, and economic benefits of forests will be affected to varying degrees by climate change. A detailed analysis of the level of genetic variability in species and populations is essential in developing climate change models (Colombo et al., 1998).

Evolutionary adaptation to new climate conditions can only occur where sufficient genetic variation exists to allow selective forces to discriminate between adaptive and maladaptive traits. Adaptation may occur more rapidly in species with shorter life cycles, as long as conditions are favourable for reproduction, than in long-lived species such as trees which will undergo a time lag response to changing conditions (Colombo et al., 1998). Forest tree species generally have high levels of genetic variability and gene dispersal rates.

On the other hand, genetic structure of Northern Ontario forests has been seriously affected by past forest management, mining, and forest fire activities. In an effort to maintain the long term viability of the forest landbase in Northern Ontario, forest companies and local government organizations have concentrated on artificial regeneration of conifer seedlings as a primary means of reforestation. To date, over nine millions of forest trees mostly conifers have been planted within the Greater Sudbury Region and surrounding areas.

The Sudbury region in Ontario, Canada has a history over the past 100 years of logging, mining, and sulphide ore smelting, releasing more than 100 million tonnes of SO₂ and tens of thousands of tonnes of cobalt, copper, nickel, and iron ores into the atmosphere from the open roast beds (1888-1929) and smelters (1888-present) (Freedman and Hutchison, 1980). These factors have caused acidification, severe metal contamination of the soils and water at sites within approximately 30 km of the smelters in the Sudbury region. Sudbury area is one of the most ecologically disturbed regions in Canada. There have been numerous studies documenting the effects of SO₂ in the Sudbury region (Cox and Hutchinson, 1980; Amiro and Courtin, 1981; Gratton *et al.*, 2000). In general, information on landscape degradation, soil toxicity, acidification, plant metal accumulation and forest composition in Northern Ontario is readily available but knowledge of genetic variation within and among forest tree populations is lacking. This genetic diversity information is crucial to ensure sustainability of the forest resource. The impoverished plant communities that are currently found in the Greater Sudbury Region (GSR) are not only structurally and floristically different from plant

communities found in uncontaminated areas in the basin, but they appear to have a different genetic make-up.

Many studies have used morphological markers to assess genetic variability within and among species and populations. Those markers are not usually reliable since phenotypic variation is often related to environmental factors. Molecular markers are an important and very powerful tool for genetic analyses of plant species. Molecular markers such as Random Amplification of Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR), and Amplified Fragment Length Polymorphism (AFLP) have been successfully used to assess the genetic diversity in many plant species (Semagn *et al.*, 2006; Sharma *et al.*, 2008). Each one of these marker systems offers a unique combination of advantages and disadvantages (Sharma *et al.*, 2008). They differ in the type of sequence polymorphism detected (insertion/deletions vs. point mutation), information content, the dominance relationships between alleles (dominant vs. codominant markers), amount of DNA required, the need for DNA sequence information in the species under analysis, development costs, the ease of use, and the extent to which they can be automated.

The overall objective of this chapter is to provide current stage of knowledge from several studies on genetic variability in planted and natural fragmented conifer populations from Northern Ontario using ISSR and microsatellite (SSR) markers.

2. Materials and methods

2.1 Genetic material

Needles from White pine (*Pinus strobus*), jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), white spruce (*Picea glauca*), and black spruce (*Picea mariana*) individual trees were sampled from natural (Na) and planted (P) populations. The locations of some sampling sites are illustrated in Figure 1. Additional samples were from the nursery used for the Sudbury land reclamation program and were considered as introductions. For each site, needles and seed samples from first and second generations trees were collected separately. In general 10% to 20 % of each population was analyzed. For each tree, 15 grams of needles were weighed in duplicates, frozen in liquid nitrogen and stored at -80 °C until DNA extraction.

2.2 Soil characterization

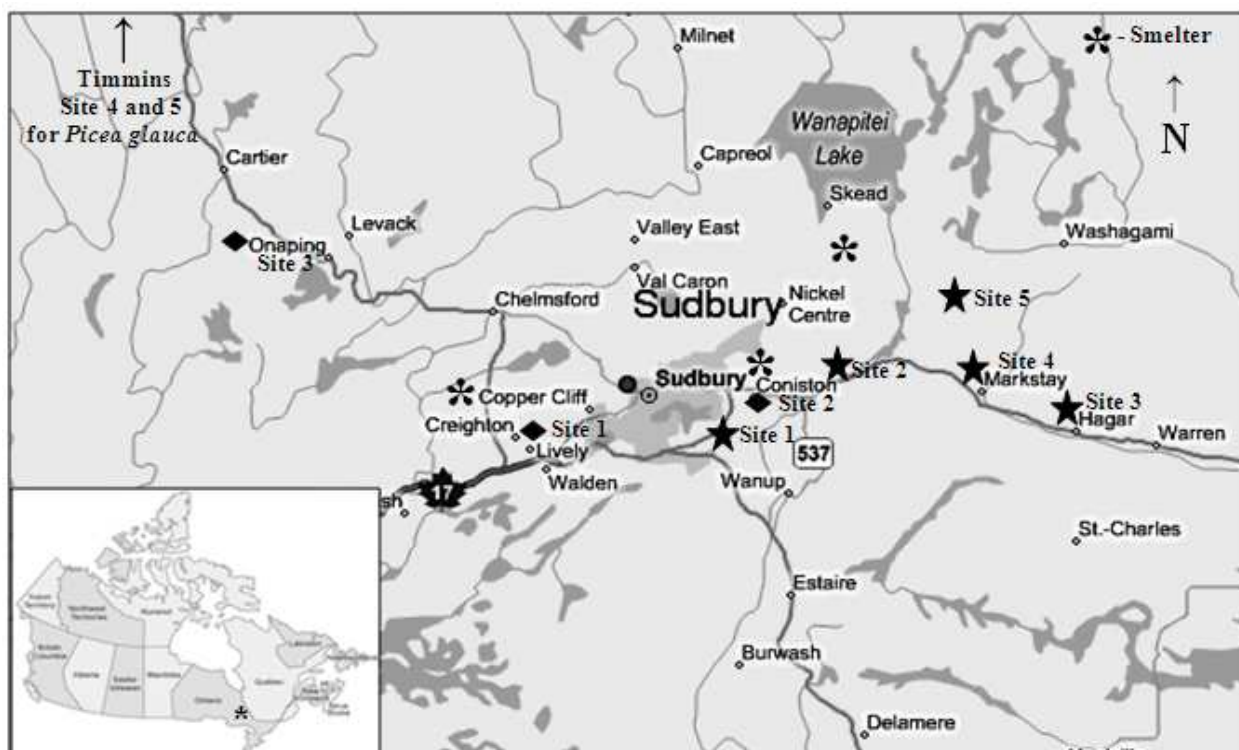
Soil samples were analyzed in collaboration with TESTMARK Laboratories Ltd. Sudbury, Ontario, Canada. The laboratory is ISO/IEC 17025 certified, a member of the Canadian Council of Independent Laboratory (CCIL) and the Canadian Association of Environmental Analytical Laboratories (CAEAL), and is accredited by the Standards Council of Canada (SSC). The laboratory employs standard QA/QC procedures, involving blank and replicate analyses and with recovery rate of $98 \pm 5\%$ in analyses of spiked samples depending on element selected, in their inductively coupled plasma mass spectrometry (ICPMS) analyses reported here. The minimum detection limits (MDL) following microwave digestion of plant tissue Aqua Regia for elements reported here, were: Aluminum 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$), Arsenic 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$), Cadmium 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$), Cobalt 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$), Copper 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$), Iron 1.0 $\mu\text{g/g}$ (10 $\mu\text{g/g}$), Lead 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$),

Magnesium 0.2 $\mu\text{g/g}$ (2.0 $\mu\text{g/g}$), Manganese 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$), Nickel 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$) and Zinc 0.05 $\mu\text{g/g}$ (0.5 $\mu\text{g/g}$). These MDLs reflect actual sample weights and dilutions; instrument detection limits were lower.

The data for the metal levels in soil samples were analyzed using SPSS 7.5 for Windows. All the data were transformed using a \log_{10} transformation to achieve a normal distribution. Kruskal-Wallis test the non-parametric analog of a one-way ANOVA was used to compare independent samples, and tests the hypothesis that several populations have the same continuous distribution. ANOVA followed by Tukey's HSD multiple comparison analysis were performed to determine significant differences ($p < 0.05$) among the sites.

2.3 DNA extraction

The total cellular DNA from individual samples was extracted from seedling tissue using the method described by Nkongolo (1999), with some modifications. The modification involved addition of PVP (polyvinylpyrrolidone) and β -mercaptoethanol to the CTAB extraction buffer. The DNA concentration was determined using the fluorochrome Hoechst 33258 (bisbensimide) fluorescent DNA quantitation kit from Bio-Rad (cat. # 170-2480) and the purity was determined using a spectrophotometer (Varian Cary 100 UV-VIS spectrophotometer).



◆ *Picea glauca* ; Site 1: Lively; Site 2: Coniston; Site 3: \approx 40 km from Sudbury HW144 North towards Timmins; Site 4: \approx 16 km from site 3 HW144 North; Site 5 (control): \approx 35 km from Site 4 HW144 North.
 ★ *Pinus strobus*: Site 1: Daisy Lake (HW17 Bypass); Site 2: Coniston (HW17); Site 3: Hagar \approx 60km from Sudbury; Site 4: Markstay \approx 38km from Sudbury; Site 5: Kukagami Road (\approx 9 km from HW17).

Fig. 1. Sudbury (Ontario) map showing locations of some sampling sites.

2.4 ISSR analysis

The ISSR amplification was carried out in accordance with the method described by Nagaoka and Ogihara (1997), with some modifications described by Mehes et al. (2007). All DNA samples were primed with each of the ten primers used (Table 1). All PCR products were loaded into 2% agarose gel in 1X Tris-Borate-EDTA (TBE) buffer. Gels were pre-stained with 4 µl of ethidium bromide and run at 3.14V/cm for approximately 120 minutes. These agarose gels were visualized under UV light source, documented with the Bio-Rad ChemiDoc XRS system and analyzed for band presence or absence with the Discovery Series Quantity One 1D Analysis Software.

The resulting data matrix of the ISSR phenotype was analyzed using POPGENE software (version 1.32) to estimate genetic diversity parameters (Yeh and Boyle, 1997a, 1997b). POPGENE is computer software used for the analysis of genetic variation among and within populations using co-dominant and dominant markers and quantitative traits. The program was used to determine the intra and inter-population genetic diversity parameters such as percentage of polymorphic loci (P%), Nei's gene diversity (h), Shannon's information index (I), observed number of alleles (Na) and effective number of alleles (Ne). The genetic structure was investigated using Nei's gene diversity statistics, including the within population diversity (H_s) and total genetic diversity (H_t) (Nei, 1973) calculated within the species using the same software. The mean and the total gene diversities, the variation among populations and gene flow were also calculated. The genetic distances were calculated using Jaccard's similarity coefficient estimated with the RAPDistance program version 1.04 (Armstrong et al., 1994).

Primer identification	Nucleotide sequence (5'→3')	G + C content (%)
ISSR Primers		
Echt 5	AGAC AGAC GC	60.00
HB 13	GAG GAG GAG GC	72.70
HB 15	GTG GTG GTG GC	72.70
ISSR 1	AG AG AG AG AG AG AG AG RG	50.00
ISSR 5	ACG ACG ACG ACG AC	64.28
ISSR 9	GATC GATC GATC GC	57.14
UBC 825	AC AC AC AC AC AC AC AC T	88.88
UBC 841	GA AG GA GA GA GA GA GA YC	45.00
17899A	CA CA CA CA CA CA AG	50.00
17898B	CA CA CA CA CA CA GT	50.00

Table 1. The nucleotide sequences of ISSR primers used to screen DNA samples of *Picea glauca* and *Pinus strobus*.

2.5 Microsatellite analysis

The microsatellite analysis involved three species (*Pinus banksiana*, *Pinus resinosa*, and *Picea mariana*). Ten microsatellite primers, synthesized by Invitrogen, were chosen for amplification of DNA from *Pinus banksiana* and *P. resinosa* populations. These primers described in Vandelight et al. (2011) include PtTX 3013, PtTX 3030, PtTX 3098, PtTX 309, PtTX 2123, PtTX 3088, RPS 2, RPS 20, RPS 25b, and RPS 84. For *Picea mariana*, the primers used are described in Dobrzeniecka et al. 2009. DNA amplification was performed following the procedure described by Mehes et al. 2009. The Popgene software, version 1.32 (Yeh and Boyle, 1997) was used to assess the intra- and interpopulation genetic diversity parameters such as the mean number of alleles (N_A) across loci, the total number of alleles (N_T) per locus and Shannon's information index (i) (Yeh and Boyle, 1997). The observed and expected heterozygosities (H_O and H_E respectively) were calculated using the Genepop software, version 3.4 (Raymond and Rousset, 1995). The probability test was computed using the Markov chain method (1000 iterations) in order to determine populations in Hardy-Weinberg Equilibrium (Genepop). Hardy-Weinberg equilibrium deviations were tested using alternative hypotheses, deficiency and excess of heterozygotes, for each locus and across loci and populations using Fisher's method. A test for null allele was also done using the EM algorithm of Dempster et al. (1977). The average effective number of migrants exchanged between populations in each generation, or gene flow (N_M) is estimated from F_{ST} (subdivision among populations).

3. Results

3.1 Soil analysis

Recovery and precision for all elements in reference soil samples were within acceptable range. The estimated levels of metal content in different sites from the Greater Sudbury Region in Canada are illustrated in Table 2. The levels of the metals measured were low in the control sites. Overall, the results indicated that nickel and copper continue to be the main contaminants of top soil (Table 2) in sites near the smelters (site 1 and 2). The values ranged from 30.9 to 1600.0 mg kg⁻¹ and from 52.3 to 1330.3 mg kg⁻¹ for nickel and copper respectively (Table 1). Arsenic concentration exceeded the OMEE (Ontario Ministry of Environment and Energy) guidelines in site 1 and manganese level exceeded the guideline in site 2. Their concentration ranged from 2.2 to 46.0 mg kg⁻¹ and 163.6 to 6610.3 mg kg⁻¹ for arsenic and manganese, respectively (Table 2).

Aluminum, iron and magnesium concentrations were significantly higher in sites 1 to 4 (top layer, Table 2) compared to the control site 5. The values ranged from 1673.3 to 9193.3 mg kg⁻¹, 2193.3 to 31433.3 mg kg⁻¹ and 349.6 to 6866.6 mg kg⁻¹ for aluminum, iron and magnesium, respectively (Table 2). Cadmium, cobalt, lead and zinc levels were within the OMEE guideline. The values for these metals ranged from 0.3 to 2.1 mg kg⁻¹, 1.6 to 37.9 mg kg⁻¹, 18.2 to 176.0 mg kg⁻¹ and 52.0 to 86.8 mg kg⁻¹ (Table 2). The control site 5 was always among the least contaminated for the metals analysis. All the metal concentrations obtained from the bottom layer (5 - 20 cm) were within the OMEE guideline (data not shown). Surprisingly, the data from tailings were similar or significantly lower than other contaminated sites. The pH for all the sites including the controls were low (acidic).

Sampling sites	Elements											pH
	Aluminum	Arsenic	Cadmium	Cobalt	Copper	Iron	Lead	Magnesium	Manganese	Nickel	Zinc	
Site 1	7360b	46b	2b	29b	1330bc	31433bc	176bc	2970b	163a	1600b	86bc	4.6
Site 2	9193b	10a	1b	37b	373ac	12933ac	46ac	6866b	6610c	318bc	52ab	4.5
Site 3	7670b	10a	0.9b	22a	170a	6476a	39ac	2706b	539b	120bd	60ac	-
Site 4	7090b	2a	0.3a	6a	52a	10233ac	18a	2996b	165a	54acd	32a	4.0
Site 5 (control)	1673a	2a	0.7b	2a	93a	2913a	88ab	349a	206a	30acd	55a	3.8
Tailings	-	-	0.3a	34b	300ac	-	46ac	-	-	292bc	59ac	-

*Means in columns with a common subscript are not significantly different based on Tukey multiple comparison test ($P \geq 0.05$). Site 1: Lively; Site 2: Coniston (close to HW17); Site 3: ≈ 40 km from Sudbury HW144 towards Timmins; Site 4: ≈ 16 km from site 3 HW144 towards Timmins; Site 5 (control): ≈ 35 km from Site 4 HW144 towards Timmins. Tailings data represent means of two tailings located near smelters.

Table 2. Metal concentrations in top layer (0 – 5 cm) of soil from the Sudbury region sites, concentrations are in mg kg⁻¹, dry weight*.

3.2 Analysis of populations using ISSR markers

Ten ISSR oligonucleotides (Table 1) were used for the amplification of spruce and pine populations. For each population in each species, the levels of polymorphism for the two generations analyzed were similar. Thus, the data were compiled and analyzed per population.

3.2.1 *Pinus strobus* (white pine)

The percentage of polymorphic loci within each population varied between 22% observed in the site 5 (control) to 36% in site 4 (Table 2). The level of genetic variation was similar between natural and planted populations in site 1. For site 2, the polymorphic loci were significantly higher in planted populations compared to the natural population. Data for the Nei' gene diversity (h) ranged from 0.05 (S5) to 0.14 (S4) with a mean of 0.19. A similar pattern was observed for the Shannon's information index (I), with the high value of 0.20 observed in S4 and a low value of 0.08 observed in S5. The observed number of alleles (N_a) and the effective number of alleles (N_e) ranged from 1.22 to 1.36 and 1.08 to 1.25 respectively. The genotype diversity among population (H_t) was 0.15 and the within population diversity (H_s) was 0.09. Mean coefficient of gene differentiation (G_{st}) was 0.366 indicating that 63.4% of the genetic diversity resided within the population. The observed structure of genetic variability shows that there is a low level of differentiation among the *Pinus strobus* populations. The overall rate of gene flow (N_m) among population was 0.87.

Population*	P (%)	Na	Ne	h	I
S1P	30	1.30	1.14	0.08	0.13
S1Na	30	1.30	1.18	0.10	0.15
S2P	34	1.34	1.21	0.12	0.18
S2Na	22	1.22	1.12	0.07	0.11
S3M	32	1.32	1.19	0.11	0.17
S4M	36	1.36	1.25	0.14	0.20
S5M	22	1.22	1.08	0.05	0.08
Mean	29	1.29	1.17	0.10	0.15

*Population: P represents Plantation and Na represents Natural populations. M represents mixed populations including natural and planted trees.

Table 2. Genetic diversity parameters of *Pinus strobus* based on ISSR data.

3.2.2 *Pinus banksiana* (jack pine)

For Jack pine, a low to moderate levels of genetic variation was revealed within each population. The percentage of polymorphic loci (P %) ranged from 14.6 % to 45.8 % with a mean of 31.6 %. The mean level of polymorphism for the eight populations from the greater Sudbury area was 27.6% while this value was higher for populations from the nurseries with an average of 42.4% detected polymorphic loci. The levels of genetic variation detected in populations from metal-contaminated areas were similar to those found in control sites. The Nei's gene diversity (h) for all jack pine populations analyzed varied from 0.046 to 0.169 with an average of 0.100, and Shannon's index (I) ranged from 0.070 to 0.250 with an average of 0.153. The mean observed number of alleles (Na) ranged from 1.146 to 1.458, while the mean effective number of alleles (Ne) varied from 1.107 to 1.31 (Table 3).

Populations	P (%)	h	I	Ne	Na
Nursery 1 (Introduction 1)	39.58	0.0961	0.1535	1.1579	1.3958
Nursery 2 (Introduction 2)	41.67	0.1380	0.2106	1.2248	1.4167
Nursery 3 (Introduction 3)	45.83	0.1687	0.2501	1.2946	1.4583
Inco 1	31.25	0.1120	0.1653	1.2035	1.3125
Inco 2	31.25	0.1171	0.1727	1.2061	1.3125
Falconbridge 1	14.58	0.0456	0.0701	1.0756	1.1458
Falconbridge 2	27.08	0.0995	0.1467	1.1758	1.2708
Falconbridge 3	20.83	0.0630	0.0982	1.1004	1.2083
Inco Tailing	35.42	0.0977	0.1552	1.1514	1.3542
Temagami (control)	29.17	0.0818	0.1284	1.1310	1.2917
Low Water Lake (control)	31.25	0.0812	0.1297	1.1256	1.3125
Mean	31.63	0.1001	0.1528	1.1679	1.3163

P represents percentage of polymorphic loci; h, Nei's gene diversity; I, Shannon's information index; Ne, effective number of alleles; Na, observed number of alleles.

Table 3. Genetic variability parameters of *Pinus banksiana* populations growing in the Sudbury area based on ISSR data.

3.2.3 *Pinus resinosa* (red pine)

The level of genetic variation was much lower in the red pine populations. For this species, the level of polymorphic loci varied from 4.55 % to 27.27 % (Table 4). The mean level of polymorphic loci for populations from the greater Sudbury region excluding the population from the nursery was only 8.3%. Like in jack pine populations, the polymorphism detected in contaminated populations was similar to that found in non contaminated site used as a control. Overall, the mean for Nei's gene diversity and Shannon's information index, were 0.034 and 0.053, respectively for all the red pine populations analyzed. The mean observed number of alleles (Na) ranged from 1.045 to 1.27 while the mean effective alleles (Ne) varied from 1.00 to 1.17 (Table 4). The highest genetic diversity values were observed in the populations used for the Sudbury reforestation program. High levels of metal content did not affect the level variation for both species.

Population	P (%)	h	I	Ne	Na
Near Falconbridge	4.55	0.0044	0.0092	1.0049	1.0455
Very near Falconbridge	13.64	0.0411	0.0638	1.0672	1.1364
Falconbridge	4.55	0.0226	0.0314	1.0450	1.0455
Coniston	9.09	0.0180	0.0309	1.0244	1.0909
Daisy Lake	9.09	0.0272	0.0433	1.0389	1.0909
Verner (control)	9.09	0.0267	0.0423	1.0398	1.0909
Introduction 1 (control)	27.27	0.0988	0.1465	1.1710	1.2727
Mean	11.04	0.0341	0.0525	1.0559	1.1104

P represents percentage of polymorphic loci; h, Nei's gene diversity; I, Shannon's information index; Ne, effective number of alleles; and Na, observed number of alleles.

Table 4. Genetic variability parameters of *Pinus resinosa* populations growing in the Sudbury area based on ISSR data.

3.2.4 *Picea glauca* (white spruce)

All the selected primers amplified 11 to 21 fragments across the six populations studied. The amplified fragment size ranged from 170 bp to 2,240 bp. The percentage of polymorphic loci within each population varied between 50% observed in the natural site 5 Na (control) to 61% in site 1, P (Table 5). Nei' gene diversity (h) ranged from 0.17 (site 1, P) to 0.21 (site 5, Na; control) with a mean of 0.19. A similar pattern was observed for the Shannon's information index (I), with the highest value of 0.32 observed in the planted population of site 1P and the lowest value of 0.26 observed in site 5Na (control). The observed number of alleles (Na) and the effective number of alleles (Ne) ranged from 1.50 to 1.61 and 1.29 to 1.37 respectively. The genotype diversity among population (H_t) was 0.19 and the within population diversity (H_s) was 0.23. The mean coefficient of gene differentiation (G_{st}) was 0.168 indicating that 83.2% of the genetic diversity resided within the population. The observed structure of genetic variability shows that there is a low level of differentiation among the *Picea glauca* populations in the target regions even when the populations located as far as 100 km from the Sudbury were included. The overall rate of gene flow (N_m) among population was 2.47.

3.2.5 *Picea mariana* (black spruce)

The genetic diversity within each population was high. For each population, the percentage of polymorphic loci was the same for the parental and the offspring generations analyzed. Thus, the data from the two generations were combined. The percentage of polymorphic loci (P%) ranged from 65% to 90 % with a mean of 75% . Nei's gene diversity (h) varied from 0.264 to 0.359 with an average of 0.310, and Shannon's index (I) ranged from 0.381 to 0.524 with an average of 0.449 (Table 6). The mean observed number of alleles (Na) ranged from 1.650 to 1.900, while the mean effective number of alleles (Ne) varied from 1.168 to 1.632 (Table 6). Among the nine populations investigated, the highest genetic diversity was observed in population 9 from lowland in Timmins while the lowest level of diversity was detected in population 4 from upland in Chelmsford. Overall, the average level of polymorphic loci was much higher in lowlands (85%) than in uplands (68%). There was no difference between metal contaminated and uncontaminated sites for genetic variation.

Population*	P (%)	Na	Ne	h	I
Site 1 (P)	61	1.61	1.37	0.22	0.32
Site 2 (P)	53	1.53	1.33	0.19	0.29
Site 3 (Na)	55	1.55	1.32	0.19	0.29
Site 4 (Na)	53	1.53	1.33	0.19	0.28
Site 5 (control) (Na)	50	1.50	1.30	0.18	0.26
Nursery	57	1.57	1.35	0.20	0.30
Mean	55	1.55	1.33	0.19	0.29

*Population: P represents Plantation and Na represents Natural populations

Table 5. Genetic diversity parameters of *Picea glauca* based on ISSR data.

Populations	P (%)	h	I	Ne	Na
Site 1	80	0.328	0.473	1.603	1.800
Site 2	85	0.350	0.508	1.630	1.850
Site 3	70	0.269	0.396	1.473	1.700
Site 4	65	0.264	0.381	1.490	1.650
Site 5	75	0.317	0.456	1.582	1.750
Site 6	70	0.274	0.402	1.482	1.700
Site 7	70	0.308	0.441	1.567	1.700
Site 8	70	0.325	0.459	1.168	1.700
Site 9	90	0.359	0.524	1.632	1.900
Mean	75	0.310	0.449	1.514	1.750

P represents percentage of polymorphic loci; h, Nei's gene diversity; I, Shannon's information index; Na, observed number of alleles; and Ne, effective number of alleles.

Table 6. Genetic variability parameters of black spruce (*Picea mariana*) populations growing in the Sudbury area based on ISSR data.

3.2.6 Genetic differentiation among populations

For *Pinus banksiana*, the mean gene diversity within populations (Hs) and the total gene diversity (Ht) were 0.100 and 0.1438, respectively. The variation among populations (Gst)

was 0.304 indicating that 30.4 % of total genetic diversity were attributed to the differences among populations. The observed structure of genetic variability shows that there is a sensitive level of differentiation among the jack pine populations in the target regions. The overall rate of gene flow (N_m) among populations was 1.144. For *Pinus resinosa* the H_s and H_t values were 0.0341 and 0.0437, respectively. About 22% of the total genetic diversity in *Pinus resinosa* was attributed to differences among populations. For *Pinus strobus*, the genotype diversity among population (H_t) was 0.15 and the within population diversity (H_s) was 0.09. Mean coefficient of gene differentiation (G_{st}) was 0.366 indicating that 63.4% of the genetic diversity resided within the population. The observed structure of genetic variability shows that there is a low level of differentiation among the *Pinus strobus* populations. The overall rate of gene flow (N_m) among population was 0.87.

For *Picea glauca* H_t and H_s were 0.19 and 0.23, respectively. The mean coefficient of gene differentiation (G_{st}) was 0.168 indicating that 83.2% of the genetic diversity resided within the population. The observed structure of genetic variability shows that there is a low level of differentiation among the *Picea glauca* populations in the target regions even when the populations located as far as 100 km from the Sudbury were included. The overall rate of gene flow (N_m) among population was 2.47.

For *P. mariana*, the mean gene diversity within populations (H_s) and the total gene diversity (H_t) were 0.310 and 0.385, respectively. The variation among populations (G_{st}) was 0.19. This indicates that 19.3% of total genetic diversity was attributed to the differences among populations. Like in *P. glauca*, the observed structure of genetic variability shows that there is a low level of differentiation among the *P. mariana* populations. The overall rate of gene flow (N_m) among populations was 2.088.

3.3 Genetic relationships among conifer populations based on ISSR analysis

3.3.1 *Pinus banksiana*, *Pinus strobus*, and *Pinus resinosa*

Because of limited genetic variation in *Pinus resinosa* samples analyzed, the genetic relatedness was analyzed only for *Pinus banksiana* and *Pinus strobus* populations. The Jaccard similarity coefficients and genetic distance were calculated using ISSR data. The genetic distance scale runs from 0 (identical) to 1 (different for all criteria). In general, the genetic distance values were low as they ranged from 0.06 to 0.21 for *Pinus strobus* and from 0.037 to 0.365 (Table 7) for *Pinus banksiana*. Overall the genetic distance values revealed that all the eleven *P. banksiana* and *P. strobus* populations were genetically closely related (Table 7). For *P. banksiana*, the two populations from control site (uncontaminated), Low Water Lake and Temagami were the most closely related. The largest genetic distance was observed between population 5 from INCO 2 and the new population used in 2006 for reclamation (called introduction 2 in the present study). The dendrogram constructed, based on ISSR data revealed a particular clustering (Fig. 2). All the populations from the greater Sudbury that we analyzed clustered together while the three newly introduced populations from nurseries were grouped in a separate cluster (Fig. 2). For *Pinus strobus* the genetic distance values ranged from 0.06 (S1P and S2P) to 0.21 (S2P and S2Na) (Table 8). Dendrogram was not constructed considering the low levels of genetic distances. For *Pinus resinosa*, the level of genetic variation was too low to calculate genetic distance among populations or to construct a dendrogram.

	1	2	3	4	5	6	7	8	9	10	11
1	0	0.132	0.229	0.243	0.321	0.321	0.333	0.250	0.259	0.247	0.280
2		0	0.186	0.273	0.365	0.345	0.356	0.277	0.286	0.274	0.306
3			0	0.219	0.341	0.321	0.333	0.250	0.280	0.268	0.280
4				0	0.225	0.250	0.198	0.175	0.185	0.195	0.207
5					0	0.134	0.146	0.190	0.221	0.188	0.179
6						0	0.085	0.085	0.096	0.084	0.073
7							0	0.120	0.108	0.096	0.108
8								0	0.038	0.049	0.038
9									0	0.037	0.049
10										0	0.037
11											0

1 represents introduction 1; 2, Introduction 2; 3, Introduction 3; 4, Inco 1 site; 5, Inco 2 site ; 6, Falconbridge 1 site; 7, Falconbridge 2 site; 8, Falconbridge 3 site; 9, Inco Tailing; 10, Temagami site; and 11, Low Water Lake site.

Table 7. Distance matrix generated using bulk sample analysis from various populations of *Pinus banksiana* ISSR data (RAPDistance version 1.04).

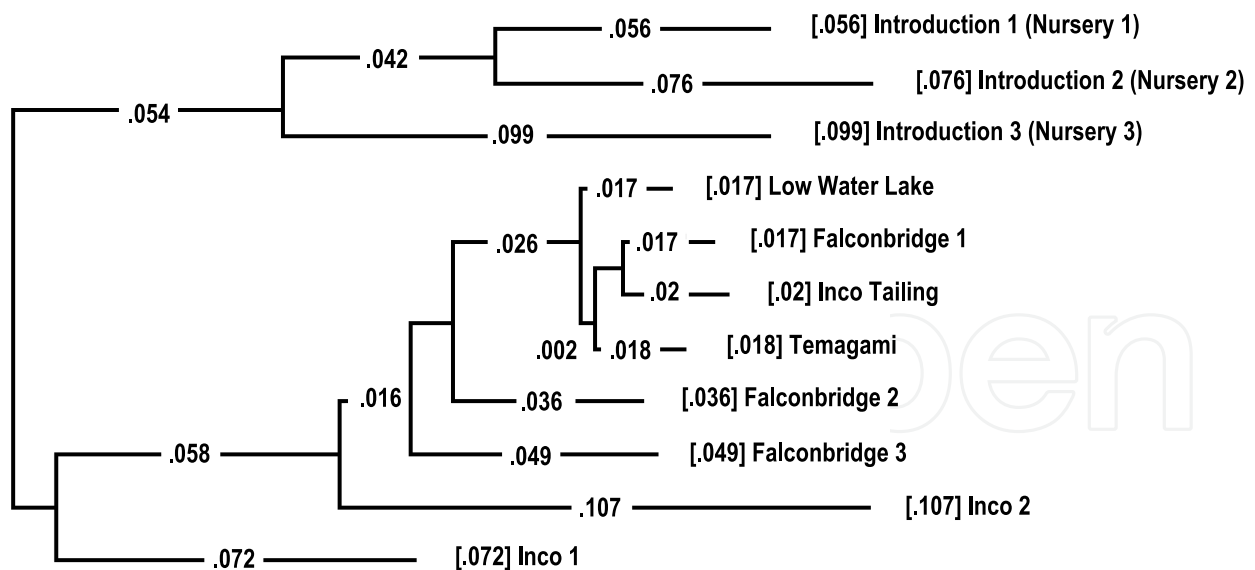


Fig. 2. Dendrogram of the genetic relationships among *Pinus banksiana* populations based on Jaccard similarity matrix using ISSR data. The values above the branches indicate the patristic distances based on the neighbor-joining (NJ) analysis.

	S1P	S1Na	S2P	S2Na	S3M	S4M	S5M
ISSR							
S1P	0.0000	0.1489	0.0667	0.1956	0.1304	0.1739	0.2128
S1Na		0.0000	0.1702	0.1778	0.1111	0.1556	0.1956
S2P			0.0000	0.2174	0.1522	0.1556	0.01956
S2Na				0.0000	0.0714	0.1191	0.2045
S3M					0.0000	0.0930	0.1778
S4M						0.0000	0.1818
S5M							0.0000

Population: P represents Plantation and Na represents Natural populations. M represents mixed populations including natural and planted trees. Site 1: Daisy Lake (HW17 Bypass); Site 2: Coniston (HW17 Hydro Dam ≈ 10 km from Bypass); Site 3: Hagar ≈ 60 km from Sudbury; Site 4: Markstay ≈ 38km from Sudbury; Site 5: Kukagami Road (≈ 9 km from HW17).

Table 8. Distance matrix generated from ISSR data using the Jaccard similarity coefficient analysis for *Pinus strobus* populations (Free Tree Program).

3.3.2 *Picea glauca* and *Picea mariana*

The genetic distance values were close to 0 as they varied between 0.02 (site 3, Na and site 4, Na) and 0.07 (site 2, P and site 5, Na) (Table 9) for *Picea glauca*. For *Picea mariana*, the genetic values ranged from 0.171 to 0.351 (Table 10). Overall the genetic distance values revealed that all the populations were genetically closely related (Table 10) for each of the *Picea* species. For *P. mariana*, the dendrogram constructed, based on ISSR data revealed a particular clustering between upland (dry) and lowlands (wet lands) (Figure 3). With the exception of site 7, no upland (dry land) population clusters with a population from a lowland (wet land). For example, the low - land (wetland) population 1 from Falconbridge clusters with the lowland (wetland) population 9 from Timmins; the upland (dry land) population 4 from Chelmsford clusters with the upland (dry land) population 8 from Timmins; the lowland (wetland) population 2 from Falconbridge clusters with the lowlands (wetland) population 5 from Cartier; and the up-land (dry land) population 3 from Capreol clusters with the upland (dry land) population 6 from Cartier.

	Site 1	Site 2	Site 3	Site 4	Site 5 (control)	Nursery
ISSR						
Site 1 (P)	0.0000	0.0520	0.0417	0.0417	0.0626	0.0209
Site 2 (P)		0.0000	0.0729	0.0729	0.0737	0.0316
Site 3 (Na)			0.0000	0.0213	0.0632	0.0417
Site 4 (Na)				0.0000	0.0632	0.0417
Site 5 (Na)					0.0000	0.0625
Nursery						0.0000

Population: P represents Plantation and Na represents Natural populations

Table 9. Distance matrix generated from ISSR data using the Jaccard similarity coefficient analysis for *Picea glauca* populations.

	Site1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Site 1	0	0.236	0.235	0.171	0.282	0.270	0.307	0.222	0.212
Site 2		0	0.324	0.263	0.230	0.307	0.256	0.350	0.351
Site 3			0	0.235	0.297	0.181	0.324	0.235	0.225
Site 4				0	0.325	0.270	0.263	0.171	0.314
Site 5					0	0.236	0.230	0.325	0.324
Site 6						0	0.263	0.222	0.264
Site 7							0	0.307	0.351
Site 8								0	0.314
Site 9									0

Table 10. Distance matrix generated using the neighbour-joining analysis from *Picea mariana* ISSR data (RAPDistance version 1.04).

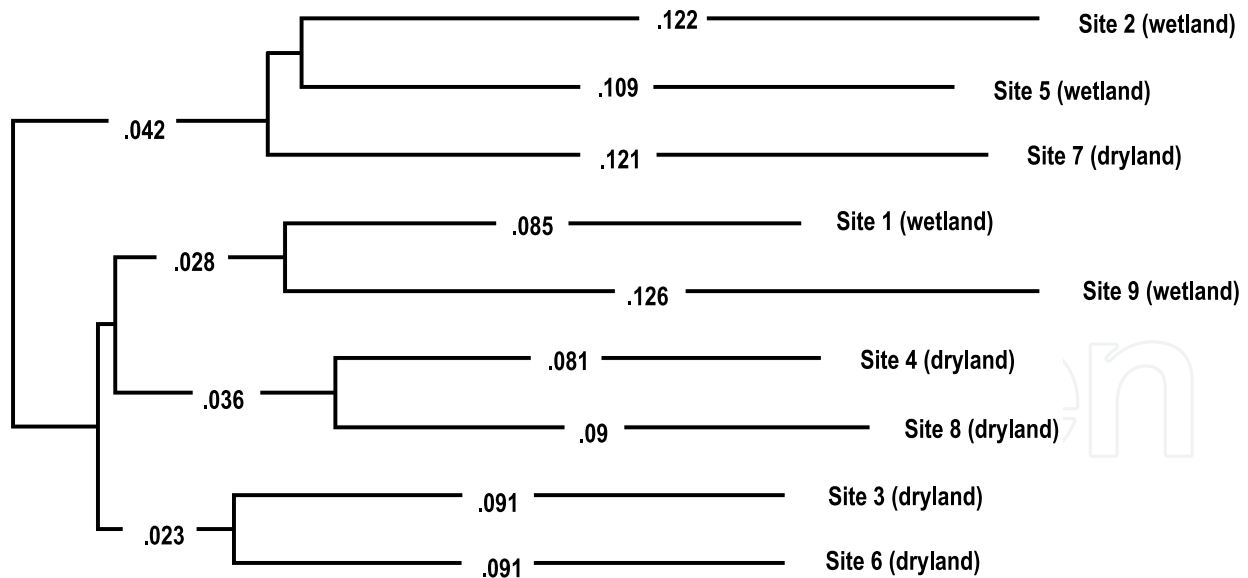


Fig. 3. Dendrogram of the genetic relationships among *Pinus banksiana* populations based on Jaccard similarity matrix using ISSR data. The values above the branches indicate the patristic distances based on the neighbor-joining (NJ) analysis.

3.4 Analysis of populations using microsatellites

3.4.1 Genetic diversity

The microsatellite loci analyzed in *Pinus banksiana* and *P. resinosa* populations are summarized in Table 11. For *P. banksiana*, the mean number of alleles per locus was 9 and the mean effective number of alleles was 3.5 (Table 11). The mean number of alleles across loci per population ranged from 3.00 to 4.67 with the samples from the INCO 3 (site 4) and the introduction 3 (population or site 11) having the highest allelic diversity. The lowest allelic diversity was observed in samples from the INCO tailing (site 7) populations (Table 11).

For *Pinus resinosa* the mean number of alleles per locus was six and the mean effective number of alleles was 2.50. The mean number of alleles across loci per population ranged from 2.33 to 3.00 for the *P. resinosa* populations (Table 11). The highest allelic diversity was observed the samples from site 2 near Falconbridge and site 7 located in Verner. The lowest allelic diversity was found in the samples from site 4 in Coniston/Wahnipitae and the newly introduced population (introduction or site 2) from nursery 2 identified as population or site 8 (Table 11).

Species/Population	N _A	N _{Ap}	HO	HE	i
<i>Pinus banksiana</i>					
Val Caron (site 1)	4.3333	3.3604	0.6667	0.6983	1.2967
Introduction 1 (site 2)	4.0000	2.7951	0.4667	0.6096	1.1051
Introduction 2 (site 3)	4.0000	2.6056	0.4000	0.5850	1.0650
Inco 3 (site 4)	4.6667	2.9764	0.7333	0.6133	1.1734
Inco 1 (site 5)	4.0000	2.9054	0.5000	0.6500	1.1727
Inco 2 (site 6)	3.6667	2.7763	0.6333	0.5367	0.9716
Inco tailing (site 7)	3.0000	2.1221	0.3333	0.5129	0.8551
Falconbridge (site 8)	3.6667	2.1973	0.5852	0.5421	0.9443
Temagami (site 9)	4.0000	2.6277	0.6333	0.6050	1.1054
Low Water Lake(site 10)	3.6667	2.7987	0.2593	0.6235	1.1098
Introduction 3 (site 11)	4.6667	3.7420	0.4000	0.7283	1.3936
Introduction 4 (site 12)	3.6667	2.0854	0.2583	0.4554	0.8578
Mean	4.0000	2.7422	0.4912	0.7194	1.5155
Standard error	±0.4678	±0.1723	±0.3679	±0.0133	±0.0939
<i>Pinus resinosa</i>					
Introduction 1 (site 1)	2.6667	2.0994	0.1000	0.4150	0.7118
Falconbridge (site 2)	3.0000	2.1847	0.5506	0.4258	0.7809
Falconbridge (site 3)	2.6667	1.8039	0.1667	0.3267	0.5926
Falconbridge (site 4)	2.6667	1.9750	0.1333	0.3748	0.6653
Coniston(site 5)	2.3333	1.7365	0.0667	0.3431	0.5497
Daisy Lake (site 6)	2.6667	1.7188	0.0667	0.3346	0.5914
Verner (site 7)	3.0000	1.5541	0.1000	0.3017	0.5459
Introduction 2 (site 8)	2.3333	1.7060	0.0667	0.2783	0.5041
Mean	2.6667	1.8473	0.0892	0.4606	0.9477
Standard error	±0.2520	±1.3076	±0.0941	±0.3992	±0.828

N_A = mean allele number per locus; N_{Ap} = mean number of polymorphic alleles per locus; HO = observed heterozygosity; HE = expected heterozygosity; I = Shannon's information index;

Table 11. Genetic diversity estimates for 12 *Pinus banksiana* and 8 *Pinus resinosa* populations from the Sudbury, Ontario region using microsatellite primers.

The observed heterozygosity (H_O) at the population level ranged from 0.26 to 0.67 and the expected heterozygosity (H_E) varied from 0.46 to 0.72 for *Pinus banksiana* populations. The samples from INCO 3 (site 4) produced the highest H_O values and the samples from Low Water Lake (site 10) used as control showing the lowest observed heterozygosity (Table 11). The degree of population differentiation (F_{ST}) was 17 % for *P. banksiana*. For *Pinus resinosa*, the observed heterozygosity (H_O) at the population level, ranged from 0.07 to 0.55. Samples from site 2 located near Falconbridge produced the highest heterozygosity and the samples from nursery 2 (introduction 2) called population or site 8 showing the lowest values (Table 11). H_E values ranged from 0.28 to 0.43. The degree of population differentiation (F_{ST}) was 23.9 % for *P. resinosa*.

For *Picea mariana*, the microsatellite analysis confirmed the high level of genetic diversity within each population but revealed no significant difference between wetland and upland populations for all the genetic parameters analyzed. Overall, 11% of the total genetic diversity was attributed to differences among populations. The mean number of alleles and effective number of alleles per locus were 10.3 and 5.6, respectively. The observed and expected heterozygosity values ranged from 0.425 to 0.732 and 0.584 to 0.768, respectively (Table 12).

Population	N_A	N_{Ap}	H_O	H_E	I
Site 1 (wetland)	5.67	5.33	0.482	0.619	1.333
Site 2 (wetland)	5.33	5.33	0.587	0.752	1.512
Site 3 (dryland)	5.67	5.67	0.652	0.768	1.572
Site 4 (dry land)	5.33	5.33	0.641	0.740	1.503
Site 5 (wetland)	6.33	6.33	0.648	0.729	1.532
Site 6 (dry land)	3.67	3.33	0.577	0.584	1.040
Site 7 (dry land)	6.00	6.00	0.559	0.744	1.541
Site 8 (dryland)	6.00	6.00	0.732	0.740	1.540
Site 9 (wet land)	5.67	5.67	0.425	0.772	1.585
Mean	5.52	5.44	0.589	0.717	1.462
Standard dev.	±0.765	±0.867	±0.094	±0.067	±0.175

N_A = mean allele number per locus; N_{Ap} = mean number of polymorphic alleles per locus; H_O = observed heterozygosity; H_E = expected heterozygosity (Nei 1973); I = Shannon's information index; F_{IS} = measure of heterozygote deficiency or excess (Wright 1978).

Table 12. Genetic diversity estimates for black spruce (*Picea mariana*) populations using microsatellite primers.

After the correction for null alleles, exact test for Hardy-Weinberg Equilibrium revealed, the majority of the populations deviated significantly from the Hardy Weinberg Equilibrium. The results revealed that the null allele frequency estimates were negligible for all populations (data not shown). The HWE deviation for these populations might be the result of other factors than null alleles. The global tests revealed significant heterozygote deficiency for most populations. Overall, the present study indicates that the long-term exposure of *P. mariana* populations to metal (more than 30 years) is not associated with the level of genetic diversity.

3.4.2 Gene flow

The gene flow estimates were considered low for both species, $N_m = 1.21$ for *Pinus banksiana* and $N_m = 0.79$ for *P. resinosa* based on Slatkin (1985). There was also no significant

difference in the inbreeding coefficients among the stands within the same species. The mean inbreeding coefficients were considered high for *P. resinosa* and low for *P. banksiana*.

4. Discussion

Loss of rare alleles, lower heterozygosity and directional selection have been concerns of plant populations (Slatkin, 1985; Bergmann and Scholz, 1989). Most of the forest ecosystems within the Sudbury area have improved considerably during the last 30 years (Dudka et al., 1995; Gratton et al., 2000). Vascular and nonvascular plants such as conifers, birches and lichens have re-invaded semi-barren landscapes. More than nine millions trees mostly conifers have been planted in the Greater Sudbury Region. Genetic diversity is the foundation for forest sustainability and ecosystem stability. Bench marking genetic diversity in forest tree populations can provide resource managers with an indicator of long-term forest sustainability and ecosystem health (Mosseler and Rajora, 1998; Rajora and Mosseler, 2001a, 2001b).

For *Pinus banksiana* and *Pinus glauca*, the levels of genetic variation were low to moderate. In fact, genetic variation in *Pinus strobus* (White pine) studied varied from 24 to 40%. The newly planted populations of *Pinus banksiana* and *Pinus glauca* revealed a higher level of genetic variation compared to natural populations. The genetic distances among the pine populations growing in the Greater Sudbury area revealed that all the populations analyzed were genetically close to each other. The highest genetic diversity values were observed in new plantations being developed by the Sudbury reforestation program (Ranger et al., 2007). The level of genetic variation was low (less than 10%) for *P. resinosa*. This was attributed to other events that took place during the history of this species in North America (Mosseler et al., 1992).

Genetic variation and genetic structure of *P. mariana* (black spruce) populations growing in wet and dry lands with different levels of metal contaminations was high in all the populations analyzed with the percentage of polymorphic loci (P %) ranging from 65% to 90%. For *Picea glauca* populations polymorphism levels ranged from 50% to 61% for ISSR markers and from 70% to 80% for RAPD markers. The level of variation in newly introduced populations of *P. mariana* and *P. glauca* from the Sudbury Reclamation program was also high. Variation within populations accounts for most of total genetic variation. Moreover, genetic tests with species-specific molecular markers revealed that all the trees from *P. mariana* and *P. glauca* planted and natural populations were pure genotypes with no introgression of other species.

In all the conifer species, metal content in soil was not associated with the level of diversity in populations analyzed. Within each species, the different populations studied were genetically closely related. Overall, the results of the present study indicate that the conifer populations from the Greater Sudbury region and other surrounding areas meet most genetic criteria of sustainability. Moreover, the levels of genetic variation observed in the targeted species were similar to data reported for other fragmented populations across Canada for the same species (Mehes et al., 2007).

Elevated accumulations of metal accumulations in soils and vegetation have been documented within short distances of the smelters in Sudbury compared to control sites (Freedman and Hutchinson, 1980; Gratton et al., 2000; Nkongolo et al., 2008). Among the sites analyzed in the present study, the highest level of metal content in soil and plant

tissues were detected in samples from populations 1 and 2 located near Falconbridge Smelters in Sudbury (Gratton et al., 2000; Nkongolo et al. 2008). These populations showed the highest level of genetic variability for *Picea mariana* for example along with the control population 9 from Timmins. The same level of genetic variation was observed in parents and progenies within the same populations. This clearly indicated that the exposure to metals for more than 30 years has no effect on genetic structure and diversity of black spruce populations in Northern Ontario. This lack of association between the level of genetic variation and metal content can be attributed to the long life span of conifer species. In fact, the populations analyzed were only the first and second generations of progenies from parents exposed to metal contamination

This is in contrast to data observed in herbaceous species such as *Deschampsia cespitosa* where the level of metal accumulation reduced significantly the level of genetic variation (Nkongolo et al. 2008). Metals impose severe stress on plants, especially in the rooting zone, which has led to the evolution of metal-resistant ecotypes in several herbaceous species like *D. cespitosa* (Cox and Hutchinson 1980). Evidence of loss of genetic variation based on enzymatic analysis at the population level caused by pollution has been demonstrated in some species (Lopes et al. 2004; Prus-Glowacki et al. 2006; van Straalen and Timmermans 2002). But, plants possess homeostatic cellular mechanisms to regulate the concentration of metal ions inside the cell to minimize the potential damage that could result from the exposure to nonessential metal ions. These mechanisms serve to control the uptake, accumulation and detoxification of metals (Foy et al. 1978). This might be the case in black spruce trees exposed to certain levels of metals.

Genetic variation is the foundation for ecosystem stability and population sustainability. In tree populations this information is an indicator of long term population sustainability and health. For example, environmental stressors, such as anthropogenic factors, can affect the genetic frequencies by increasing mutation or selection. This further leads to differences among populations and increase uniformity within a population, thus increasing homozygosity and inbreeding (Dimsoski and Toth, 2001). Studies of genetic variation of impacted and unimpacted populations have defined a positive relationship between the exposure to the stressor and diversity.

Using various types of markers, several authors have reported differences in genetic structure of plants growing in contaminated areas (Muller-stark, 1985; Scholz and Bergmann, 1984). Enzymatic studies of *Picea abies* (Norway spruce) revealed genetic differences between groups of sensitive trees in polluted areas (Scholz and Bergmann, 1984). Higher heterozygosity was reported in tolerant plants of European beech in *Pinus sylvestris* (Scots pine) in Germany and Great Britain (Muller- starck, 1985; Geburek et al., 1987). Berrang et al., (1986) also reported a high heterozygosity in *Populus tremuloides* (Trembling aspen) and *Acer rubrum* (Red maple) populations in the USA.

No significant differences were observed among natural and planted *Picea glauca* populations. All the populations revealed high levels of polymorphic loci for the ISSR markers. This suggests that the *Picea glauca* populations are likely sustainable in long term. For *Pinus strobus*, the levels of genetic variations were in general low to moderate. The newly introduced populations revealed higher levels of polymorphic loci compared to natural populations. This confirms that the land reclamation by planting *Pinus strobus* trees and other pine species is increasing the sustainability of pine populations in the Sudbury region.

Genetic distance values were calculated according to the Jaccard similarity coefficient. In general, the genetic distance values revealed that the different *Pinus spp.* and *Picea spp.* populations were genetically closely related. Overall, the genetic distance analysis showed a high level of homogeneity among populations which could be due to the species characteristics. In fact, the relative small genetic distance values reported in the present analysis are consistent with other studies on *Picea glauca* populations in various provinces that used various molecular markers and allozymes (Rajora *et al.*, 2005; Tremblay and Simon, 1989; Alden and Loopstra, 1987). In general, the genetic similarity among the populations suggests that these populations could have originated from a common source. In addition, *Picea glauca* is an anemophilous species and its pollen is transported over great distances. The fact that *Picea glauca* populations are fairly distributed should promote the exchange of genes among populations. Hence, it is rare to find alleles that are unique to a given populations, and the frequencies of the main alleles are generally similar from one population to another (Rajora *et al.*, 2005; Tremblay and Simon, 1989; Alden and Loopstra, 1987).

In Sudbury (Canada) during the last 25 years, production of nickel, copper and other metals has been maintained at high levels while industrial sulphur dioxide (SO₂) emissions have been reduced by approximately 90% through combination of industrial technological developments and legislated controls. This has allowed for some degree of recovery to occur such as improved air quality and natural recovery of damaged ecosystems during this period of reduced emissions at Sudbury. The recovery has been further done through the reforestation program by planting over 9 million trees such as conifers in the Sudbury region. On the other hands, the African Copper belt, on the border between Zambian and DR-Congo, are among the ten most polluted areas worldwide (The Blacksmith institute, 2008; Banza *et al.*, 2009). Like in many other regions producing heavy metals, such as Senegal, Tanzania, China, Russia, Romania, India, Philippines, Thailand, Indonesia etc., there are virtually no controls on the discharge of pollutants from mining and smelters. There are no land reclamation programs and environment degradations from past mining activities have not been addressed. Studies on the effect of metal contamination on genetic diversity of plant populations in those regions are limited. Prus-Glowack *et al.* (2006) demonstrated in a small scale study in Poland that the stress resulting from gaseous pollution and contamination of the soil with heavy metals exerts a significant effect on phenotype of individuals and on genetic structure of *Pinus sylvestris* L populations. Such data needs to be validated at larger scale using molecular markers.

5. Conclusion

The present study indicates that *Pinus spp.* and *Picea spp.* populations from the Sudbury region, Ontario, are genetically variable. Metal contamination levels were not associated with genetic variation in *Picea glauca* populations. Overall, the results indicate that the conifer populations from the Greater Sudbury region and other surrounding areas meet most genetic criteria of sustainability. This conclusion was confirmed by molecular analysis using ISSR, SSR markers, and cytological studies. The effects of metals, if any, may require several generations to be detected. The reclamation of Sudbury forest lands with new populations increases the sustainability specifically for *Pinus* (Pine) species. Since Sudbury is not among the ten most polluted areas in world, a replication of this study in areas with

higher soil metal content is recommended to validate the effects of metal populations in tree populations.

6. Acknowledgement

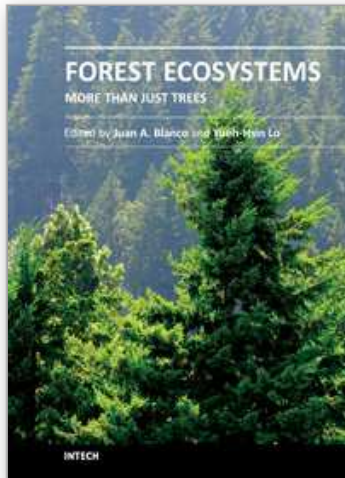
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Forest Ecosystems - More than Just Trees

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The common idea for many people is that forests are just a collection of trees. However, they are much more than that. They are a complex, functional system of interacting and often interdependent biological, physical, and chemical components, the biological part of which has evolved to perpetuate itself. This complexity produces combinations of climate, soils, trees and plant species unique to each site, resulting in hundreds of different forest types around the world. Logically, trees are an important component for the research in forest ecosystems, but the wide variety of other life forms and abiotic components in most forests means that other elements, such as wildlife or soil nutrients, should also be the focal point in ecological studies and management plans to be carried out in forest ecosystems. In this book, the readers can find the latest research related to forest ecosystems but with a different twist. The research described here is not just on trees and is focused on the other components, structures and functions that are usually overshadowed by the focus on trees, but are equally important to maintain the diversity, function and services provided by forests. The first section of this book explores the structure and biodiversity of forest ecosystems, whereas the second section reviews the research done on ecosystem structure and functioning. The third and last section explores the issues related to forest management as an ecosystem-level activity, all of them from the perspective of the other parts of a forest.

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