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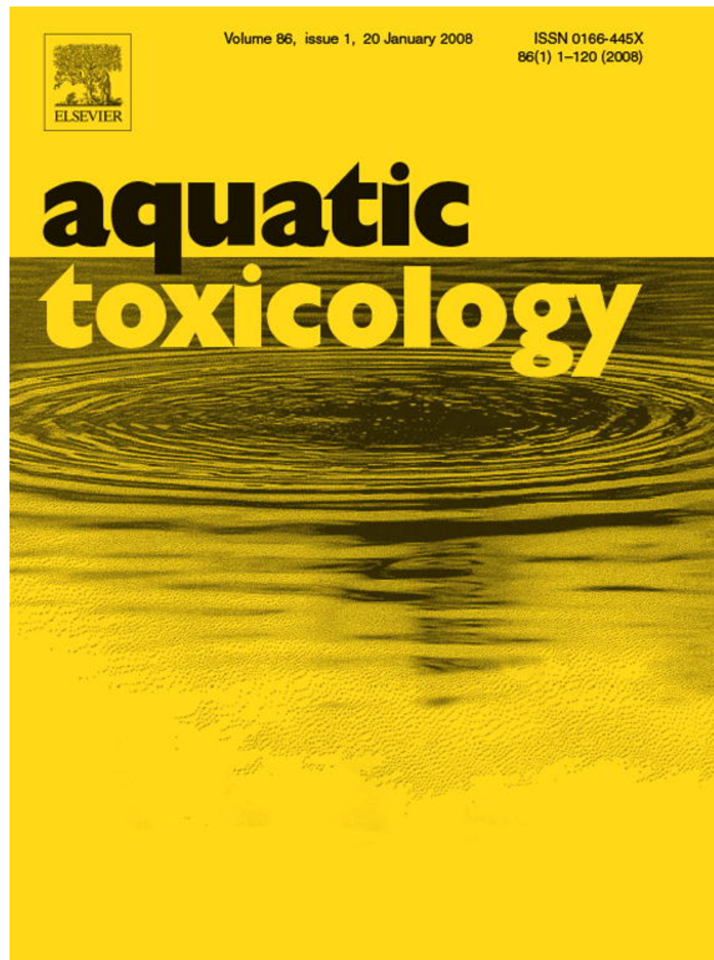

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Evolutionary ecotoxicology of wild yellow perch (*Perca flavescens*) populations chronically exposed to a polymetallic gradient

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Abstract

Depending on such factors as the intensity and duration of the exposure, and the genetic diversity and connectedness of the starting population, exposure to elevated metal concentrations can result in population level alterations such as demographic bottlenecks or metal-induced selection. These processes can be revealed using a population genetic approach, and have important implications with respect to population persistence. The main objective of this study was to examine the role of metal contamination in driving evolutionary changes by documenting patterns of genetic diversity within and among populations of wild yellow perch (*Perca flavescens*) in two major mining regions that have been subjected to metal emissions from smelters for at least 80 years; Rouyn-Noranda, Québec and Sudbury, Ontario. Yellow perch populations from ten lakes representing a gradient of metal contamination in each of the two lake systems were evaluated concurrently to reveal relationships between metal contamination and genetic diversity. These replicated sympatric observations allowed us to evaluate correlations and infer causal relationships between metal exposure and evolutionary responses in this species. Within-population gene diversity over all loci was negatively correlated with liver cadmium contamination ($P < 0.001$; $r^2 = 0.47$). Similarly, a negative correlation between gene diversity and liver copper contamination was observed at a single locus (*Pfla L1*, $P = 0.005$; $r^2 = 0.33$), suggesting a local effect of copper contamination. Internal relatedness, an index of individual diversity, presented the opposite tendency as the more contaminated individuals were more diverse than were the less contaminated ones in contaminated and reference populations. Our results thus suggest that the selective response to contamination has been large enough to substantially reduce the within-population genetic diversity, despite the fact that the less inbred individuals may be favoured by selection within any given population. Overall, our results reveal that >50 years of metal contamination have significantly impacted patterns of genetic diversity observed among populations of wild yellow perch in mining areas and as such, may have affected the capacity of populations to respond to future environmental changes.
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Keywords: Evolutionary ecotoxicology; Population genetics; Wild fish; Yellow perch; Metal contamination; Lakes; Cadmium; Copper

1. Introduction

Anthropogenic toxicants from many sources are known to have a multitude of consequences to natural populations (Posthuma and Van Straalen, 1993; Robb, 1994; Belfiore and Anderson, 2001; Mineau, 2005). Among these, physiological and behavioural endpoints have been extensively explored in the past, but relatively few studies have examined longer term effects on the genetic composition of populations. Understanding the effects of environmental contaminants on the genome is crucial for preserving the evolutionary potential of natural populations, as genetic diversity provides their potential to adapt to environmental changes (Gillespie and Guttman, 1989; Maes et al., 2005; Frankham et al., 2002). Indeed, the interaction between genetic diversity and fitness is a key element in evolutionary biology and the importance of understanding this process for conservation biology is also well acknowledged (DeSalle and Amato, 2004).

Contaminants may have opposite effects on genetic diversity. On the one hand, an increase in genetic variation has been reported when the toxicant is mutagenic (Baker et al., 2001; Chen et al., 2003; Berckmoes et al., 2005; Eeva et al., 2006). Moreover, differential selection associated with contaminants could theoretically contribute to increased genetic

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diversity under the hypothesis that tolerance to contaminants may increase as a function of individual heterozygosity (Van Straalen and Timmermans, 2002). On the other hand, although it may increase the average fitness of the population in the short-term, selection may be accompanied by a loss of genetic variation if the selective differential associated with tolerance to contaminants has been large (Baker et al., 2001). Many studies have suggested that decreases in individual heterozygosity may in turn affect population growth and recruitment as such decreases may be associated with decreased resistance to diseases, growth rate and fertility (Leberg, 1990; Reed and Frankham, 2003; Hartl and Clark, 1997). Thus, as pointed out by McMillan et al. (2006), rapid adaptation to novel environmental stressors is no guarantee of long-term population sustainability.

The main objective of this study was to examine the role of metal contamination in driving evolutionary changes by documenting patterns of genetic diversity within and among populations of wild yellow perch (*Perca flavescens*) in two major mining regions that have been subjected to metal emissions from smelters for at least 80 years; Rouyn-Noranda (RN), Québec and Sudbury (S), Ontario. A copper smelter has been in operation since 1927 in the city of Rouyn-Noranda, and although emissions are currently controlled, lakes located downwind from this smelter are contaminated with metals such as Cd, Cu and Zn due to historical atmospheric deposition (Couillard et al., 2004). Similarly, lakes in the Sudbury region have been affected by historical inputs from nickel mining and smelting operations. The selected lakes included some pristine lakes with low ambient metal levels, below the Canadian Water Quality Guidelines for these metals; some highly contaminated lakes, with metal concentrations many times over the Canadian Guidelines; and a broad range of lakes between these extremes (Campbell et al., 2003). Moreover, some of the most contaminated lakes included in this study were fishless some decades ago and now only yellow perch are abundant (Scheider et al., 1975, V. Bourret personal observation). These two regions offer an excellent framework to explore the effects of metal contamination on wild aquatic populations. They both present polymetallic (mostly Cd, Cu, Zn and Ni) gradients in lakes surrounding their industrial centers (Giguère et al., 2005; Pyle et al., 2005).

By sampling yellow perch from a large number of lakes along two polymetallic gradients of contamination in different geographic regions and by determining their levels of hepatic Cd and Cu (as metals representative of regional anthropogenic inputs and indicative of the extent of environmental contamination), we sought to test: (1) whether yellow perch from the two regions are genetically distinct; (2) whether there are significant differences in levels of genetic diversity in populations inhabiting lakes with contrasting metal levels (i.e., whether metal contamination exerted a selective pressure on populations along the polymetallic gradients), and (3) whether individual genetic diversity is associated with the concentration of accumulated metal within individual fish (i.e., whether fish with high liver metal concentrations within a lake population exposed to the same contamination differ genetically from those with lower metal concentrations). To address these issues, we used basic population genetics parameters and assessed relationships between the levels of contamination and differentiation both between populations and among individuals within populations.

2. Materials and methods

2.1. Fish sampling

Based on previous studies (reviewed in Pyle et al., 2005), ten lakes were selected in each mining region on the basis of their level of contamination by copper and cadmium, in order to cover a broad range of lakes along the spatial metal gradient present in these regions (Fig. 1). Sampling was carried out between the end of July and the beginning of August in both 2004 and 2005. In each lake, a minimum of 50 yellow perch of all sizes available were collected using a seine net; in contaminated lakes we increased the sample size to 100 so as to allow us to investigate relationships between within-population gene diversity and levels of contaminants. Each fish was measured (total length, TL), weighed (Wt), and its sex determined. We then calculated Fulton's condition factor (FCF; (weight (g)) $\times$ (total length (cm))$^{-3}$ $\times$ 1000). Opercular bones were collected for age determination, and the liver as well as fin clips were collected for tissue metal analysis and DNA extraction, respectively. We targeted only the liver as a way to simplify the already extensive analyses because according to a study previously done by our group, from a practical point of view, the liver seems to be the organ best suited for biomonitoring, since its accumulated concentrations most closely reflected Cd and Cu exposure (Giguère et al., 2004).

2.2. Liver metal concentrations

Liver samples were placed in 10 mL Teflon vials (Cole-Parmer) and weighed after lyophilisation. Dry samples were digested in 4 mL of analytical grade nitric acid in a microwave oven at an output of 750 W for intervals of 30 s, 20 s, 15 s, and finally 10 s. The Teflon vial caps were sealed tightly to prevent sample evaporation during the microwave procedure. The samples were allowed to cool between intervals to prevent excess pressure within the vials. Certified reference material from the National Research Council of Canada (TORT2) was used to monitor analytical accuracy and recovery, and blanks were inserted every 10 samples to control for contamination. Recoveries averaged 96.3 $\pm$ 4.5% for Cd and 93.7 $\pm$ 4.5% for Cu (mean $\pm$ S.E.). Digests were then diluted in deionised MilliQ water and concentrations of cadmium (Cd) and copper (Cu) were measured by inductively coupled plasma atomic emission spectrophotometry (ICP-AES) or by inductively coupled plasma mass spectrometry (ICP-MS) when the concentrations were too low for detection by ICP-AES. Although tissue nickel was also measured, it is not reported in this paper, since its concentration was below detection limits in several samples and available data were judged unreliable based on poor reproducibility and variable recovery rates.

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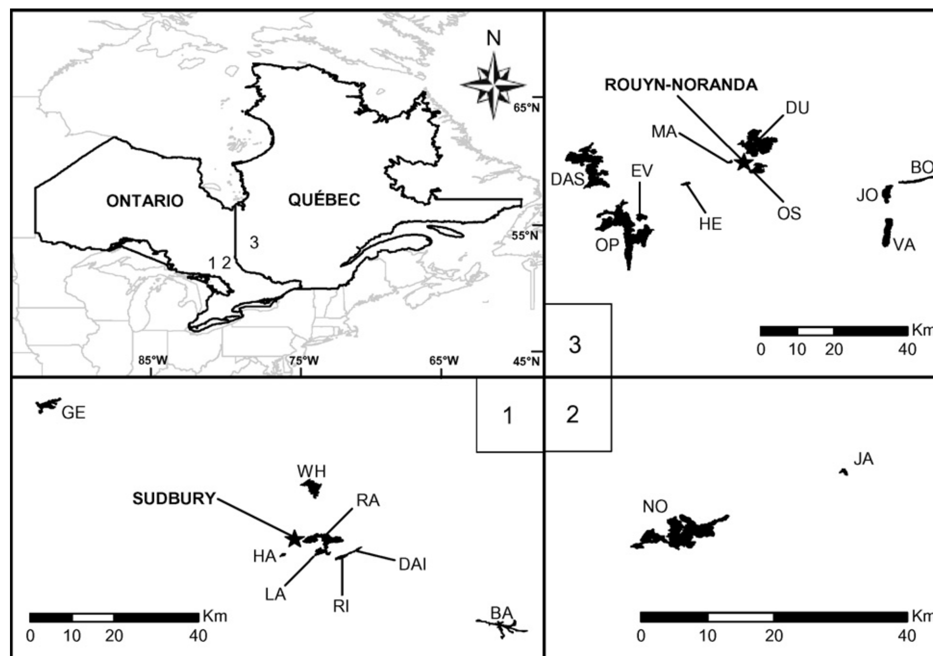

Fig. 1. Location of the 20 lakes in this study (10 in Ontario and 10 in Québec). Lakes sampled in Ontario are: Geneva (GE), Whitson (WH), Hannah (HA), Ramsey (RA), Laurentian (LA), Richard (RI), Daisy (DAI), Barlow (BA), Nosbonsing (NO) and James (JA). Lakes sampled in Québec are: Dasserat (DAS), Opasatica (OP), Évain (EV), Hélène (HE), Marlon (MA), Dufault (DU), Osisko (OS), Vaudray (VA), Joannès (JO) and Bousquet (BO).

2.3. Microsatellite genotyping

Fin clips were used to extract DNA following the salt-extraction method developed by Aljanabi and Martinez (1997). Samples were then screened for variation at nine microsatellite loci. Simplex polymerase chain reaction (PCR) amplifications were performed for six microsatellite loci developed for yellow perch (*Pfla L1*, *Pfla L3*, *Pfla L4*, *Pfla L5*, *Pfla L6*, *Pfla L9*; Leclerc et al., 2000) and three for walleye (*Sander vitreus*) (*Svi L10*, Wirth et al., 1999; *Svi 17*, Borer et al., 1999; *Svi E*, Eldridge et al., 2002). PCRs were performed in 12.5 μL reaction volumes composed of double-distilled H_2O, 1.0 μL 10× reaction buffer (10 mM Tris–HCl pH 9.0, 1.5 mM $MgCl_2$, 0.1% TritonX-100, 50 mM KCl), 50–100 ng of template DNA, 0.3 μL dNTP (or deoxynucleosides, the monomers that DNA polymerase uses to form DNA) (10 mM final concentration for each dNTPs), 0.16 μM fluorescently labelled (forward) primer, 0.32 μM (final concentration) of reverse primer, and 0.4 U *Taq* DNA polymerase. The PCR program used involved the following steps: 95 °C for 5 min; 35 cycles at 95 °C for 30 s, 45 s at a marker-specific annealing temperature (see Table 1) and 45 s at 72 °C; and a final 10 min at 72 °C. Amplified products were resolved on an ABI 3100 genetic analyser (Applied Biosystem Inc.) using GeneScan™-500 LIZ™ as a size standard and scored using GENESCAN 3.7 and GENOTYPER 3.7 software (Applied Biosystem Inc.).

2.4. Genetic data analyses

2.4.1. Genetic diversity

Genetic diversity within each population was quantified using standard descriptive statistics for each locus and globally: observed and expected heterozygosities (H_O and H_E) as well as allelic diversity (A) using GENEPOP 3.4 (Raymond and Rousset, 1995) and Nei's (1977) estimator of gene diversity using FSTAT 2.9.3 (Goudet et al., 2002). Global and per locus allelic richness ($\hat{A}$) were corrected (using the rarefaction method of FSTAT 2.9.3) to the smallest sample ($n = 50$) to increase the power of detecting differences in $\hat{A}$ (Leberg, 2002). Conformity to Hardy–Weinberg equilibrium (HWE) expectations for each locus and across all loci, and linkage disequilibrium (LD) between all locus pairs were also tested using GENEPOP 3.4. When applicable, the sequential Bonferroni correction for multiple tests was applied to maintain the table-wide significance level at $\alpha = 0.05$ (Rice, 1989). For other statistical tests (see below), the 0.05 level of significance was used unless otherwise stated.

2.4.2. Neutrality test

The rationale of most neutrality tests is that while genetic drift and migration affect the whole genome to the same extent, selection is targeted to a few loci only, and thus a locus that presents a significantly different pattern than the remainder of the genome might be located in a chromosomal region under selection. Schlotterer (2002) proposed a test of neutrality to be assessed on microsatellites: the *LnRH* method. The test assumes the stepwise mutation model, neutrality and mutation-drift equilibrium and therefore expects the variance in diversity to be a good estimator of microsatellite variability. The test statistics calculate the ratio of the genetic variabilities of two populations for each locus to identify those that differ significantly in variability from the remainder of the genome (Schlotterer, 2002). Maple v7.0 (2001) was used to calculate the gene diversity ratio between all possible pairs of populations and a Fisher *F*-statistic

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Table 1
Microsatellite loci characteristics: structure of repeat motif, primer sequence, allele size range, PCR annealing temperature (T_A) in °C, number of alleles observed (A) and reference publication are provided

Locus	Species	Repeat motif	Primer sequence (5′–3′)	Size range (bp)	T_A (°C)	A	Reference
Pfla L1	*Perca flavescens*	$(GA)_{27}$	F: AAGCAGCCTGATTATATATC R: CAGACAATTAAACATGCAAC	90–126	50	12	Leclerc et al. (2000)
Pfla L3	*Perca flavescens*	$(TG)_{18}$	F: GCCGAATGTGATTGAATG R: CGCTAAAGCCAACTTAATG	115–190	53	20	Leclerc et al. (2000)
Pfla L4	*Perca flavescens*	$(TC)_{37}$	F: AAAGGGAAAAGGCTACGGTG R: ATCAGCAGTGCTTATGTTTG	80–135	53	15	Leclerc et al. (2000)
Pfla L5	*Perca flavescens*	$(GT)_{27}$	F: TGAGAGCCCATGAATTAC R: GCAAACACAGCCAATTTAG	126–160	53	10	Leclerc et al. (2000)
Pfla L6	*Perca flavescens*	$(TG)_{18}$	F: GCATACATATAAGTAGAGCC R: CAGGGTCTTCACTATACTGG	135–180	53	16	Leclerc et al. (2000)
Pfla L9	*Perca flavescens*	$(TG)_{24}$	F: GTTAGTGTGAAAGAAGCATCTGC R: TGGGAAATGTGGTCAGCGGC	210–280	59	14	Leclerc et al. (2000)
Svi 5	*Sander vitreus*	$(AC)_{18}$	F: CTTAATTCCCCCAGCAAC R: TGTGTGCATCATTATCACTG	160–220	52	15	Eldridge et al. (2002)
Svi 17	*Sander vitreus*	$(AC)_{13}$	F: GCGCACTCTCGCATAGGCCCTG R: CGTTAAAGTCCTTGGAAACC	140–190	52	11	Borer et al. (1999)
Svi L10	*Sander vitreus*	$(CA)_{33}$	F: GGTAATGTATTTTCAGTTATTGC R: GCTGTTCTCCAAGTAAAGCC	190–280	51	39	Wirth et al. (1999)

was used to identify the loci that differed significantly from neutrality in their gene diversity ratios.

2.4.3. Differentiation

Genic differentiation (G; Guo and Thompson, 1992) at individual loci between all pairs of samples and significance values over all loci were obtained using Fisher's method (Ryman and Jorde, 2001) available in GENEPOP 3.4. We compared global and pair-wise measures of genetic differentiation based on allelic identity (F-statistics, e.g. θ_{ST}; Weir and Cockerham, 1984) and allele size (R-statistic, e.g. R_{ST}; Michalakis and Excoffier, 1996) to highlight the relative importance of drift (θ_{ST}) vs. mutation (R_{ST}) for population differentiation with the allele size randomization test of Hardy et al. (2003) using 1000 permutations with SPAGeDi 1.2 (Hardy and Vekemans, 2002). If the null hypothesis of $F_{ST} = R_{ST}$ were rejected ($\alpha = 0.05$), R_{ST} measures would be considered the better indicator of genetic differentiation between study sites, because of the contribution of stepwise mutation model (SMM)-like mutations in the genetic differentiation. Here, acceptance of the null hypothesis led to the calculation of genetic differentiation among lakes or populations using the F_{ST} of Weir and Cockerham (θ) as calculated with GENETIX (Belkhir et al., 2001) after 1000 permutations for significance. An analysis of molecular variance (AMOVA) was performed using ARLEQUIN 3.01 (Schneider et al., 2000) in order to assess the relative importance of regional (Sudbury vs. Rouyn-Noranda) and population genetic differences within each region.

2.4.4. Internal relatedness

As a measure of individual genetic diversity, we used internal relatedness (IR; Amos et al., 2001), which is based on the relatedness measure of Queller and Goodnight (1989), except that at each locus, two alleles rather than two pairs of alleles are compared. Over several loci, the resulting values are approximately normally distributed and centered on zero, with negative values suggesting relatively outbred individuals and high positive values being suggestive of inbreeding (Garant et al., 2005). IR values were computed using an EXCEL (Microsoft Inc.) macro written in Visual Basic provided on the William Amos website (http://www.zoo.cam.ac.uk/zoostaff/amos/, Department of Zoology, Cambridge University, UK).

2.4.5. Effective population size and bottleneck test

Estimates of contemporary (parental generation) inbreeding effective population sizes (Ne) were produced using the linkage disequilibrium method on single temporal samples. This method was developed by Hill (1981) and Bartley et al. (1992) and is based on the expectation that smaller populations will accumulate more disequilibrium over time. Estimates were performed on each sampled population using NEESTIMATOR 1.3 (Peel et al., 2004). We considered that the linkage disequilibrium method was the most appropriate for our study because (i) the methodology requirements are appropriate for the sampling employed in this study (Waples, 1991; Bartley et al., 1992); (ii) we did not have temporal sampling to estimate Ne from temporal methods when only one generation separates samples (Waples, 1989; Tallmon et al., 2004); and (iii) it does not appear to be seriously affected by a reduction in population size (Waples, 2005).

We also tested for recent and major reduction in population size using BOTTLENECK 1.2.02 (Piry et al., 1999), which should be particularly sensitive to recent population bottlenecks. Populations that have experienced a bottleneck event usually present a correlated reduction in allele numbers (k) and

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gene diversity (He). However, the resulting gene diversity in a recently bottlenecked population should be higher than the expected equilibrium gene diversity (Heq) that is computed from the observed number of alleles (*k*), under the assumption of a constant-size (equilibrium) population (Luikart et al., 1998). Bottleneck significance was tested with the Wilcoxon sign-rank test (Luikart et al., 1998), under a two-phase mutation model (TPM; more appropriate for microsatellites; Di Rienzo et al., 1994), with 95% one-step (SMM) and 5% infinite allele models (IAM), and 12% variance of multiple-step mutations (Luikart et al., 1998) for 5000 iterations as recommended by Piry et al. (1999).

2.5. *Statistical analysis*

2.5.1. *Population differentiation and metal concentrations*

Mantel tests (Mantel, 1967) and partial Mantel tests (Legendre and Legendre, 1998) were computed in R 2.2.1 (The R core development team) to examine the relationships between genetic differentiation, metal contamination, geographic distance and lake areas (all parameters expressed as pair-wise distances in matrices). We used pair-wise F_{ST} as a ratio ($F_{ST}/(1-F_{ST})$) to test for isolation by geographic distance between populations based on lakes coordinates (Rousset, 1997). Lake areas were calculated using ArcGIS 9 (ESRI Inc.) and matrices of metal contamination were built based on differences (absolute values) of mean tissue metal concentration between populations for Cd and Cu. To first test for effects of geographic distance and lake area on genetic differentiation, simple Mantel tests were applied on two matrices for 10,000 randomizations. We then computed partial Mantel tests for 10,000 randomizations to examine the individual impact of cadmium and copper contamination on the extent of genetic differentiation while controlling for geographic distance or lake area.

2.5.2. *Within-population gene diversity and levels of contaminants*

We first tested whether single variables, namely total length, weight, age, Fulton's condition factor and tissue metal concentration (Cd and Cu) reported as means for every population were correlated with overall (global effect of Heterozygosity-Fitness-Correlation, HFC) and individual (local HFC effects) loci gene diversity of populations (*H*) as well as allelic richness, both computed using FSTAT 2.9.3 (Goudet et al., 2002) (see Pemberton, 2004 for a review of HFC). If univariate analysis revealed a significant correlation, we then used multiple regression models to test for the inclusion of more than one variable. We also tested whether both overall and single-locus gene diversity were correlated to lake area and effective population size (Ne). Univariate statistics were computed in SAS 9.1 (SAS Institute Inc.) as correlations to first test for significant relationships and then as regressions to explore the relative individual effects of correlated variables on the dependent variables of gene diversity.

Secondly, we tested for the existence of an association between levels of contaminants (both Cu and Cd) within individuals and individual gene diversity (measured by IR). Here, individuals were classified into four quartiles according to their liver metal concentrations, and comparisons among those groups were performed using the LSMEANS procedure available in SAS. We then performed a less stringent analysis since LSMEANS did not reveal a significant difference among groups despite the fact that a visual trend was observed in the data (see Section 3). Thus, we tested whether there was a linear relationship among groups using the method of linear contrast. This analysis was performed both by pooling individuals of all populations into a single analysis as well as separately for each population.

3. Results

3.1. *Samples and hepatic metal concentrations*

Liver digests and measurements of Cd and Cu concentrations were performed on a total of 1393 fish collected over 2 summers in Rouyn-Noranda (RN) and Sudbury (S) (Fig. 2). Thus, we analysed 50 fish in 12 lakes and 100 fish from 8 highly contaminated lakes with an expected high level of contamination, except for Lake Dufault where 93 fish were analysed. In accordance with previous studies (Couture et al., 2008a,b; Pyle et al., 2008), mean liver Cd concentrations in contaminated populations from Rouyn-Noranda were higher than those from Sudbury, and reciprocally, the highest liver Cu concentrations were recorded in Sudbury fish (Fig. 2).

3.2. *Genetic data analyses*

3.2.1. *Genetic diversity*

Locus *Pfla L4* was excluded from all analyses because of scoring problems related to amplification and locus *Svi L10* was discarded because of generalized strong deficits in heterozygotes. Therefore, seven loci were considered for all further analyses. All loci were moderately polymorphic, with the total number of alleles per locus ranging from 11 to 20 (mean = 14), and expected heterozygosity across all samples ranging from 0.21 to 0.50 (mean = 0.37) (Table 2). Allelic richness corrected to $n = 50$ using the rarefaction method ranged from 1 to 20 alleles per locus per population.

The null hypothesis of the Hardy–Weinberg equilibrium was not rejected after correcting the significance level or the number of pair-wise comparisons of populations and loci ($\alpha = 0.00037$, $k = 135$), without any locus-sample showing significant deviation. Fisher's exact test of genotypic linkage disequilibrium did not reveal significant linkages after correction ($\alpha = 0.00013$, $k = 393$) and only 28 significant linkages if considering the standard level of significance of 5%, a value close to expectation by chance alone ($n = 20$). Therefore, we accepted the null hypothesis of independence of the seven loci kept for analyses.

3.2.2. *Neutrality test and population differentiation*

The neutrality test revealed a lower proportion of significant standardized *lnRH* than expected by chance (1 of 19 compar-

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Table 2
Summary of genetic diversity at 9 microsatellite loci among, from left to right, 10 populations from Sudbury and 10 from Rouyn-Noranda

			Sudbury										Rouyn-Noranda									
		N	99	100	100	100	50	50	50	50	50	50	93	100	100	100	50	50	50	50	50	50
Locus	A		WH	DAI	HA	LA	RA	RI	GE	BA	JA	NO	DU	OS	BO	JO	MA	VA	OP	DAS	EV	H_E
Pfla L1	12	$\hat{A}$**	3	3.7	3.8	4.9	6	5	3	7	5	5	5.2	2	3.9	3.9	4	3	5	2	6	5
		F_{IS}	−0.02	−0.06	−0.07	−0.02	−0.08	−0.11	0.22	−0.03	0.17	−0.01	0.01	0.05	−0.01	−0.02	−0.15	−0.04	−0.10	−0.24	0.20	0.06
		H_E	0.23	0.16	0.18	0.55	0.47	0.29	0.33	0.68	0.58	0.44	0.48	0.15	0.31	0.33	0.52	0.46	0.33	0.50	0.50	0.51
		H_O	0.23	0.17	0.19	0.56	0.50	0.32	0.26	0.70	0.48	0.44	0.47	0.14	0.31	0.34	0.60	0.48	0.36	0.62	0.40	0.48
Pfla L3	20	$\hat{A}$	5	8.5	4.4	3	7	10	5	10	6	10	5.5	3	6.2	5.2	4	1	8	9	7	8
		F_{IS}	−0.07	−0.03	0.06	0.03	−0.01	0.02	−0.10	0.18	0.06	0.04	0.10	0.04	−0.02	0.10	−0.07	NA	0.05	−0.08	−0.09	0.09
		H_E	0.63	0.76	0.63	0.53	0.63	0.78	0.35	0.34	0.55	0.48	0.30	0.18	0.45	0.31	0.19	0.00	0.57	0.48	0.48	0.31
		H_O	0.68	0.78	0.59	0.51	0.64	0.76	0.38	0.28	0.52	0.46	0.27	0.17	0.46	0.28	0.20	0.00	0.54	0.52	0.52	0.28
*Pfla L4****	15	N	98	44	46	96	49	47.0	30	31	49	47	73	82	88	76	44	40	43	42	42	39
		$\hat{A}$	7.2	9.2	4.9	8.3	9.4	7.8	4	8	6.5	12	9.8	4	9.8	7.9	8.7	3.7	9.4	9.5	6	6.8
		F_{IS}	0.07	0.92	0.31	0.30	0.69	0.83	1.00	1.00	0.64	0.47	0.85	0.89	0.71	0.81	0.71	0.93	0.91	0.89	0.78	0.80
		H_E	0.73	0.82	0.41	0.81	0.84	0.76	0.59	0.84	0.72	0.89	0.84	0.68	0.87	0.83	0.77	0.37	0.78	0.85	0.75	0.77
		H_O	0.68	0.07	0.28	0.57	0.27	0.13	0.00	0.00	0.29	0.47	0.12	0.07	0.25	0.16	0.23	0.03	0.07	0.10	0.17	0.15
Pfla L5	10	$\hat{A}$	2.9	4.2	3.5	3.9	4	4	2	4	3	4	5.1	3	3	3	3	2	5	5	6	4
		F_{IS}	−0.04	0.04	−0.03	0.03	−0.18	−0.05	−0.03	0.02	−0.06	0.01	−0.10	−0.01	0.04	0.00	−0.16	−0.21	0.11	0.00	0.04	0.06
		H_E	0.11	0.54	0.63	0.55	0.53	0.55	0.50	0.28	0.15	0.52	0.68	0.34	0.58	0.53	0.64	0.45	0.63	0.44	0.73	0.63
		H_O	0.11	0.52	0.65	0.53	0.62	0.58	0.52	0.28	0.16	0.52	0.74	0.34	0.56	0.53	0.74	0.54	0.56	0.44	0.70	0.60
Pfla L6	16	$\hat{A}$	2.8	3.8	5.9	4	5	4	1	5	2	8	5.3	3	4.6	2.5	5	3	5	7	5	6
		F_{IS}	0.06	−0.07	0.03	−0.06	0.15	0.27	NA	0.11	−0.08	−0.04	−0.06	−0.27	0.00	0.10	−0.19	−0.06	0.08	0.14	0.10	0.03
		H_E	0.15	0.38	0.40	0.37	0.38	0.41	0.00	0.63	0.15	0.61	0.51	0.52	0.39	0.44	0.52	0.15	0.70	0.65	0.71	0.66
		H_O	0.14	0.41	0.39	0.39	0.32	0.30	0.00	0.56	0.16	0.64	0.54	0.66	0.39	0.40	0.62	0.16	0.64	0.56	0.64	0.64
Pfla L9	14	$\hat{A}$	3.5	4.3	5.2	4	6	3	2	7	5	9	3.9	2	5.6	2	3	3	7	7	5	6
		F_{IS}	0.10	0.00	0.08	−0.08	−0.15	0.11	−0.03	0.05	0.11	0.13	0.05	−0.05	−0.02	−0.05	−0.08	0.25	0.00	0.01	−0.14	0.10
		H_E	0.48	0.42	0.20	0.43	0.42	0.40	0.08	0.74	0.61	0.51	0.23	0.10	0.26	0.10	0.17	0.13	0.42	0.53	0.44	0.53
		H_O	0.43	0.42	0.18	0.46	0.48	0.36	0.08	0.70	0.54	0.44	0.22	0.11	0.26	0.11	0.18	0.10	0.42	0.52	0.50	0.48
Svi 5	15	$\hat{A}$	3	5.5	4.6	3.6	4	5	4	5	4	7	3.2	2	3.4	2.5	2	1	5	5	5	4
		F_{IS}	−0.07	0.00	0.12	−0.04	0.11	−0.03	0.02	−0.05	−0.08	0.29	−0.01	−0.02	−0.08	0.17	0.00	NA	−0.06	0.26	0.17	−0.04
		H_E	0.25	0.27	0.33	0.12	0.13	0.27	0.59	0.30	0.20	0.22	0.04	0.05	0.24	0.10	0.02	0.00	0.19	0.13	0.39	0.33
		H_O	0.26	0.27	0.29	0.12	0.12	0.28	0.58	0.32	0.22	0.16	0.04	0.05	0.26	0.08	0.02	0.00	0.20	0.10	0.32	0.34
Svi 17	11	$\hat{A}$	2	3.9	1.8	4	6	5	3	5	3	5	3.1	1	2.9	2.5	3	1	4	4	5	3
		F_{IS}	0.17	0.08	−0.01	−0.05	0.02	0.24	0.29	−0.09	0.20	0.01	−0.01	NA	0.28	−0.03	−0.02	NA	−0.03	−0.05	−0.05	−0.01
		H_E	0.31	0.34	0.02	0.65	0.67	0.39	0.39	0.59	0.53	0.30	0.05	0.00	0.10	0.07	0.08	0.00	0.12	0.13	0.15	0.06
		H_O	0.25	0.31	0.02	0.68	0.66	0.30	0.28	0.64	0.42	0.30	0.05	0.00	0.07	0.07	0.08	0.00	0.12	0.14	0.16	0.06
*Svi L10****	39	$\hat{A}$	15.5	11.3	9	13.4	15	13	5	20	14	19	8.9	5.7	8.3	6.6	10	6	9	10	9	10
		F_{IS}	0.32	0.28	0.06	0.32	0.50	0.24	0.18	0.16	0.22	0.30	0.19	0.11	0.40	0.60	0.20	0.29	0.27	0.10	0.34	0.26
		H_E	0.92	0.75	0.77	0.91	0.91	0.70	0.75	0.90	0.87	0.88	0.69	0.72	0.66	0.57	0.68	0.64	0.77	0.71	0.78	0.78
		H_O	0.63	0.54	0.73	0.62	0.46	0.54	0.62	0.76	0.68	0.62	0.56	0.64	0.40	0.23	0.54	0.46	0.56	0.64	0.52	0.58
*All loci***		$\hat{A}$	3.2	4.9	4.2	3.9	5.4	5.1	2.9	6.1	4	6.9	4.5	2.3	4.2	3.1	3.4	2	5.6	5.6	5.6	5.1
		H_E	0.31	0.41	0.34	0.45	0.46	0.44	0.32	0.51	0.40	0.44	0.33	0.19	0.33	0.27	0.31	0.17	0.42	0.41	0.49	0.43
		H_O	0.30	0.41	0.33	0.46	0.48	0.41	0.30	0.50	0.36	0.42	0.33	0.21	0.33	0.26	0.35	0.18	0.41	0.41	0.46	0.41

*N the number of individuals genotyped from each population on the first line (except for locus *Pfla L4*, where N is given above other locus data), and per locus in each populations: **Allelic richness ($\hat{A}$) corrected to $n = 50$ using the rarefaction method of FSTATS 2.9.3 (Goudet et al., 2002), correlation value of heterozygote deficit (F_{IS}) and expected (H_E) and observed (H_O) heterozygosities. ***Indicate locus showing significant deviation from Hardy–Weinberg equilibrium ($P = 0.05$). ****For *All loci*, statistics represent compilation of the seven loci that were retained for analyses (loci *Pfla L4* and *Svi L10* were excluded).

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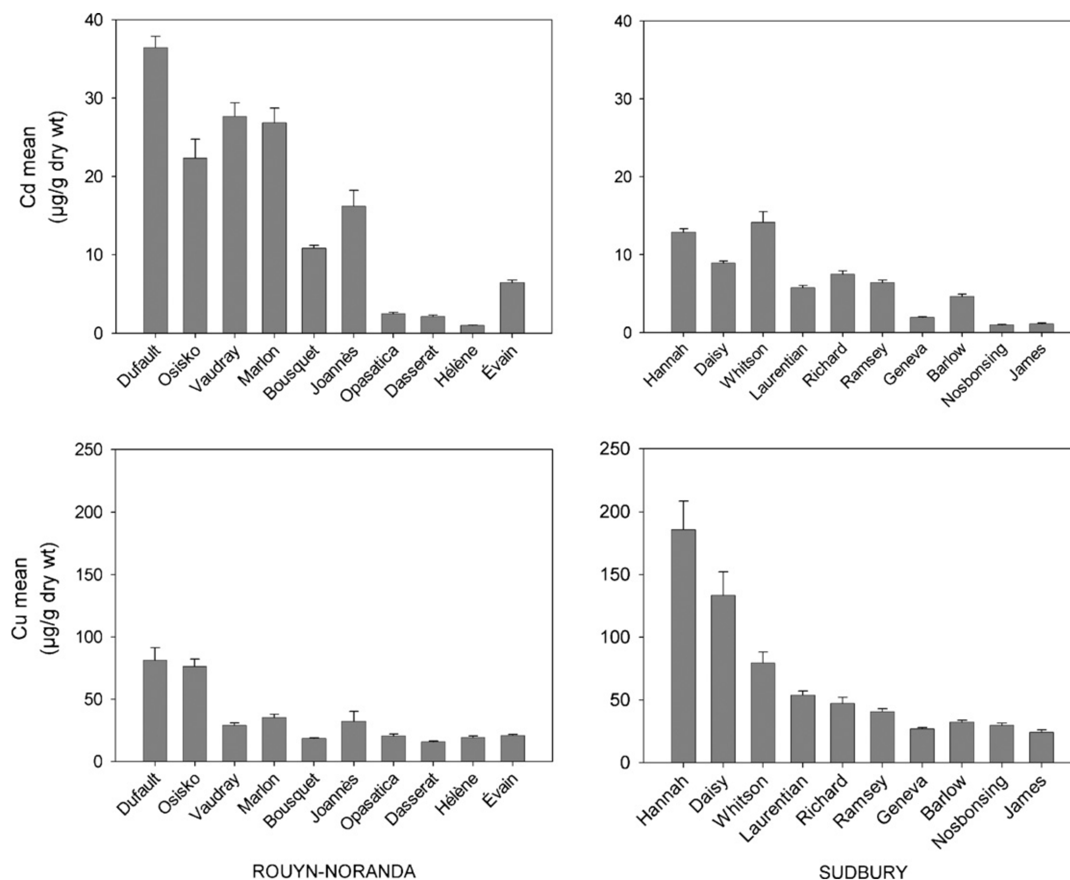

Fig. 2. Concentrations of Cd (top panels) and Cu (bottom panels) (mean ± S.E., µg/g dry weight), in liver samples from individuals of populations from Rouyn-Noranda, Québec (left panels) and Sudbury, Ontario (right panels).

isons) on 5 out of 7 loci. Loci *Pfla L6* and *Svi 17* showed more significant values than expected by chance under the standard 5% significant level but none under the 1% level. Moreover, they did not present significant *P*-values for the two-way Fisher test. Thus, the neutral hypothesis could not be rejected for any loci under the assumption of the *lnRH* test, and therefore all were retained for subsequent analyses of population differentiation.

Multilocus R_{ST} values were not significantly higher than the simulated distribution of R_{ST} values (ρR_{ST}) ($P = 0.23$) (Table 3). This result indicated that the *F*-statistic (θ_{ST}) was more appropriate for assessing differentiation between populations, and that there was no apparent effect of long-term historical isolation on population differentiation (Hardy et al., 2003). In a global test over all loci, significant genic differentiation was observed ($P < 0.001$), which translated into a global F_{ST} value of 0.362, reflecting an overall pronounced level of differentiation among populations. Global F_{ST} values within each region also indicated moderate to strong differentiation among populations at this scale (RN = 0.162, $S = 0.262$). Pairwise F_{ST} values (estimated by θ) were all significant except for two comparisons (Daisy and Richard in Sudbury, Évain and Hélène in Rouyn-Noranda), and ranged from 0.010 and 0.680 (Table 4). The AMOVA results further confirmed the observed population structure, whereby 29.5% of the overall genetic variance was attributable to differences between regions and 15.6% to differences among populations within a region (Table 5).

Table 3
Summary of the allele size permutation test of Hardy et al. (2003) for population differentiation

Locus	θ_{ST}	R_{ST}	ρR_{ST}	*P*-value
Pfla L1	0.347	0.494	0.286	0.004**
Pfla L3	0.337	0.465	0.294	0.157
Pfla L5	0.273	0.178	0.267	0.891
Pfla L6	0.250	0.048	0.204	0.995
Pfla L9	0.483	0.293	0.407	0.695
Svi 5	0.151	0.135	0.149	0.465
Svi 17	0.582	0.429	0.488	0.650
All loci	0.362	0.389	0.331	0.233

θ_{ST} and R_{ST} estimates for each locus employed and across all loci *P*-values of significance tests ($R_{ST} > \rho R_{ST}$) with asterisks ($^{**}P < 0.01$ level).

3.2.3. Effective population size and bottleneck test

Estimates of contemporary effective population sizes (Ne) with the linkage disequilibrium method ranged from 20.4 (Geneva) to infinity (nine populations). No evidence of population bottlenecks was detected according to BOTTLENECK: after correction ($\alpha = 0.0025$, $k = 20$). No significant departure from mutation-drift equilibrium was observed for any sampled population, based on the Wilcoxon sign-rank test, under the

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Table 4
Number of loci significantly ($\alpha = 0.05$) different for each population pair based on the genic differentiation test (above the diagonal) and pair-wise measures of genetic differentiation based on allelic identity (θ_{ST}) (below the diagonal)

	Sudbury										Rouyn-Noranda									
	HA	WH	DAI	LA	RA	RI	BA	GE	JA	NO	OS	DU	JO	BO	VA	MA	OP	DAS	EV	HE
HA		6	7	7	7	7	7	7	7	7	6	7	7	7	7	7	7	7	7	7
WH	0.300		7	6	7	7	6	7	6	7	7	7	6	6	7	7	6	6	7	7
DAI	0.092	0.340		6	0	4	7	7	7	6	7	7	7	7	6	6	6	6	7	7
LA	0.160	0.292	0.077		7	2	7	7	6	5	6	6	6	6	6	6	6	6	7	6
RA	0.099	0.334	*NS	0.070		5	7	7	7	5	7	7	7	7	7	6	5	6	7	7
RI	0.144	0.305	0.049	0.010	0.044		7	7	5	4	6	6	6	7	6	6	5	6	7	7
BA	0.336	0.419	0.238	0.248	0.223	0.209		7	5	6	6	7	7	7	7	7	6	6	7	7
GE	0.299	0.430	0.296	0.324	0.283	0.327	0.427		6	7	7	7	6	7	5	7	7	7	7	7
JA	0.311	0.447	0.221	0.244	0.225	0.215	0.151	0.458		6	7	7	6	5	5	5	6	5	5	5
NO	0.445	0.420	0.305	0.294	0.283	0.275	0.206	0.507	0.308		7	7	7	7	7	6	4	5	6	6
OS	0.610	0.578	0.486	0.485	0.492	0.509	0.477	0.680	0.569	0.144		5	5	6	5	4	5	6	7	6
DU	0.527	0.471	0.426	0.411	0.414	0.411	0.353	0.595	0.440	0.073	0.101		5	6	5	1	4	4	4	4
JO	0.578	0.508	0.500	0.468	0.494	0.483	0.437	0.629	0.468	0.262	0.345	0.195		2	4	5	4	5	6	5
BO	0.540	0.465	0.462	0.424	0.448	0.431	0.396	0.580	0.411	0.243	0.351	0.214	0.011		5	6	4	5	5	4
VA	0.595	0.536	0.493	0.465	0.497	0.493	0.448	0.659	0.489	0.279	0.375	0.264	0.084	0.076		4	3	5	5	5
MA	0.547	0.517	0.434	0.416	0.425	0.415	0.329	0.625	0.431	0.066	0.163	0.015	0.245	0.251	0.335		5	4	6	5
OP	0.522	0.484	0.418	0.373	0.393	0.368	0.312	0.572	0.355	0.146	0.312	0.137	0.119	0.101	0.214	0.128		3	2	2
DAS	0.503	0.468	0.380	0.355	0.353	0.348	0.303	0.555	0.382	0.066	0.147	0.061	0.198	0.190	0.246	0.068	0.067		3	2
EV	0.470	0.393	0.383	0.346	0.353	0.336	0.282	0.511	0.326	0.127	0.269	0.106	0.089	0.071	0.152	0.118	0.034	0.078		0
HE	0.479	0.387	0.392	0.359	0.367	0.353	0.292	0.523	0.343	0.132	0.264	0.111	0.073	0.060	0.116	0.135	0.062	0.086	*NS	

* NS asterisks indicate non-significant comparison.

assumption that all loci fit the two-phase mutation model TPM (data not shown).

3.3. Statistical analysis

3.3.1. Population differentiation and levels of contaminants

Simple Mantel tests showed significant correlation between genetic differentiation (θ_{ST}) and geographic distance ($P < 0.0001$; $r^2 = 0.487$) and non-significant correlation between θ_{ST} and lake areas ($P = 0.884$). Therefore, partial Mantel tests were used to further explore relationships between population differentiation and metal contamination (Cd and Cu) while controlling for geographic distance. These tests did not show a significant relationships with Cd contamination ($P = 0.210$) but revealed marginally significant relationships for Cu contamination ($P = 0.063$) (Table 6), whereby 5.2% ($r^2 = 0.052$) of overall genetic differentiation among populations was explained by this metal concentration. Partial tests computed on individual loci revealed that this relationship was driven by *Pfla L1*, which was the only significant locus ($P = 0.021$) and indicated that 8.0% ($r^2 = 0.080$) of genetic differentiation at this locus could be explained by differences in Cu contamination among lakes.

Table 5
Hierarchical partitioning of genetic variance (AMOVA) between both geographical regions (Rouyn-Noranda and Sudbury), among populations within regions and within individual populations

Source of variation	df	Percentage of variation	*P*
Between regions	1	29.47	***
Among populations within regions	18	15.59	***
Within populations	2764	54.94	***

Significance at the *** $P < 0.001$ level.

3.3.2. Within-population gene diversity and levels of contaminants

Univariate tests did not show significant correlations between overall within-population gene diversity or allelic richness with total length ($P = 0.200$), weight ($P = 0.152$), age ($P = 0.174$), FCF ($P = 0.942$), effective population size ($P = 0.146$), or lake area ($P = 0.712$). However, a strong negative correlation was found between overall gene diversity and Cd ($P = 0.0005$, $r^2 = 0.472$) (Fig. 3). A negative, albeit weaker correlation was also found

Table 6
Results of simple and partial Mantel tests showing the relationships between genetic differentiation (θ_{ST}), geographic distance (geo. dist., shortest distance) and differences in cadmium contamination between paired populations (absolute values)

Locus	Mantel test/partial Mantel test	*P*-value significance	r^2
All loci	Differentiation vs. geographic distance	<0.0001	0.487
	Differentiation vs. Cu controlling for geo. dist.	**0.063**	**0.052**
	Differentiation vs. Cd controlling for geo. dist.	0.2098	0.007
Pfla L1	Differentiation vs. geographic distance	0.0023	0.105
	Differentiation vs. Cu controlling for geo. dist.	**0.0207**	**0.080**
	Differentiation vs. Cd controlling for geo. dist.	0.9737	0.034

Geographic distances were log transformed before Mantel tests. Significant or marginal correlations are showed in bold characters.

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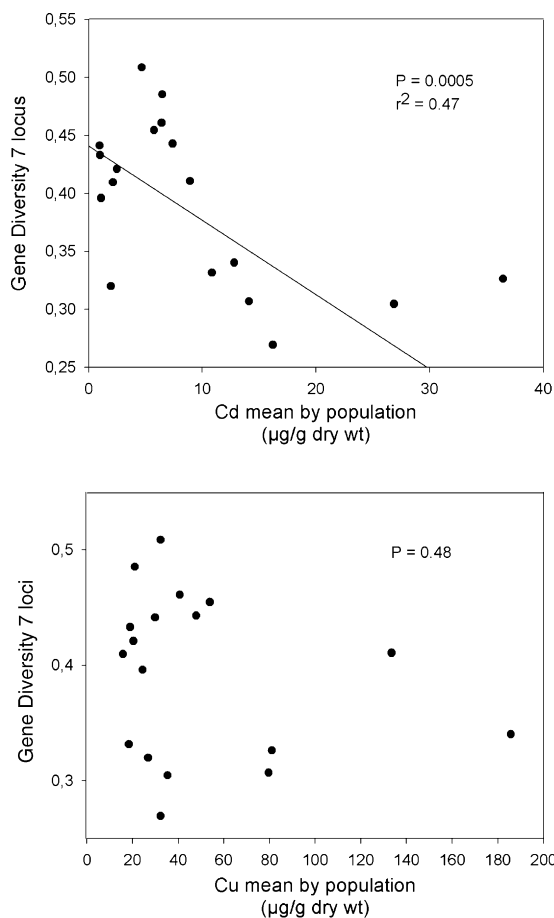

Fig. 3. Relationship between overall gene diversity of populations calculated from 7 microsatellite loci vs. (top panel) mean liver Cd concentration and (bottom panel) mean liver Cu concentration. *P*-values and adjusted regression are indicated.

between allelic richness and Cd ($P = 0.0085$, $r^2 = 0.289$). Tests performed on individual loci revealed that four out of seven loci contributed significantly to this pattern with Cd (*Pfla L3*, $P = 0.0133$, $r^2 = 0.256$; *Pfla L9*, $P = 0.0028$, $r^2 = 0.365$; *Svi 5*, $P = 0.0184$, $r^2 = 0.231$; *Svi 17*, $P = 0.0028$, $r^2 = 0.366$). In contrast, there was no significant association between either overall gene diversity or allelic richness and Cu contamination ($P = 0.480$; 0.501). However, a highly significant correlation between gene diversity and Cu contamination was observed for locus *Pfla L1* only ($P = 0.005$, $r^2 = 0.326$) (Fig. 4). Tests using multiple regression analysis failed to reveal any significant contribution from additional variables to the observed patterns of genetic diversity.

3.3.3. Within-individual gene diversity and levels of contaminants

Pooling all individuals from all populations into an integrated analysis using the LSMEANS procedure, we found that individual genetic diversity (IR) was not significantly different among groups formed on the basis of Cu ($P = 0.655$) but a marginally significant trend was observed for Cd ($P = 0.056$). Moreover, a significant linear relationship was detected among those groups for Cd contamination ($P = 0.036$) with the linear contrast test, which indicated that IR was positively correlated with individual Cd contamination (Fig. 5). The latter analysis performed separately for each population revealed that the overall significant relationship was mainly driven by the significant or marginal positive linear correlation between IR and Cd contamination for four populations from the two lakes with highest level of Cd contamination, Dufault ($P = 0.034$) and Vaudray ($P = 0.062$), and two lakes with low levels of contamination, Dasserat ($P = 0.044$) and James ($P = 0.044$) (Fig. 6). On the other hand, there was no significant linear relationship detected among pooled groups for Cu contamination ($P = 0.493$).

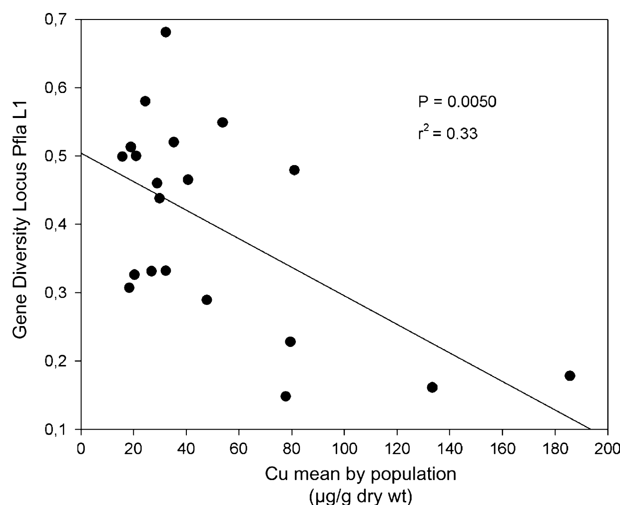

Fig. 4. Relationship between gene diversity of populations calculated from locus *Pfla L1* and population mean liver Cu concentrations. *P*-value and adjusted regression are indicated in the top-right corner.

4. Discussion

The main objective of this study was to examine the role of metal contamination in driving evolutionary change by documenting patterns of genetic diversity among populations of wild yellow perch in two major mining regions that were subjected

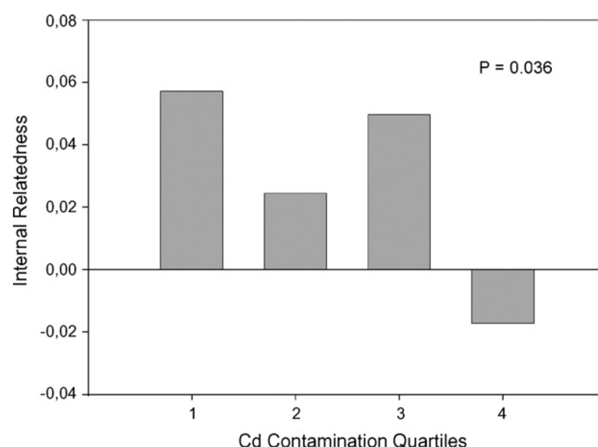

Fig. 5. Individuals' mean internal relatedness (IR) for each contamination group based on liver Cd concentration. The groups represent 4 quartiles formed using 1376 individuals (all samples pooled), the most contaminated being the 4th quartile. *P*-value of linear contrast is indicated.

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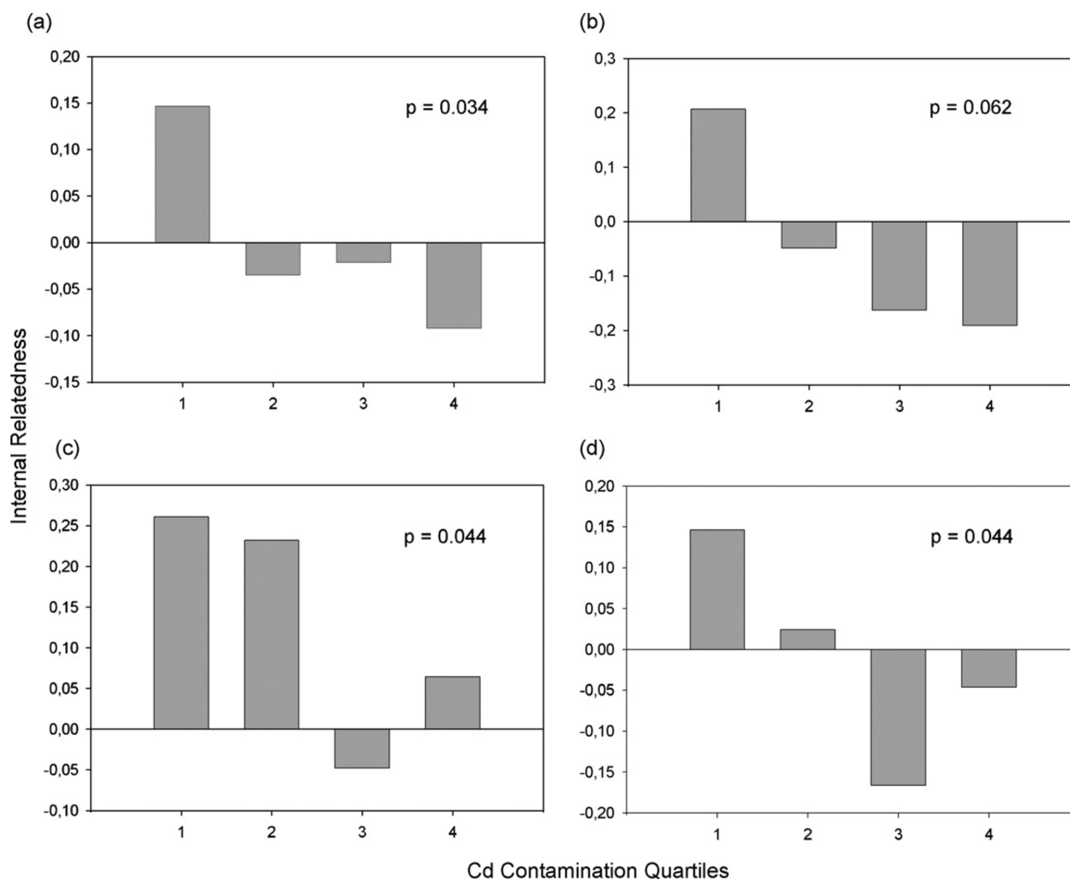

Fig. 6. Individuals' mean internal relatedness (IR) for each group formed based on their liver Cd concentration in four populations where the relationship was significant: (a) Dufault, (b) Vaudray, (c) James and (d) Dasserat. The groups represent 4 quartiles formed using all samples from each population, the most contaminated being the 4th quartile. *P*-values of linear contrasts are indicated.

to high metal emissions from smelters for over 50 years (∼1930 to ∼1985). The emissions are currently much better controlled, but lakes in the affected regions have not yet fully recovered (Gunn et al., 1995; Perceval et al., 2006). More specifically, we tested whether (i) contamination was involved in genetic differentiation between populations; (ii) within-population genetic diversity varied as a function of metal contamination, and (iii) whether individual genetic diversity was associated with the metal concentrations within individuals. Overall, our results revealed that metal contamination has had a significant impact at both the population and individual levels of genetic structuring, although apparent differential effects were observed between Cu and Cd. This study demonstrates that metal contaminants associated with mining activities have evolutionary impacts on the genetic makeup of yellow perch populations from northern Ontario and Québec.

As mentioned in Section 1, lakes in the two study regions were subjected to contamination by more than one metal. Based on the metal concentration gradients (i.e., the ratio of maximum to minimum concentrations), the major contaminants in the Rouyn-Noranda area are zinc and cadmium. Homeostatic control of internal zinc concentrations in yellow perch is very effective (Giguère et al., 2004, 2005), and these and other studies (Couture et al., 2008a; Pyle et al., 2008) have concluded that it is unlikely that indigenous perch are directly affected by zinc. The situation is different for cadmium, however, for which tissue metal levels respond directly to the gradient in ambient cadmium. Indeed, several lines of evidence suggest that cadmium is the metal primarily responsible for the biological deterioration of the lakes in the Rouyn-Noranda area (Campbell et al., 2003; Borgmann et al., 2004). In the Sudbury lakes, copper and nickel are the metals that demonstrate the greatest concentration gradient, although aqueous, but not sediment, cadmium concentrations in some lakes approach the high values reported in Rouyn-Noranda lakes (Couture et al., 2008a). For invertebrates in the Sudbury region, Borgmann et al. (2001) concluded that nickel was the metal most likely to cause toxicity. This metal was also demonstrated to accumulate in Sudbury yellow perch (Rajotte and Couture, 2002; Pyle et al., 2005; Giguère et al., 2006) as well as in fish from Rouyn-Noranda in the Spring (Couture et al., 2008a) and was related to effects on condition and metabolism in fish from both regions (Pyle et al., 2008; Couture et al., 2008b). In the following discussion we have focused on hepatic Cd and Cu as predictors of metal-induced effects. However, given the polymetallic nature of the contamination and the positive correlations reported for several hepatic metals in fish from these regions (Cd, Cu, Ni, Se and Zn; Couture et al., 2008a), we cannot establish definite cause → effect relationships.

First, partial Mantel tests controlling for the effect of geographic distance did not show a significant relationship between

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the extent of population differentiation and Cd or Cu contamination when performed over all loci. However, the analysis performed on individual loci revealed that a significant association between population structuring observed at locus *Pfla L1* and Cu contamination. Despite the fact that the neutrality test did not identify *Pfla L1* as an outlier locus potentially under selection, the above results suggest that selection imposed by Cu contamination may be partially responsible for the different pattern of population differentiation observed at this marker. This hypothesis was further supported by the results we observed when testing for an association between within-population genetic diversity at single loci and levels of metal contamination. Again, a highly significant negative correlation between gene diversity and Cu contamination was observed for locus *Pfla L1* only. Whereas many studies have demonstrated similar effects of pollution on genetic diversity either at the population or the individual level (see below), we are aware of only one study where a particular chemical agent impacted patterns of population differentiation. Thus, in a study of natural populations of brown rats resistant to a rodenticide, Kohn et al. (2003) showed that resistance was apparently due to directional selection in these populations and was assigned to a specific locus (*Rw*) containing a known microsatellite marker. Their results also showed that the extent of population differentiation was more pronounced at this marker than others. We thus propose that the differential pattern of population structuring and genetic diversity observed at *Pfla L1* and only for Cu could be indicative of the directional selective effect of this metal on a chromosomal region comprising this locus (Hansson and Westerberg, 2002). Admittedly, however, future experimental studies will be needed to rigorously test this causal effect.

Secondly, we observed a strong negative correlation between overall gene diversity and Cd concentration independent of fish age, length, weight, condition factor, effective population size and lake area. In contrast, no such global effect was observed for Cu, indicating a differential evolutionary impact of these two contaminants on the genetic composition of yellow perch populations. This study adds to the results of previous work performed on other species, which also revealed that contamination may cause a significant reduction of genetic diversity in impacted populations. Reductions of genetic diversity have been associated with a wide range of contaminants, either inorganic (Peles et al., 2003; Kim et al., 2003; Berckmoes et al., 2005; Maes et al., 2005; Keane et al., 2005), or organic (Baker et al., 2001; Chen et al., 2003; Theodorakis et al., 2006). For instance, in a study on the European eel (*Anguilla anguilla*), Maes et al. (2005) reported a reduced genetic variability in polluted eels at allozyme loci in addition to a significant negative correlation between a metal bioaccumulation index (for nine metals) and condition. Moreover, Keane et al. (2005) reported that the mean genetic similarity of dandelions (*Taraxacum officinale*) was always significantly higher at urban sites than at rural sites. They also observed a significant negative correlation between the number of genotypes at any given site and increasing amounts of airborne particular matter. Arguably, there are also many studies that failed to reveal significant effects of contamination on within-population genetic diversity (Berckmoes et al., 2005; Roark et al., 2005; McMillan et al., 2006). While these results may truly reflect a lack of such effects, many confounding factors may have hampered the detection of a significant effect of contamination. McMillan et al. (2006) argued that there are several reasons why population genetics change and why their consequences cannot be predicted simply by the magnitude of contaminant exposure. For example, population expansion and successful immigration of new individuals may help populations to buffer against losses of genetic diversity. Berckmoes et al. (2005) concluded that genetic diversity in seven wood mouse (*Apodemus sylvaticus*) populations was not affected by metal contamination and they proposed two hypotheses that could have contributed to this conclusion, even though they had some evidence of contamination-related genetic structuring. These authors first suggested that the pollution-induced stress was not intense enough or that insufficient time had passed to induce a population genetics response. Then, they proposed that important gene flow among populations could have masked the effects of metals on local populations. Here, the effects of such potential confounding factors were mitigated by the fact that we analysed a large number of populations among which the amount of gene flow is apparently very low, as revealed by the high F_{ST} values that we observed. For instance, applying the standard relationship linking F_{ST} to gene flow (Nm estimate ($F_{ST} = 1/(4Nm + 1)$)), the mean F_{ST} value of 0.362 we observed suggests that the mean level of gene flow among all populations analysed is roughly 0.5 migrant per generation. Moreover, lakes were selected to reflect a strong gradient of contamination on the basis of both duration (up to 50 years) and intensity of exposure to metal contaminants.

In studies where a significant impact of contaminants on genetic diversity has been reported, the causal effect has been associated with two main explanations: a demographic bottleneck induced by pollution, or selective pressure exerted by contaminants (Bickham et al., 2000; Baker et al., 2001; Keane et al., 2005). Here, using our analyses of locus diversity, we did not find any significant evidence for a population bottleneck in any lake sampled, apparently suggesting that contamination has not caused a pronounced and very rapid population decline. Admittedly, however, the small number of loci that we used coupled with probability adjustments to account for simultaneous multiple tests may not have offered sufficient power to detect recent bottlenecks (Cornuet and Luikart, 1996). Moreover, the stepwise mutation model (SMM) is statistically conservative (Luikart and Cornuet, 1998; Spencer et al., 2000); the heterozygosity excess test is sensitive to the severity of the population size reduction, and should detect only historical population bottlenecks that were severe (Luikart and Cornuet, 1998). Thus, we cannot rigorously exclude the possibility that reduced diversity in lakes contaminated by Cd was indeed caused by population bottlenecks on the basis of this test alone. In fact, it is known that habitat quality in some of these lakes was much worse in the past than it is today, and that perch populations in those lakes were reduced to very small numbers (Yan and Welbourn, 1990). Furthermore, we did not find any significant correlation between contemporary estimates of population effective size or lake area (which in lakes of similar morphometry and nutrient

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status could represent a surrogate for census size; see Johnson et al., 1992) with gene diversity. This result suggests that present demographic conditions cannot explain the low level of diversity observed in contaminated populations. Consequently, we propose that the most likely explanation for the pattern of genetic diversity we observed is linked to the important demographic reductions associated with metal contamination that occurred in the past when environmental conditions were harsher. While our results do not allow us to conclude that demographic decline in contaminated lakes was associated with intense selective pressures acting on unfit genotypes (but see below), they do suggest that metal pollution has had an evolutionary impact on perch populations since we observed a significant loss of genetic variation in these populations. Theory predicts, and empirical studies have shown, that a reduction of genetic diversity may in turn reduce individual fitness due to inbreeding effects, and reduce as well the evolutionary potential to adapt to environmental change (Theodorakis, 2001; Van Straalen and Timmermans, 2002; Reed and Frankham, 2003).

Previous physiological studies suggest that metal contamination has caused significant phenotypic change in the yellow perch populations we studied. In their study of Sudbury lakes, Rajotte and Couture (2002) reported that nucleoside diphosphate kinase activity, an indicator of biosynthesis, was higher in the most metal-contaminated fish. This suggested an increased rate of protein turnover and a bioenergetic cost of metal exposure, which corresponded to a lower growth rate in these fish. Furthermore, their results suggested that yellow perch from the most metal-contaminated lakes exhibited lower aerobic capacities. A more recent study by Audet and Couture (2003) revealed lower muscle aerobic and anaerobic capacities and higher liver anaerobic capacities in a contaminated population compared to a control one. Direct effects of metals on the yellow perch endocrine systems, which were detected in a pattern linked to accumulated metal concentrations (i.e., along the metal exposure gradient), have been reported in lakes sampled in the Rouyn-Noranda area and also in Sudbury (Gravel et al., 2005; Rasmussen et al., 2008). Adult and 1^+ perch collected from the more contaminated lakes exhibited an attenuated cortisol stress response *in vivo*, whereas young-of-the-year did not. Lower levels of the thyroid hormones T3 and T4, key hormones for regulation of intermediary metabolism and osmoregulation, were measured in adult yellow perch from contaminated lakes (Levesque et al., 2003; Gravel et al., 2005). However, in order to assess whether these phenotypic differences are the result of evolutionary change (change in the genetic architecture), the quantitative genetic parameters (heritability, genetic (co)variance) would have to be determined in controlled experiments. Such experiments would also allow testing whether the observed phenotypic differences between populations from contaminated and non-contaminated lakes have been driven by divergent selection, for instance by comparing the extent of quantitative genetic differentiation at phenotypic traits (Q_{ST}) with that of neutral expectations quantified at microsatellite loci (F_{ST}) (Spitze, 1993).

Further evidence that Cd contamination may exert differential selective pressures associated with levels of genetic diversity was suggested by the test of association between levels of an individual's genetic diversity (as measured by the internal relatedness parameter) and its Fulton condition factor and its metal contamination level. No significant relationship was observed with FCF, or for levels of accumulated Cu. However, this analysis did reveal that individual genetic diversity (IR) was positively correlated with individual Cd contamination. The analysis performed separately for each population revealed that the overall significant relationship was driven by a positive correlation between IR and Cd contamination in four populations. Overall, while these results do not allow us to generalize the positive association between levels of individual genetic diversity and tolerance to metal contamination, they do provide at least partial evidence that less inbred perch are more tolerant to Cd contamination in some circumstances. In such cases then, one would predict that individual fitness should increase with individual genetic diversity, and consequently, selective pressures exerted by Cd contamination should favour the maintenance of higher genetic diversity within contaminated populations. For instance, Peles et al. (2003) also observed significant higher individual diversity in earthworm (*Lumbricus rubellus*) populations inhabiting contaminated sewage sludge, and suggested that heterozygosity may be selectively advantageous to individuals that are exposed to multiple toxicants. Here, however, we observed a pronounced reduction in genetic diversity as a function of increased levels of Cd contamination. Taken as a whole, these results suggest that the selective response to contamination has been large enough to substantially reduce the effective population size and thus within-population genetic diversity, despite the fact that the less inbred individuals may be favoured by selection within any given population. Further experiments to assess the relative survival or growth of more diverse vs. less diverse individuals would allow a more rigorous assessment of the importance of Cd contamination as a selective force acting on different genotypes.

5. Conclusions

With reference to our three original objectives, we have demonstrated (1) that yellow perch from the Rouyn-Noranda and Sudbury regions are genetically distinct, (2) that overall genetic diversity decreases along a gradient of increasing Cd contamination and that Cu contamination may be involved in reducing the diversity at one of the seven loci we analysed, and (3) that within a lake population, increased metal tolerance (indicated by higher tissue Cd concentrations) is associated with higher genetic diversity. Overall, our results indicate that measurable evolutionary impacts of pollution can be observed in just an average human lifetime, thus adding to the growing evidence that human activities are not only affecting the demography and the ecology of wild species, but also their evolutionary trajectory (Ashley et al., 2003; Smith and Bernatchez, 2008). Namely, since genetic diversity provides the potential to adapt to selective forces (Gillespie and Guttman, 1989), and despite the recent recovery of many lakes impacted by toxic industrial emissions (Gunn et al., 1995), our results support the hypothesis that Cu, but more importantly Cd contamination, may have at least par-

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tially compromised the evolutionary potential of those species composing aquatic communities from these regions with respect to future environmental change. Finally, given that these regions are also affected by zinc and nickel contamination, investigation of the possible impacts of these metals on the genetics of wild aquatic species is also needed to better understand the consequences for chronically exposed populations. Also, diversity and gene expression of specific genes involved in metabolic activities or detoxification mechanisms should be the focus of the next steps towards improving our understanding of selective pressures exerted by metal contamination. Indeed, as stated by Staton et al. (2001), a single gene approach may provide the strongest model system for the genetic assessment of environmental impacts on natural population where there is strong evidence of gene/contaminant interaction.

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