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Effect of Metal Contamination on the Genetic Diversity of *Deschampsia cespitosa* Populations from Northern Ontario: An Application of ISSR and Microsatellite Markers

Sophie Gervais and Kabwe Nkongolo
Laurentian University, Sudbury, Ontario, Canada

1. Introduction

Sudbury, Ontario (Canada) has been subjected to intense sulphur dioxide fumigation, soil contamination by aerial metal fallout and acid precipitation since the discovery of nickel and copper deposits in the late 1800s (Cox and Hutchinson, 1979; Bush and Barrett, 1993). The discovery of silver ore in Cobalt, Ontario (Canada) in the early 1900s, has been associated with arsenide and sulfarsenide mineral contamination of the soil in the region (Dumaresq, 1993). Although both these sites have been able to recover to some extent over the past 30 years due to emission reductions and remediation efforts (Dudka et al., 1995; Nkongolo et al., 2008), fact remains that such highly contaminated mine tailings often have metal concentrations that are increased to a level that are toxic for the majority of plants (Jiménez-Ambriz et al., 2007). The toxic metal pollutants, accompanied by the detrimental physical disturbances in the environment can influence plant survivorship, recruitment, reproductive success, mutation rates and migration, all of which affect the genetic diversity of the exposed populations (Deng et al., 2007). To date, hundreds of metal-tolerant genotypes have been identified from a wide variety of plant species surviving on such metal contaminated soils with many different life stories, pollinating systems and life-spans at an unexpectedly high rate (Mengoni et al., 2000; Wu et al., 1975). This type of rapid and widespread adaptation to metal pollution suggests that the evolution of metal tolerance is one of the major strategies for plant colonization of mining spoils (Deng et al., 2007).

Several investigations on the genetic diversity among metal tolerant populations relative to their non-metal tolerant counterparts have been carried out. Despite founder effect and selection, studies on *Silene paradoxa* (Mengoni et al., 2000) and *Agrostis stolonifera* (Wu et al., 1975) demonstrated that the genetic diversity of the contaminated population was the same as that of the uncontaminated populations. Other studies reported a high heterozygosity in tolerant plants such as European beech (Muller-Starck, 1985), Scots pine (Geburek et al., 1987), trembling aspen and red maple (Berrang et al., 1986). Contrary to these results, a reduction of genetic diversity has been found in other tolerant populations such as *Armeria maritime* (Vekemans and Lefèbvre, 1997).

In regards to *Deschampsia cespitosa*, two studies have examined the level of genetic diversity of populations growing on metal contaminated soils relative to those growing on uncontaminated soils. In their study, Bush and Barrett (1993) examined variation at 19 putative isozyme loci. Their population samples included individuals from 8 uncontaminated habitats, 5 from mine sites around Sudbury, Ontario and another 5 from Cobalt, Ontario. Results demonstrated lower levels of diversity in both Sudbury and Cobalt population relative to uncontaminated sites. In a subsequent study, Nkongolo et al. (2001) examined the metal contamination of the soil and associated genetic variation of *D. cespitosa* populations from Sudbury and Cobalt based on RAPD molecular markers. Results showed that the *D. cespitosa* plant has metal tolerant capabilities which allow it to survive and thrive on these mine tailings containing elevated levels of copper, nickel cobalt, aluminum, cobalt, nickel and arsenic. Although a high level of aneuploidy was observed, no link between genetic characteristics and metal contamination was made.

The methods used to infer the level of genetic diversity in these previous studies present certain disadvantages. Isozyme analysis reflects alterations in the DNA sequence through changes in amino acid composition. These changes will often alter the protein charge thereby producing a change in electrophoretic mobility which is useful in evaluating level of variation between individuals and populations on the basis of gene loci coding for specific enzymes (Hamrick, 1989). However, not all alteration in the DNA sequence will produce changes in amino acid composition therefore certain amino acid substitutions may also go undetected. Also, the limited number of isozymes (about 30) reflects only a small portion of the coding genome. The main weakness of RADP analysis is its poor reproducibility which can be caused by its sensitivity to reaction conditions such as the concentration of MgCl, *Taq* polymerase and dNTP (Qian et al., 2001). Since its introduction in 1994, the Inter-Simple Sequence Repeat (ISSR) random marker system has grown in popularity and has superseded RAPD method (Zietkiewicz et al., 1994). The ISSR marker system is based on the use of a 15-20 bp primers designed to be complimentary to microsatellite sequences found throughout the eukaryotic genome, therefore providing information at a number of different loci. Also, as a result of the longer lengths of ISSR primers in comparison to RAPD primers, the required annealing temperatures are higher and as such, non-specific binding is reduced and banding patterns have higher reproducibility (Qian et al. 2001).

Microsatellite markers have emerged as the genetic marker of choice over the last decade (Buschiazzo and Gemmell, 2006). These single-locus, hypervariable and abundant markers characterized by a Mendelian mode of inheritance, co-dominant nature, high reproducibility and easy application are a very powerful tool for assessing population genetic parameters and DNA profiling (Gaitán-Solís et al., 2002; Jones et al., 2001). Studies have reported that microsatellites are more variable than RFLPs or RAPDs and therefore have the potential to show polymorphism in species otherwise characterized by low levels of genetic diversity.

Although the information revealed by microsatellites regarding the genetic parameters of a population is highly informative, the development of these markers *de novo* is extremely expensive, labour intensive and time consuming (Saha et al., 2006). Several studies have examined the possibility of cross-species transferability of microsatellite primers pairs developed in closely related species and have reported the conservation of some loci across genera and sometimes even families (Saha et al., 2006). This method considerably reduces primer development costs while providing useful information for comparative linkage relationships between species.

The main objectives of the present study were to determine any association between metal content in the soil and the genetic variation in *D. cespitosa* populations growing in Northern Ontario using ISSR and microsatellite markers.

2. Materials and method

2.1 Sampling sites

Soil samples and leaves of *Deschampsia cespitosa* were collected from a total of nine sites located within three regions of Northern Ontario exhibiting various concentrations of metal contamination in the soil, including Sudbury, Cobalt and Manitoulin Island (Figure 1, Table 1). The sampling sites from the Sudbury region were located in Coniston, Copper Cliff, Falconbridge and Walden. The sampling sites from the Cobalt region were located on three different abandoned mine sites designated Cobalt-3, Cobalt-4 and Cobalt-5 (Figure 1). The two sampling sites from the Manitoulin Island region were located in Little Current and at the Mississagi Lighthouse. These nine sampling sites were the same as those previously characterized by Nkongolo et al. (2001) as having significant accumulation of several metals within the soil. Cobalt-4 was the most contaminated site whereas the two sampling sites from the Manitoulin Island region were the least contaminated, thus serving as control populations in this study. A total of 40 individuals were collected from each sampling area, representing approximately 5 to 20% of the entire plant population observed on site.

2.2 Metal analysis

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 ± 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 µg/g (0.5 µg/g), Arsenic 0.05 µg/g (0.5 µg/g), Cadmium 0.05 µg/g (0.5 µg/g), Cobalt 0.05 µg/g (0.5 µg/g), Copper 0.05 µg/g (0.5 µg/g), Iron 1.0 µg/g (10 µg/g), Lead 0.05 µg/g (0.5 µg/g), Magnesium 0.2 µg/g (2.0 µg/g), Manganese 0.05 µg/g (0.5 µg/g), Nickel 0.05 µg/g (0.5 µg/g) and Zinc 0.05 µg/g (0.5 µg/g). These MDLs reflect actual sample weights and dilutions; instrument detection limits were lower.

The data for the metal levels in soil and tissue samples were analyzed using SPSS 7.5 for Windows. All the data were transformed using a $\log_{10}$ transformation to achieve a normal distribution. Variance-ratio test was done which make certain assumptions about the underlying population distributions of the data on which they are used; for example that they are normal. If the assumptions of the parametric test were violated, nonparametric test was used in place of parametric test. 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).

2.4 ISSR analysis

All DNA samples were primed with each of the nine primers (ISSR 17898B, UBC 818, UBC 823, UBC 825, UBC 827, UBC 835, UBC 841, UBC 844, and UBC 849) (Mehes et al., 2007). 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 PCR products were loaded into 2% agarose gel in 1X Tris-Borate-EDTA (TBE) buffer. Gels were pre-stained with 4µl 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.

ISSR assays of each population were performed at least twice. Only reproducible amplified fragments were scored. For each sample, the presence or absence of fragments was recorded as 1 or 0, respectively and treated as a discrete character. Pairwise comparison of banding patterns was evaluated using RAPDistance, version 1.04 (Armstrong et al. 1994). The data were analyzed to generate Jaccard's similarity coefficients and genetic distances. These similarity coefficients were used to construct dendrograms, using neighbour-joining analysis (Saitou and Nei 1987). Analysis of molecular variance (AMOVA) was applied, to estimate variance components for ISSR phenotypes. Variations were partitioned among individuals (within regions) and between regions. Levels of significance were determined using nonparametric permutational methods with the Winamova program (Excoffier et al., 1992).

2.5 Microsatellite analysis

A total of 31 microsatellite primers, developed in several members of the *Poaceae* family (Table 2), were screened for cross-species consrvation in *Deschampsia cespitosa*. Of these, 5 were from *Elymus caninus* (Sun et al., 1999), 7 were from *Avena sativa* (Li et al., 2000), 3 were from *Triticum aestivum* (Röder et al., 1995) and 17 were developed in *Hordeum vulgare* (Liu et al., 1996; MacRitchie and Sun, 2004; Struss et al., 1998). The microsatellites primers used in this study were selected based on the phylogenetic relationship between the species of origin and *D. cespitosa*, the polymorphic index of the alleles within their respective species and, in some cases, previous reports of cross-species conservation of the microsatellite locus.

DNA amplification was performed following the procedure described by Mehes et al. (2010). Of the 31 microsatellite primer pairs screened, only those that successfully amplified a clear, reproducible, distinguishable band, demonstrated microsatellite characteristics and showed a certain degree of polymorphism were used in the study. The agarose gels were documented using the Bio-Rad ChemiDoc XRS system and analyzed with the Discovery Series Quantity One 1 D Analysis Software. Nine microliters of 3X loading buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol) was added to the

remainder of the 18 µl of the amplified products and 2.5 µl of 3X loading buffer was added to the mixture of 5 µl of water and 1.5 µl of 10 bp ladder (Invitrogen). The samples and the 10 bp ladder (Invitrogen) were denatured at 99ºC for 10 min and snap cooled for 2 min on ice prior to loading on denaturing gels. The PCR products were electrophoresed on a 0.4 mm denaturing 6% polyacrylamide gels containing 8 M Urea and 1X TBE buffer at constant power of 73 W, 2 100 V and 90 mA and were equilibrated to 55ºC (DNA Sequencing System, FisherBiotech, Fisher Scientific). The amplifications products were visualized with the Silver Staining Sequence DNA Sequencing System according to the manufacturers protocol (Promega Corporation). Resolved fragments were sized by The Quantity One and Genescan softwares.

Fig. 1. Map of Northern Ontario illustrating the three regions, Manitoulin Islands, Sudbury and Cobalt, where samples of *Deschampsia cespitosa* were collected for this study.

The presence and absence of alleles yielded by the microsatellite primer pairs were scored as 1 or 0, respectively in order to determine the polymorphism of each locus. Such designations were carried out with the Quantity One software by establishing the alleles of interest and comparing them to the 10 bp ladder which served as a marker system. The data was computed into the Popgene software, version 1.32 (Yeh et al., 1997) and used to determine the intra- and interpopulation genetic diversity parameters such as number of alleles per locus, mean number of alleles across loci, percentage of polymorphic loci and Shannon's information index. In order to determine whether the observed allelic proportions met Hardy-Weinberg expectations, the populations were tested using an exact test (Guo and Thompson, 1992) by the computer program GENEPOP version 1.2 (Raymond and Rousset,

1995). Hardy-Weinberg Equilibrium deviations were further tested with heterozygote deficiency and excess alternative hypotheses. Wright's *F* statistics including inbreeding coefficients and fixation index (Weir and Cockerham, 1984) as well as gene flow were determined using Popgene. The PowerMarker software, version 3.25 (Liu and Muse, 2005) was used to calculate the genetic distances between the populations based on the Cavalli-Sforza and Edwards Chord's Distance (1967). Finally, the relationship between the matrices based on microsatellite data and geographical location was calculated using the Pearson's correlation coefficient and the significance of the correlation was determined by the Mantel test (Mantel, 1967) using XLSTAT version 7.5 software package (www.xlstat.com).

Region	Site	Latitude	Longitude	Altitude
Manitoulin Island	Little Current	N°45°58.755′	W°81° 54.950′	180m
	Mississagi Lighthouse	N°45°53.493′	W°83° 13.552′	176m
Sudbury	Coniston	N°46°28.703′	W°80° 50.862′	252m
	Copper Cliff	N°46°30.246′	W°81° 02.819′	292m
	Falconbridge	N°46°34.619′	W°80° 48.701′	323m
	Walden	N°46°25.842′	W°81° 04.627′	260m
Cobalt	Cobalt-1	N°47°22.524′	W°79°41.008′	313m
	Cobalt-2	N°47°23.206′	W°79° 39.683′	302m
	Cobalt-3	N°47°21.725′	W°79° 38.861′	347m

Table 1. Geographic coordinates of the nine *D. cespitosa* sampling sites located throughout Northern Ontario.

Species of origin	Locus	Primer Sequence (5′→3′)	Repeat	Number of alleles	Tm (°C)	Expected size (bp)
Elymus caninus	ECGA22	gaaggtgactaggtccaac atagtctcggtcaggctc	$(CT)_{27}$	13	54	166
	ECGA125	tgcttccaacttgctca tgcatctgtgtgtccaca	$(AG)_{23}$	10	54	204
	ECGA126	gtcactagtggatcgtgcc gatttggtgtcgttctgatc	$(GA)_{15}$	9	54	186
	ECGA114	cttatatcttgtgggttatcat gatctgatacgtgacatctca	$(TC)_{15}$	8	54	129
	ECGA210	cgacaactagtggatcaaa gaagtactctcgagaagctt	$(GA)_{22}$	6	54	196
Avena sativa	AM1	ggatcctccagcctgttga ctcatccgtatgggcttta	$(AG)_{21}(CAGAG)_{6}$	5	46	204
	AM3	ctggtcatcctcgccgttca catttagccaggttgccaggtc	$(AG)_{35}$	5	51	280
	AM4	ggtaaggtttcgaagagcaaag gggctatatccatccctcac	$(AG)_{34}$	6	48	166
	AM14	gtggtgggcacggtatca tgggtggcgaagcgaatc	$(AC)_{21}$	4	48	133

	AM22	attgtatttgtagccccagttc aagagcgacccagttgtatg	$(AC)_{22}$	8	46	138
	AM30	tgaagatagccatgaggaac gtgcaaattgagtttcacg	$(GAA)_{14}$	6	43	203
	AM31	gcaaaggccatatggtgagaa cataggtttgccattcgtggt	$(GAA)_{23}$	6	47	186
Triticum aestivum	WMS2	ctgcaagcctgtgatcaact cattctcaaatgatcgaaca	$(CA)_{18}$	4	50	132
	WMS6	cgtatcacctcctagctaaactag agccttatcatgaccctacctt	$(GA)_{40}$	4	50	205
	WMS30	atcttagcatagaagggagtggg ttctgcaccctgcctgat	$(AT)_{19}(GT)_{15}$	7	60	206
Hordeum vulgare	GMS005	actacgtccagtcgtttcc tgaacaccacgggttcatc	$(GT)_{19}T(CT)_2(GT)_{13}$	10	55	170
	GMS006	tgaccagtaggggcagtttc ttcttctccctccccac	$(GA)_2ATA(GA)_{19}$	14	55	154
	GMS046	atgtatttatcacccacccagc aaggcattagaaccggcac	$(GA)_{13}$	15	60	156
	GMS056	gagaaacgcagctgtggc gtcaccgaggccttcctc	$(GA)_{11}$	13	60	137
	GMS114	aaccagtgggttttaacccc tgccaccacatgcatacac	$(GT)_{11}$	11	55	152
	HVM3	acaccttcccaggacaatccattg agcacgcagagcaccgaaaaagtc	$(AT)_{29}$	3*	55	188
	HVM5	aacgacgtcgccacacac aggaacgaagggagtattaagcag	$(GT)_6(AT)_{16}$	4**	55	202
	HVM7	atgtagcggaaaaaataccatcat cctagctagttcgtgagctacctg	$(AT)_7$	2**	55	174
	HVM20	ctccacgaatctctgcacaa caccgcctcctctttcac	$(GA)_{19}$	5*	T1	151
Hordeum vulgare	HVM27	ggtcggttcccggtagtg tcctgatccagagccacc	$(GA)_{14}$	+	T1	192
	HVM40	cgattccctttcccac attctccgccgtccactc	$(GA)_6(GT)_4(GA)_7$	3**	T1	160
	HVM44	aaatctcaggttcgtgggca ccacggagaccacctcactt	$(GA)_8$	4*	T1	114
	HVM51	tctaaattaccttcccagcca aagcagacatgtaggaggtca	$(GA)_3(GGGA)_3(GA)_8$	4*	T1	151
	HVM60	caatgatgcggtgaactttg cctcggatctatgggcctt	$(AG)_{11}(GA)_{14}$	3*	T1	115
	HVM65	agacatccaaaaaatgaacca tggtaacttgtcccccaaag	$(GA)_{10}$	5*	T1	129
	HVM 68	aggaccggatgttcataacg caaatcttccagcgaggct	$(GA)_{22}$	+	T1	204

*: Number of alleles expected in the *Avena* species
**: Number of alleles expected in the *Elymus* species
+: Polymorphic in four crossed mapping populations of barley
T1: Touchdown PCR with 18 cycles 64°C-55°C and 30 cycles at 55°C (Liu et al., 1996)

Table 2. List of the microsatellite primer pairs screened for transferability in *D. cespitosa.*

3. Results

3.1 Heavy metal analysis

Recovery and precision for all elements in reference soil samples were within acceptable range. The estimate levels of metal content in different sites are illustrated in Figure 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 soil in Coniston while cadmium, cobalt, copper, lead, zinc and to some extent nickel, were found in high concentration in Cobalt sites. The Cobalt 4 site is by far the most contaminated of all the sites (Fig. 2). For example, the average mean level of zinc at Cobalt 4 is at least twenty-one fold than that of Sites from Sudbury. Cobalt-4 was also among the sites with the highest accumulation of copper, lead, and nickel.

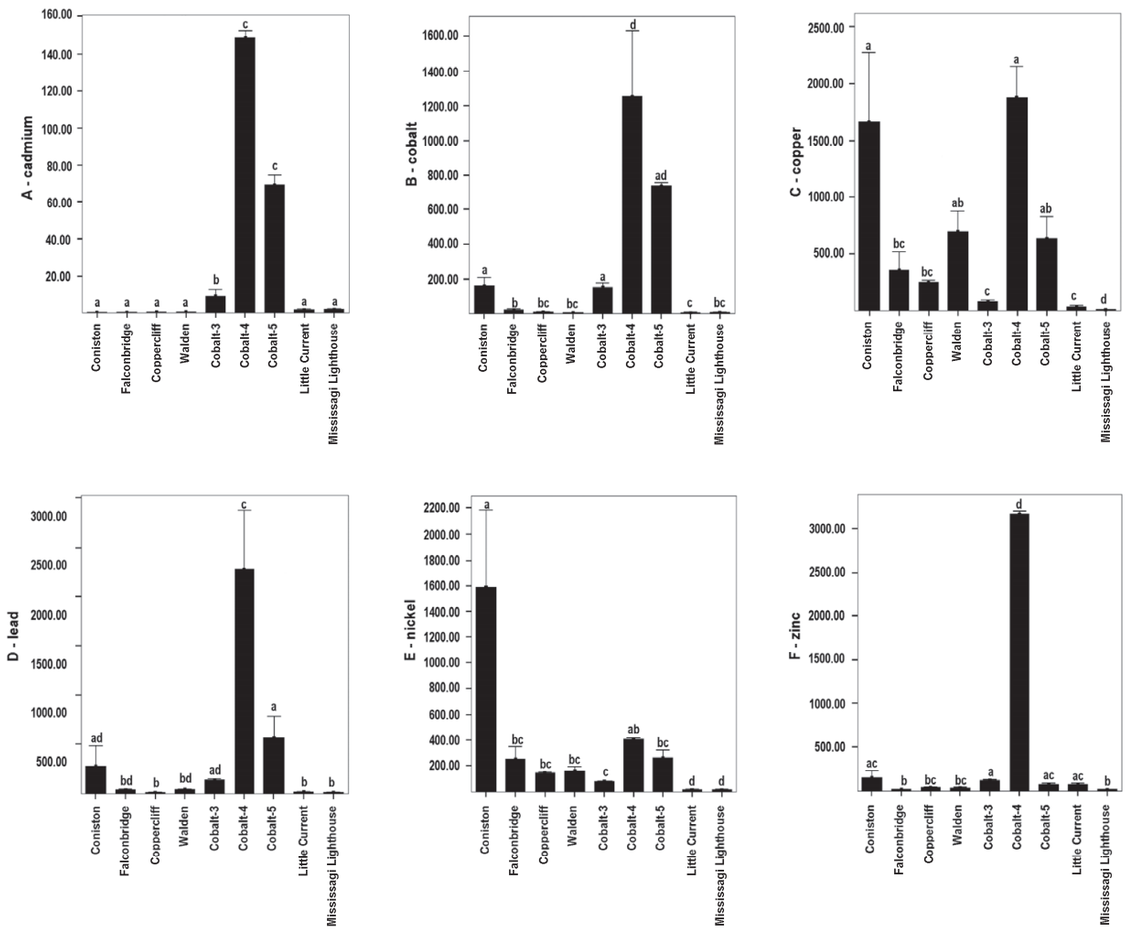

Fig. 2. Cadmium, cobalt, copper, lead, nickel, and zinc concentrations in soil samples collected from the Sudbury, Cobalt, and Manitoulin Island regions. Means with common notations are not significantly different as indicated by Tukey HSD analysis ($p > 0.05$).

Unlike the Sudbury sites, which are located near smelters, Cobalt-4 site is located in the concrete remains of the foundation of an abandoned mine site. This could explain the extraordinarily high heavy metal accumulation in that area. This particular site also does not appear to have been detoxified or rehabilitated like the Sudbury sites have been. Cobalt 3 showed relatively lower level of heavy metal accumulation than Cobalt 4 and 5 and was typically in the middle of the spectrum of contaminated sites. The two control sites, Little Current and Mississagi Lighthouse were always among the least contaminated for the metals analysis. Three of the Sudbury sites, Falconbridge, Coppercliff, and Walden were also consistently among the least contaminated. Coniston was found to be on average significantly more contaminated than the other Sudbury sites (Fig. 2).

3.2 ISSR analysis

Five of the nine primers screened (Table 3) produced good amplification products ranging from 160 bp to 1300 bp. They include 17898B, UBC 818, UBC 827, UBC835 and UBC 841 had good amplifications products (Figure 8). The level of polymorphic loci among populations was 63 %. This value was much lower than the polymorphisms level (90%) reported in RAPD analysis of the samples from the same sites (Nkongolo et al., 2001). The polymorphism within each population varied between 44% observed in Cobalt-5 to 92% in Falconbridge (Table 4). The overall values for the three regions were compared. The highest level of polymorphism was observed in samples from the Sudbury region (Coniston, Falconbridge, Copper Cliff, Walden) with an average of 74 %, followed by the Manitoulin Island region (Little Current and Mississagi Lighthouse) with an average of 69%. The lowest level of polymorphic loci was observed in samples originating from the Cobalt region (Cobalt-3, Cobalt-4, and Cobalt-5) with a mean value of 46%. Interestingly, the Cobalt region was also the site which exhibited the highest accumulation of metals in the soil.

Primer Identification	Nucleotide sequence (5′→ 3′)	Amplification	Fragment size range (bp)
ISSR 17898B	CACACACACACAGT	Good	200-1300
UBC 818	CACACACACACACACAG	Good	400-1100
UBC 823	TCTCTCTCTCTCTCTCC	Absent	-----
UBC 825	ACACACACACACACACT	Fair	650-1000
UBC 827	ACACACACACACACACG	Good	400-1000
UBC 835	AGAGAGAGAGAGAGAGYC	Good	200-1000
UBC 841	GAAGGAGAGAGAGAGAYC	Good	160-850
UBC 844	CTCTCTCTCTCTCTCTRC	Fair	500-650
UBC 849	GTGTGTGTGTGTGTGTYA	Fair	300-500

Table 3. Nucleotide sequences of the primers used to produce ISSR profiles by amplification of genomic DNA from nine populations of *Deschampsia cespitosa*.

Region	Site	Total number of bands	Number of polymorphic bands	Polymorphic bands (%)	Mean polymorphism per region (%)
Sudbury	Coniston	75	54	72	74 (Sudbury)
	Falconbridge	72	66	92	
	Copper Cliff	60	40	67	
	Walden	72	47	65	
Cobalt	Cobalt-3	75	36	48	46 (Cobalt)
	Cobalt-4	69	32	46	
	Cobalt-5	81	36	44	
Manitoulin Island	Little Current	66	46	70	69 (Manitoulin)
	Mississagi Lighthouse	87	59	68	

Table 4. Levels of polymorphisms within *Deschampsia cespitosa* populations from Northern Ontario generated with ISSR primers.

The between- populations variance contributed 13.6 % of the total variance while the within-population variance accounted for 71.2%. Using a nonparametric test, we found that between-population differences were significant (Table 5). No single locus appears to be specific to contaminated sites.

Source of variation	df	MS	Variance Component	% Total	P
Among regions	2	1.224	0.034	13.60	0.001
Populations within region	6	0.562	0.053	15.23	0.001
Individuals within populations	63	0.277	0.281	71.17	0.001

Table 5. Analysis of molecular variance (AMOVA) for ISSR variation among *Deschampsia cespitosa* populations from several locations in Northern Ontario.

In general, the genetic distances among the nine *D. cespitosa* populations from Northern Ontario values varied from 0.059 to 0.488. The scale utilized denotes a 0 for identical populations to a 1 for populations that are different for all criteria (Table 6). The closest genetic distance value (0.06) was observed between the populations from Cobalt-5 and Cobalt-3. The two most genetically distant populations were Cobalt-5 and Falconbridge (0.49). The genetic distance data also showed that the four *D. cespitosa* populations from the Sudbury area (Coniston, Falconbridge, Copper Cliff and Walden) were closely related. These data also revealed that the *D. cespitosa* populations from the Cobalt region (Cobalt-3, Cobalt-4, and Cobalt-5) were closely related to the *D. cespitosa* population from Little Current (Table 6, Fig. 3). The results were supported by the cluster analysis that illustrated that the four *D. cespitosa* populations from the Sudbury region clustered together and the three Cobalt populations clustered with the populations from Little Current and Mississagi Lighthouse (Fig. 3). Overall, the molecular analysis using ISSR markers showed that the *D. cespitosa* populations from Northern Ontario are different but closely related.

	1	2	3	4	5	6	7	8	9
1	0.000	0.147	0.189	0.1944	0.366	0.391	0.375	0.308	0.400
2		0.000	0.222	0.278	0.476	0.452	0.488**	0.425	0.395
3			0.000	0.114	0.341	0.317	0.390	0.325	0.333
4				0.000	0.308	0.282	0.359	0.289	0.297
5					0.000	0.184	0.059*	0.189	0.333
6						0.000	0.237	0.211	0.350
7							0.000	0.194	0.385
8								0.000	0.222
9									0.000

1 represents D. cespitosa population from Coniston; 2: D. cespitosa population from Falconbridge; 3: D. cespitosa population from Copper Cliff; 4: D. cespitosa population from Walden; 5: D. cespitosa population from Cobalt-3; 6: D. cespitosa population from Cobalt-4; 7: D. cespitosa population from Cobalt-5; 8: D. cespitosa population from Little Current; 9: D. cespitosa population from Mississagi Lighthouse.

Table 6. Distance matrix generated using the neighbor-joining analysis from *Deschampsia cespitosa* ISSR data (RAPDistance version 1.04).

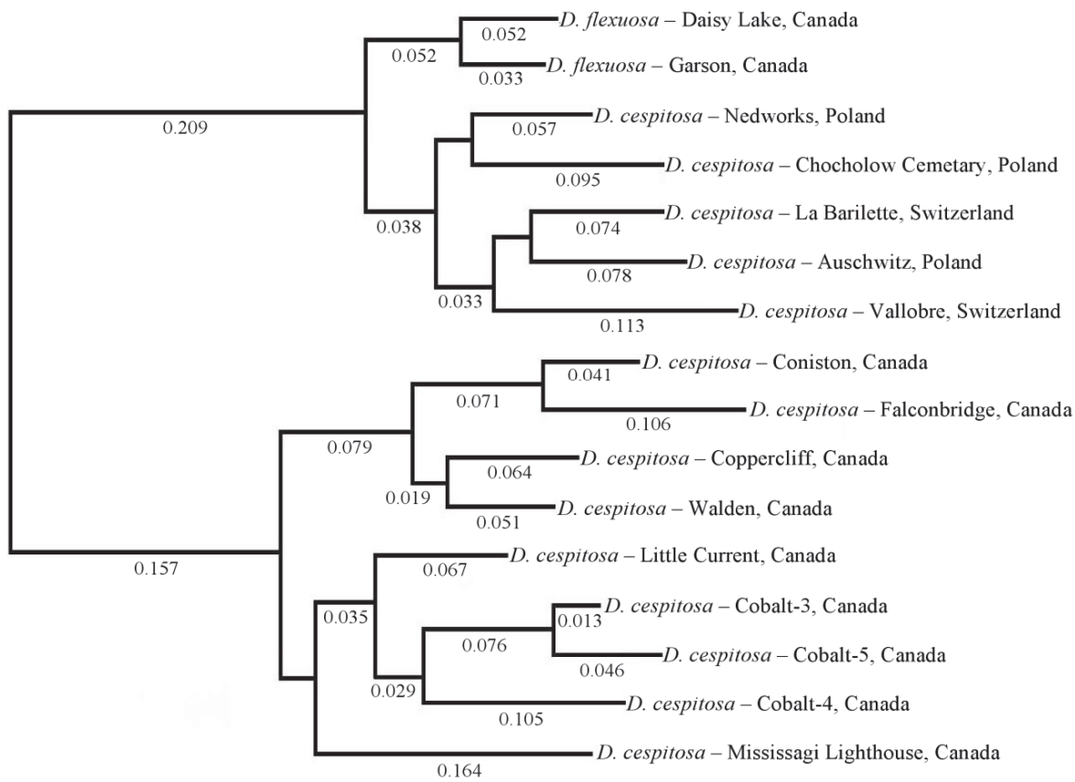

Fig. 3. Dendrogram of the genetic relationships between the nine *D. cespitosa* populations from Northern Ontario using data generated from the Jaccard similarity matrix from ISSR profiles. The values below the branches indicate the patristic distances based on the neighbor-joining analysis. The *D. cespitosa* populations from Europe and the *D. flexuosa* populations (Northern Ontario) were used as outgroups.

3.3 Nuclear microsatellite analysis

Only primer pairs HVM3, HVM5, HVM20, HVM65 and WMS6 successfully amplified a clear, reproducible, distinguishable band within an acceptable range of the expected size while demonstrating polymorphism. This represents only 14% of the microsatellite primer pairs screened for transferability within *D. cespitosa*. All alleles detected were scored according to the guidelines previously outlined. Every possible microsatellite loci pair, in every population, was surveyed for association following the null hypothesis that genotypes at one locus are independent from genotypes at the other locus. Based on the results, no evidence of linkage disequilibrium between the microsatellite loci used in this study was observed. Only data related to genetic diversity are discussed in the present report.

3.3.1 Genetic diversity

Genetic diversity within each individual, population, region and locus was assessed using standard descriptive statistics. The five polymorphic microsatellite markers detected a total of 40 alleles. The mean number of alleles per locus across populations was 2.1, 2, 7.3, 4.6 and 2.6 for locus HVM 3, HVM 5, HVM 20, HVM 65 and WMS 6 respectively. The mean number of alleles per populations across loci was 3 for Little Current, 3.2 for Mississagi Lighthouse and Walden, 3.6 for Falconbridge and Copper Cliff, 3.8 for Coniston and Cobalt-5, 4 for Cobalt-4 and 5.2 for Cobalt-3. At the population level, the observed mean heterozygosity (H_O) and the expected mean heterozygosity (H_E) ranged from 0.413 and 0.48 in the Mississagi Lighthouse population to 0.65 and 0.76 in the Cobalt-3 population. At the regional level, the H_O and the H_E ranged from 0.40 and 0.46 in the Cobalt region to 0.34 and 0.42 in the Manitoulin Island region. Finally, the H_O and the H_E observed by individual loci ranged from 0.28 and 0.44 for HVM3 to 0.85 and 0.99 for HVM20. Shannon's Information Index (i) was also calculated as an additional measure of genetic diversity. Values varied from 0.54 at the HVM5 locus to 2.58 at the HVM20 locus with a mean of 1.417 (Table 7).

Inbreeding is defined as the non-random uniting of gametes which results in a decrease of heterozygotes. The level of inbreeding within a population is determined by the inbreeding coefficient, F_{IS}, where -1 (all individuals heterozygous) ≤ F_{IS} ≤ 1 (no observed heterozygotes). The inbreeding coefficients (F_{IS}) were determined for each population per loci based on the null hypothesis of no inbreeding represented as F_{IS} = -1. All nine populations exhibited a negative F_{IS} value at loci HVM3, HVM5 and HVM20 indicating an excess of heterozygotes. Only Walden and Cobalt-3 populations presented negative F_{IS} values at locus HVM65. Finally, six of the nine populations exhibited negative F_{IS} values at the WMS6 locus. As a result, the overall inbreeding coefficient for *D. cespitosa* populations were -0.18, -0.08, -0.07, -0.35, -0.19, -0.07, -0.12, -0.21 and -0.17 for Coniston, Falconbridge, Copper Cliff, Walden, Cobalt-3, Cobalt-4, Cobalt-5, Little Current and Mississagi Lighthouse, respectively.

The fixation index (F_{ST}) is a measure of the extent of genetic differentiation among populations due to genetic drift. Values can range from 0.0, indicating no differentiation, to 1.0, indicating complete differentiation. However, because the observed maximum is usually much less than 1.0, a value between 0.0 and 0.05 is considered as little genetic differentiation, 0.05 and 0.15 as moderate genetic differentiation, 0.15 and 0.25 as great

genetic differentiation and values above 0.25 as very great genetic differentiation. The F_{ST} values were 0.079 for HVM5 and 0.088 for HVM20, indicating moderate genetic differentiation. Locus HVM65 and locus WMS6 demonstrated great genetic differentiation with 0.22 and 0.22 F_{ST} values, respectively (Table 8). Finally, HVM3 locus represented a very great deal of genetic differentiation with an F_{ST} value of 0.311 (Table 8).

The F_{ST} values were subsequently used to estimate the level of gene flow ($N_{m)}$ for each locus according to Nei (1987), where $N_m = 0.25(1-F_{ST})/F_{ST}$. The mean level of gene flow was 0.933 with individual N_m values ranging from 0.5424 for the HVM65 locus to 2.347 for the HVM5 locus (Table 8). Genetic distance coefficients were calculated according to the Cavalli-Sforza and Edwards chord distance (D_C). This particular scale ranges from 0, indicating no genetic difference to 1, indicating differences at all conditions criteria. The genetic distance coefficients varied between 0.57 between the Coniston and Cobalt-3 populations and 0.18 between the Falconbridge and Copper Cliff populations (Table 9). Based on these values, an un-rooted Neighbour-Joining phylogenetic tree was constructed with 101-bootstrap. The resulting tree illustrates three major clades (Fig. 4). The first is composed of the Little Current population, the second comprises all four Sudbury region populations and the Mississagi Lighthouse population and the third includes all three Cobalt region populations. Within the second clade, the Mississagi Lighthouse population is the most distantly related, while the Falconbridge and Copper Cliff populations and the Walden and Coniston populations form clusters. Within the third clade, the Cobalt-5 population appears to be the most distantly related.

The Mantel test results reveal a significant correlation between the two distance matrices ($r = 0.514$, $p = 0.01$) suggesting a congruence between the genetic distance generated from microsatellite data and the geographical distance between populations.

Population	N_A*	H_O*	H_E*	I*	F_{IS}*
Coniston	3.8	0.680	0.582	1.0572	-0.179
Falconbridge	3.6	0.580	0.538	0.9689	-0.083
Copper Cliff	3.6	0.500	0.468	0.8506	-0.071
Walden	3.2	0.700	0.529	0.8887	-0.346
Cobalt-3	5.2	0.760	0.645	1.2141	-0.190
Cobalt-4	4.0	0.680	0.640	1.1305	-0.066
Cobalt-5	3.8	0.600	0.540	0.9720	-0.118
Little Current	3.0	0.620	0.518	0.8401	-0.210
Mississagi Lighthouse	3.2	0.480	0.413	0.7379	-0.174
Mean	3.71	0.542	0.622	0.9622	-0.142
Standard Error	± 0.05	±0.025	±0.031	±	± 0.01

*Genetic diversity descriptive statistics. NA, mean number of alleles across all loci; HO, observed heterozygosity; HE, expected heterozygosity; I, Shannon's information index; FIS, inbreeding coefficient.

Table 7. Genetic diversity estimates for Deschampsia cespitosa populations.

Locus	A	Obs. Gen	N_E	H_O	H_E	I	PIC	F_{ST}	N_m
HVM3	6	5	2.1	0.444	0.280	0.750	0.350	0.311	
HVM5	2	2	2	0.467	0.340	0.543	0.294	0.079	2.347
HVM20	17	45	7.3	0.989	0.851	2.584	0.905	0.088	
HVM65	11	24	4.6	0.478	0.666	2.056	0.824	0.219	0.542
WMS6	4	8	2.6	0.722	0.570	1.287	0.648	0.224	
Mean			3.72	0.620	0.542	1.444	0.604	0.184	0.933
Standard Error			±1.011	±0.105	±0.105	±0.865	±0.19	±0.09	

*Genetic diversity descriptive statistics. A, total number of alleles; Obs. Gen, total number of observed genotypes; NE, effective number of alleles; HO, observed heterozygosity; HE, expected heterozygosity; I, Shannon's information index; PIC, polymorphic information content; FST, fixation index (Wright 1978); Nm, gene flow estimate as per FST=0.25(1-FST)/FST (Nei 1987).

Table 8**.** Genetic diversity parameters for the five microsatellite primer pairs of *Deschampsia cespitosa*.

	1	2	3	4	5	6	7	8	9
1	0.0000	0.2524	0.2628	0.1836*	0.5657**	0.5145	0.5210	0.4696	0.4001
2		0.0000	0.1996	0.2630	0.5183	0.4907	0.5582	0.4720	0.4639
3			0.0000	0.3141	0.5338	0.5007	0.5486	0.4413	0.4385
4				0.0000	0.5557	0.4787	0.5227	0.4960	0.4285
5					0.0000	0.3463	0.4672	0.5261	0.5090
6						0.0000	0.4286	0.4909	0.5004
7							0.0000	0.4387	0.4053
8								0.0000	0.4292
9									0.0000

1 represents D. cespitosa population from Coniston; 2 D. cespitosa population from Falconbridge; 3 D. cespitosa population from Copper Cliff; 4 D. cespitosa population from Walden; 5 D. cespitosa population from Cobalt-3; 6 D. cespitosa population from Cobalt-4; 7 D. cespitosa population from Cobalt-5; 8 D. cespitosa population from Little Current; 9 D. cespitosa population from Mississagi Lighthouse. * Most genetically similar populations (i.e. Coniston and Walden). **Most genetically different populations (i.e. Coniston and Cobalt-3).

Table 9. Cavalli-Sforza and Edward's chord's distance matrix (1967) generated from microsatellite data used in neighbor-joining analysis of *Deschampsia cespitosa* populations.

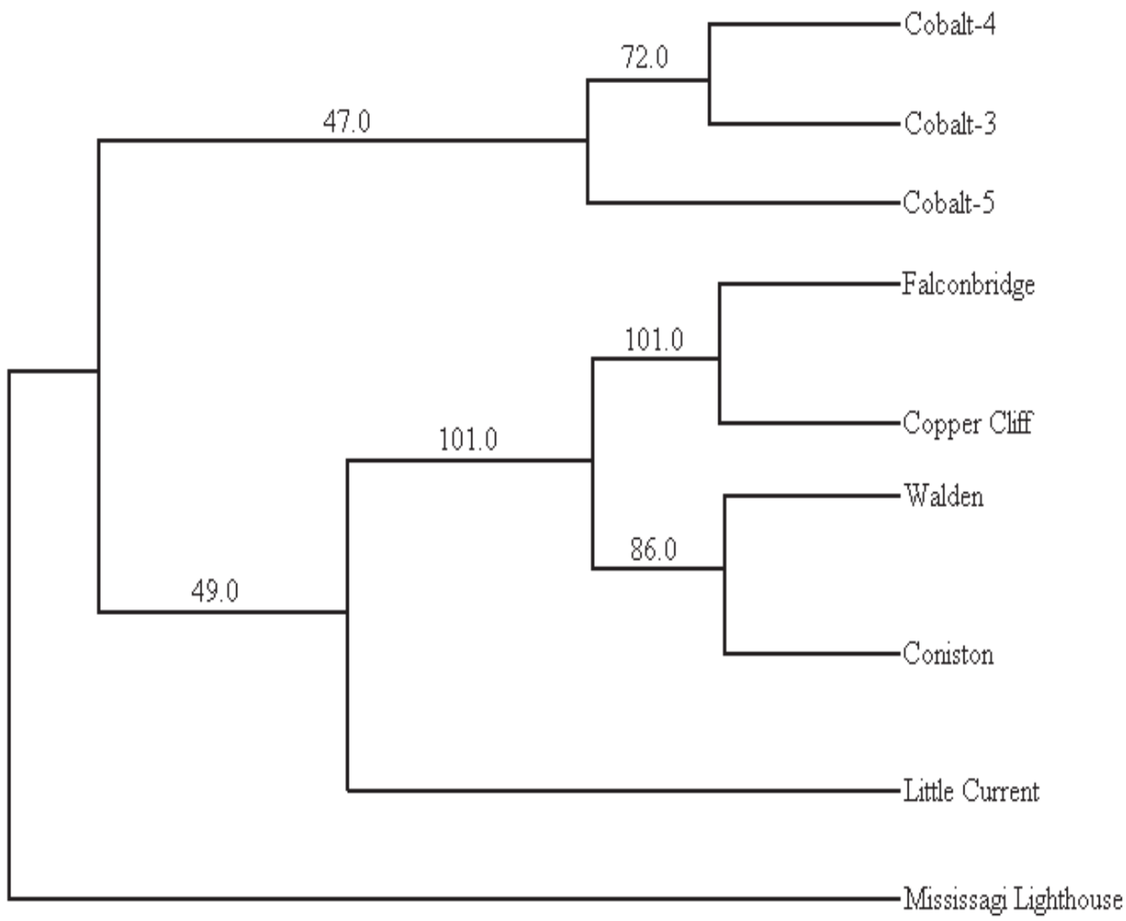

Fig. 4. Dendrogram of the genetic relationship between the nine populations of *Deschampsia cespitosa* from Northern Ontario using the data generated from Chord's distance (Cavalli-Sforza and Edwards, 1967) based on the microsatellite profiles. This is an un-rooted tree based on neighbor-joining (NJ) analysis constructed with 101-bootstrap.

4. Discussion

4.1 ISSR analysis

In general, the efficiency of a molecular marker technique depends on the amount of polymorphism it can detect among the set of accessions investigated. In the present study, the level of polymorphism detected with the ISSR system was lower than that observed with the RAPD method. Similar results were obtained by Fang and Roose (1997) who showed that RAPD PCR had identified a higher level of variation in *Citrus spp.* than ISSRs. Other studies conducted by Nagoaka and Ogihara (1997), Nkongolo et al. (2005), Raina et al. (2001) and Qian et al. (2001) have shown that ISSRs reveal a higher level of variation than RAPD markers in other plant species. Technically, RAPD and ISSR markers are different systems targeting different areas of the genome. RAPD markers cover the entire genome, revealing length polymorphism in coding or non-coding regions as well as repeated or single-copy sequences (Williams et al. 1993). Unlike RAPD products, the origin of the amplification products in ISSR-PCR is known to be from the sequences

between two simple-sequence repeat primer sites where length variation does not necessarily reflect simple-sequence length polymorphism (Zietkiewicz et al. 1994). The level of variation detected with each system greatly depends on the primers used, thus making comparisons regarding levels of polymorphisms generated with RAPD and ISSR primers rather inconsistent.

The ISSR analysis revealed great variation in regards to the genetic relatedness of the samples analyzed. In general, the genetic distance values revealed that *D. cespitosa* from the nine Northern Ontario sites are genetically close. The genetic distance values between Cobalt-3 and Cobalt-5 indicate that these two populations are quite genetically similar. This suggests that these sites were likely seeded with the same genetic materials. The relative small genetic distance values among Sudbury populations and their clustering on the dendrogram are consistent with the previous RAPD data (Nkongolo et al., 2001). These findings also corroborate with the speculations from several ecologists that these populations might be the result of a single colonization event (Winterhalder, 2002; Personal communication). In general, the genetic similarity between the nine *D. cespitosa* populations from Northern Ontario may suggest that these populations could have originated from a common source. Furthermore, based on the genetic distance data, the theory of Cobalt and Little Current populations as the source of *D. cespitosa* which colonized the Sudbury area around 1972 can not be rejected.

Previous genetic analysis of these populations aiming at establishing relationships among these nine sites using RAPD markers was inconclusive (Nkongolo et al., 2001). Also, the study conducted by Bush and Barrett (1993) using isozyme markers indicated that the Sudbury and Cobalt samples showed enough variation to reject the theory of Cobalt *D. cespitosa* colonizing Sudbury. Although isozymes and ISSRs allow the analysis of genetic variability in plant species, fundamental differences exist between these two methods. Isozyme analysis reflects alterations in the DNA sequence of coding regions in the genome leading to changes in amino acid composition which can go undetected (Hamrick 1989). ISSRs target microsatellites sequences located throughout the entire eukaryotic genome, most of which are selectively neutral areas. These areas are known to evolve rapidly and as such, have been deemed good tools for any study in genetic diversity in many organisms (Blair *et al.*, 1999).

Attempts were made in the present study to use environmental conditions for appropriately interpreting genetic information. The effects of novel and toxic environments have been examined in considerable detail in the study of life history evolution. There are theoretical reasons for expecting the genetic variance of a life history character to increase when the population is challenged with a novel environment, an expectation that has been upheld empirically by numerous studies (Service and Rose, 1985; Holloway et al., 1990). If metal tolerance is controlled by many genes as suggested by Von Frenkell-Insam and Hutchinson (1993) and McNair (1993), it is very likely that allelic frequency in an out-crossing and perennial species like *D. cespitosa* will be maintained over time resulting in a neutral genetic variation. The high level of genetic variability within *D. cespitosa* populations from the Sudbury region could be ascribed in part to these conditions.

If the toxic stress continues at a sub-lethal level for many generations, resistance could develop, resulting in a decrease in genetic variation through selection. This might be the case of *D. cespitosa* populations from Cobalt where the high accumulation of heavy metal for several years appears to have significant impacts on the genetic structure of the *D.*

cespitosa populations in that region by processes which are assumed to have selective effects. 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, 1979). 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). Selection of metal-resistant genotypes has been demonstrated to occur rapidly, within one or two generations, in populations that contain the necessary genetic information (Wu et al. 1975). These authors identified two factors that may affect the plant's ability to tolerate metals; the intensity of the contamination and the amount of time the population has been exposed to the toxic levels. The populations of *D. cespitosa* in Cobalt have been there for a much greater amount of time than the populations in Sudbury and the Cobalt soil is more contaminated than the Sudbury soil. This has resulted in a decrease and possibly a loss of alleles at some loci and many rare alleles that has lead ultimately to a lower genetic diversity in *D. cespitosa* populations from the Cobalt region. Evidence of a loss of genetic diversity at the population level caused by pollution has been demonstrated in other species (Lopes et al., 2004; Van Straalen and Timmermans, 2002). The low level of genetic variation in samples from *D. cespitosa* population from Cobalt-3 could be explained by the fact that this abandoned mine waste site that was likely as contaminated as Cobalt-4 and Cobalt-5 sites has been covered with a clay cap. This fresh clay that was brought in was not as contaminated as the surrounding area.

There is also a slight possibility that the small level of polymorphic loci detected in Cobalt samples could be caused by a founder effect. This is a form of genetic bottleneck occurring where new populations are established by a small number of individuals, or by a group of individuals whose genetic variation is not representative of the parent population. However, the possibility of a founder effect occurring only in Cobalt populations and not in Little Current where the *D. cespitosa* population has been isolated for several generations is quite small (Peter Beckett, 2006, personal communication). It should also be pointed out that *D. cespitosa* is an out-crossing species which produces a lot of seeds every year. These characteristics alone usually negate possible founder effects in many species (Hedrick et al., 1976). Muller et al. (2004) also indicated that high frequency of heavy metal tolerance in natural populations can reverse the effects of an initial genetic bottleneck.

4.2 Microsatellite analysis

The various application of microsatellite markers are the direct result of their hypervariability, co-dominant nature, abundance throughout the genome and reproducibility. Their primary disadvantage lies is their time consuming and expensive *de novo* development and, as such, has been restricted to a few agriculturally important crops**.** In light of this, a growing number of studies have examined the ability of microsatellite primer pairs to amplify across closely related species. In this study, we were able to identify several polymorphic microsatellite loci in *Deschampsia cespitosa* via SSR primer pairs developed in *Hordeum vulgare* (barley) and *Triticum aetivum* (wheat). The five microsatellite primer pairs, namely HVM3, HVM5, HVM20, HVM65 and WMS6 were originally selected because of their high polymorphic index and documented conservation across species. Primers HVM3, HVM20 and HVM65, originally developed in barley showed conservation

in *Avena* species with polymorphic information content (PIC) of 0.44, 0.77 and 0.72, respectively. In addition, a PIC of 0.15 for HVM3 and 0.50 for HVM20 was reported in oat cultivars (Li et al., 2000). The HVM5 primer originally developed from barley, showed conservation while displaying polymorphism in *Elymus* and *Pseudoroegneria* species (MacRitchie and Sun, 2004). WMS6, originally developed in wheat, had been documented as conserved and polymorphic in both barley and rye (Röder et al., 1995). In *Deschampsia cespitosa*, these primers ranged from having 2 alleles at the HVM5 locus with a PIC of 0.29 to 17 alleles at the HVM20 with PIC values of 0.91. Our findings are in accordance with other studies conducted by Gaitán-Solís et al. (2002), and Saha et al. (2004), which all report the occurrence of cross-species microsatellite primer pair transferability at an elevated rate. Such success depends heavily on the conservation of priming sites within the flanking regions and as such the evolutionary relatedness of the species sampled. Particular to the Poaceae family, believed to have radiated about 60 million years ago, genetic mapping has revealed remarkable conservation of gene content and gene order. Studies have shown that the linear organization of genes in some nine different genomes varying in chromosome number, from 5 to 12 and nuclear DNA amount, from 400 to 6000Mb, can be described in terms of only 25 rice linkage blocks (Gale and Devos, 1998). We also report shorter SSRs at HVM3, HVM5 and HVM 20 loci in *D. cespitosa* than in the species of origin with a difference in size of 36bp, 33bp and 5bp respectively. HVM65 has an allelic range of 196-220 in *D.cespitosa* whereas its size in barley is noted as 129 bp, a difference of 63 bp. Previous studies have shown that mutations at microsatellite loci are not solely restricted to the hypervariable region and can occur in the flanking regions at nonnegligeable rate, both of which can contribute to variations or lack thereof in allele size (Chapuis and Estoup, 2006). As such, inferring complete sequence homology based solely on the presence of amplification product is premature.

4.2.1 Genetic diversity

Mine tailings are typically a difficult medium for plant establishment and growth as these sites often contain elevated levels of metals, low nutrients and organic matter as well as being subjected to wind and water erosion. Some plant species and/or adapted populations have successfully colonized these toxic environments, however such inhospitable conditions often leave these areas with only scattered patches of vegetation (Mining in the Yukon). *Deschampsia cespitosa* has shown a remarkable ability to colonize and dominate such plots of land with great success having naturally colonized over 1000s of hectares of barren lands around Sudbury, following the constructions of the Super Stack in 1972. As a direct result of the mining activity in both regions, Cobalt and Sudbury present extremely hostile environments that are believed to have imposed strong selection pressures on colonizers resulting in reductions in genetic diversity. Detailed analysis of our nine populations with microsatellite markers reveals the observed mean heterozygosity (Ho) and the expected mean heterozygosity (He) ranged from 0.413 and 0.48 in the Mississagi Lighthouse population to 0.645 and 0.76 in the Cobalt-3 region. In addition, genetic diversity measures based on Nei's and Shannon's index demonstrated a similar patter with values ranging from 0.39 and 0.84 in Mississagi Lighthouse to 0.61 and 1.21 in Cobalt-3. As far as soil analysis, the Cobalt-4 site was shown to be the most contaminated of all the sites with significantly higher levels of arsenic, lead, zinc, cadmium and cobalt whereas the Mississagi Lighthouse and Little Current consistently

grouped as the sites with the significantly least amount of metals. Cobalt-3 typically placed in the middle of the spectrum among contaminated sites. Therefore, based on our microsatellite data, the level of genetic diversity at the population level does not decrease in terms of increased metal contamination. These findings are in conjunction with the results reported by Bush and Barrett (1993) on isozyme diversity that indicate that mine populations were no less polymorphic than uncontaminated populations. The retention of such elevated levels of genetic diversity within these mining populations can be attributed to number of selective, reproductive and demographic factors. As described by Bourret et al. (2007) if tolerance to the adverse environmental condition increases as a function of individual heterozygosity and/or if the contaminant is a mutagen, genetic variation within the affected population will remain elevated and may increase. Also, this species is a wind-pollinated outbreeder and, as a result, founders from such outbreeding populations are likely to heterozygous at many loci. In turn, this enhances the gene pool of small, founding populations by increasing the probability that at least the common alleles in the source population are represented in the new population (Bush and Barrett, 1993). Examination of the Hardy-Weinberg equilibrium across the nine populations revealed only two deviating populations, Walden and Cobalt-3 which were identified as having a significant heterozygote excess with values of 0.0021 and 0.0060 ($p<0.05$), respectively. Further analysis revealed inbreeding coefficients (F_{IS}) ranging from -0.346 in Walden to -0.19 in Cobalt-3 to -0.066 in Cobalt-4. These presences of these negative values across all nine populations imply a substantial amount of outbreeding, which as discussed earlier is in agreement with the reproductive pattern of the species. These findings also explain the occurrence of such highly heterozygote saturated populations because as stated above, outbreeders are more likely to be heterozygous at many loci (Bush and Barrett, 1993). Finally, the degree of differentiation among population (F_{ST}) was measured and was found to vary between moderate genetic differentiations with a value of 0.096 at locus HVM5 to very high with a value of 0.298 at locus HVM3. The mean degree of population differentiation was 0.194 in the *Deschampsia* population analyzed, indicating that 19.4% of the total genetic diversity is attributed to differences among populations.

4.2.2 Gene flow

Gene flow was examined to give an estimate of the average migration between all the populations studied per generation. The mean level of gene flow (N_M) in *Deschampsia cespitosa* based on our microsatellite analysis was 1.04 which is interpreted as the absolute number of individuals exchanged between populations. The level of genetic differentiation of 0.19 is regarded as high genetic differentiation between populations. It is also inversely proportional to Nm because as gene flow between populations increase, the genetic differentiation between these populations would decrease as a direct result. The low level of gene flow can be explained by the geographic distance between the nine populations, as the two closest sites are 2.1km away from each other and the two most distant sites are 319 km, despite the wind-pollinating reproductive strategy of the species.

4.3 Phylogenetic relationship

Studies by Bush and Barrett (1993) support the hypothesis that the metal-tolerant populations of *D. cespitosa* evolved at least twice in recent evolutionary history based on

isozyme analysis. Secondary to their work, RAPD analysis (Nkongolo et al., 2001) of these same populations reveal a relatively small genetic distance between the four Sudbury populations which suggest that they are the results of a single colonization event. The Cavalli-Sforza and Edward's (1967) chord distance, D_C, was used to estimate the genetic distance among our nine *D. cespitosa* populations. This particular algorithm is relatively unaffected by the presence of null alleles with low to moderate frequency and it relies on allele frequencies in order to determine the geometric placement of populations in a multidimensional sphere, rather than a mutational model (Chapuis and Estoup, 2007; Khasa et al., 2006). The distance matrix based on our microsatellite data revealed that the Walden and Coniston populations were the most genetically closely related populations even though their geographic locations were not the closest (18.5km), whereas the populations from Coniston and Cobalt-3 exhibited the greatest genetic distance despite the fact that these two populations were not the furthest geographically (133.7km). The four *Deschampsia* populations from the Sudbury region clustered together along with the Mississagi Lighthouse population. These findings are partly in accordance with the findings of Nkongolo et al. (2000) based on RAPD analysis, which also identified the four Sudbury population as a single cluster along with Little Current as well as the ISSR data which also clustered the four Sudbury populations. As such, this lends support to the theory that these four populations are the result of a single colonization event. The dendrogram also clustered the three Cobalt populations, which is not similar to the groupings of Nkongolo et al. (2001). In fact, the analysis of microsatellite and ISSR data suggests a very close genetic relationship between Cobalt-3 and Cobalt-4, followed by Cobalt-5. This is in disagreement with the proposed hypotheses that Cobalt-3 population arose from an unspecified seed mix (Nkongolo et al. 2001). The data described in the present study tend to lend additional supports to the allozyme findings of Bush and Barrett (1993) which suggest that Cobalt and Sudbury have independent evolutionary histories. Finally, the Little Current population appears as very genetically distantly related from the Sudbury grouping. As such, it does not lend support to the Hutchinson theory which describes the possible colonization of Sudbury region through the railway (Nkongolo et al., 2001). Finally, based on the work of isozymes (Bush and Barrett, 1993), RAPDs (Nkongolo et al., 2001), ISSRs and microsatellites, the Mississagi Lighthouse and Little Current populations never cluster together, despite both being located on the island. The Mantel test did show a correlation between the genetic matrix and the geographic distance matrix, although this relationship does not seem to be based on the concentration of metal contaminants in the soil.

In conclusion, monitoring the genetic diversity of *D. cespitosa* populations has been useful in detecting trends that should alert ecologists to potential problems. The high genetic variability detected in the *Deschampsia* populations from Sudbury and Cobalt suggests that these are healthy populations with the evolutionary potential to respond favourably and adapt to changes or disturbances in the environment.

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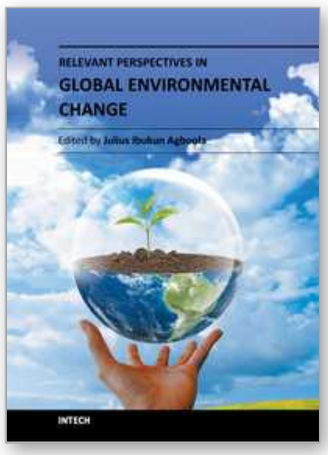

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Over the years, environmental change has sharpened significant dynamic evolution and knowledge in organizational structures of organisms, from cellular/molecular to macro-organism level including our society. Changes in social and ecological systems due to environmental change will hopefully result in a shift towards sustainability, with legislative and government entities responding to diverse policy and management issues concerning the building, management and restoration of social-ecological systems on a regional and global scale. Solutions are particularly needed at the regional level, where physical features of the landscape, biological systems and human institutions interact. The purpose of this book is to disseminate both theoretical and applied studies on interactions between human and natural systems from multidisciplinary research perspectives on global environmental change. It combines interdisciplinary approaches, long-term research and a practical solution to the increasing intensity of problems related to environmental change, and is intended for a broad target audience ranging from students to specialists.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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