Genetics of mine invasions by *Deschampsia cespitosa* (Poaceae)

ELIZABETH J. BUSH AND SPENCER C.H. BARRETT
Department of Botany, University of Toronto, Toronto, ON M5S 3B2, Canada

Received February 2, 1993

BUSH, E.J., and BARRETT, S.C.H. 1993. Genetics of mine invasions by *Deschampsia cespitosa* (Poaceae). Can. J. Bot. **71**: 1336–1348.

Deschampsia cespitosa is a self-incompatible, tussock-forming, perennial grass with a scattered distribution in Ontario, primarily along the shores of the Great Lakes. In recent decades, *D. cespitosa* has colonized metal-contaminated sites in the mining regions of Sudbury and Cobalt. Isozyme variation in populations from contaminated and uncontaminated sites were compared to investigate the genetic consequences of mine invasion. Central Ontario populations are diploid ($2n = 26$); however, the complexity of electrophoretic patterns suggested that *D. cespitosa* is a diploidized tetraploid with considerable gene duplication. Innovative approaches were therefore required for quantitative assessment of isozyme variability within and among populations. Eighteen populations of *D. cespitosa* were assayed for variation at nine enzyme systems, representing 19 putative isozyme loci. Populations included eight from various uncontaminated habitats, five from mine sites around Sudbury, and five from Cobalt. Lower levels of diversity were evident in both Sudbury and Cobalt populations relative to uncontaminated populations. The results corroborated the prediction that colonization of contaminated habitats reduces levels of genetic variability, particularly where populations are recently established. Strong selection on mine sites will also compound stochastic loss of genetic diversity associated with colonization. The distribution of isozyme variation among populations of *D. cespitosa* was also used to infer colonization history. Cobalt and Sudbury populations were clearly differentiated by unique alleles at a number of enzyme systems, providing evidence for the independent origin of metal-tolerant populations in the two mining regions. Estimates of outcrossing frequency revealed no significant difference between a mine and an uncontaminated population; both populations exhibited high levels of outcrossing.

Key words: colonization, mine invasion, genetic variation, *Deschampsia cespitosa*.

BUSH, E.J., et BARRETT, S.C.H. 1993. Genetics of mine invasions by *Deschampsia cespitosa* (Poaceae). Can. J. Bot. **71** : 1336–1348.

Le *Deschampsia cespitosa* est une herbe pérenne, auto-incompatible, qui forme des touffes et est dispersée en Ontario; on la retrouve surtout le long des rives des Grands-Lacs. Au cours des récentes décades, le *D. cespitosa* a colonisé les sites contaminés par les métaux dans les régions minières de Sudbury et de Cobalt. Les auteurs ont comparé les patrons isozymiques des populations de sites contaminés et de sites non-contaminés afin d'étudier les conséquences génétiques de l'invasion des sites miniers. Les populations du centre de l'Ontario sont diploïdes ($2n = 26$); cependant la complexité des patrons électrophorétiques suggère que le *D. cespitosa* serait un tétraploïde diploïdisé, comportant une importante duplication de gènes. Devant cette constatation, il a fallu prendre des mesures originales pour évaluer quantitativement la variabilité isozymique à l'intérieure et entre les populations. Ils ont examiné 18 populations du *D. cespitosa* en suivant la variation de neuf systèmes enzymatiques représentant présumément 19 lieux isozymiques. Parmi ces populations, on en compte huit provenant d'habitats non-contaminés, cinq de sites miniers de la région de Sudbury et cinq de celle de Cobalt. Comparativement aux populations de sites non-contaminés, on retrouve moins de diversité chez les populations de Sudbury et de Cobalt. Ces résultats confirment ce à quoi on s'attendait, à savoir que la colonisation des habitats contaminés réduit l'ampleur de la variabilité génétique, surtout là où les populations sont fraîchement établies. La forte sélection qui s'exerce sur les sites miniers va également composer la perte stochastique de diversité génétique associée avec la colonisation. Les auteurs ont également utilisé la variation isozymique entre les populations du *D. cespitosa* pour en déduire l'historique de la colonisation. Les populations de Cobalt et de Sudbury se distinguent clairement par des allèles uniques chez un nombre de systèmes enzymatiques, ce qui démontre que les populations qui tolèrent les métaux dans ces deux régions minières ont des origines distinctes. Les estimations de fréquences des croisement externes ne révèlent aucune différence significative entre une population provenant d'une mine et celle provenant d'une région non-contaminée; les deux populations montrent des croisements externes très fréquents.

Mots clés : colonisation, invasion des sites miniers, variabilité génétique, *Deschampsia cespitosa*.

[Traduit par la rédaction]

Introduction

Colonizing species have long been recognized as excellent organisms for the study of microevolution (Baker and Stebbins 1965; Parsons 1983). The rapid life cycles and prolific reproduction of many weedy plants, in concert with their invasion of disturbed habitats, provide opportunities for studying evolutionary processes on a human time scale. Investigations of the levels of genetic variation in colonizing populations have been of particular interest, since they will determine to a large extent the capacity of populations to respond genetically to environmental change (Brown and Marshall 1981; Barrett and Richardson 1986; Gray 1986; Barrett and Husband 1989; Warwick 1990; Barrett 1992). The theoretical groundwork for the genetic consequences of colonization is well developed (Wright 1931; Mayr 1963; Nei et al. 1975; Sirkkomaa 1983; Watterson 1984; Maruyama and Fuerst 1984, 1985*a*, 1985*b*). The most elementary prediction is that stochastic losses of genetic variation will occur during long-distance colonization as a consequence of restricted founder size and restrictions on gene flow resulting from geographic isolation. A decrease in genetic diversity may involve both a loss of alleles and reduced heterozygosity. Reductions in allelic richness are most affected by the size of the initial population bottleneck, whereas reduced heterozygosity depends more upon the length of time that founding populations remain small following the initial colonization event (Nei et al. 1975).

Dispersal to distant sites can involve colonization of novel environments, imposing new selection pressures on founding populations. Anthropogenic stresses have been instrumental in altering many natural habitats and we can assume that novel

Printed in Canada / Imprimé au Canada

selection pressures may also operate on colonizers of disturbed environments (Baker 1974; Barrett 1988; Bradshaw and McNeilly 1991). The colonization of metal-contaminated soils around mines provides one of the best examples of strong directional selection in a novel habitat. The rapid evolution of metal-tolerant plant populations has become a paradigm for studies of natural selection and microevolution (Bradshaw et al. 1965). The rapidity of the evolutionary response occurs because individuals that lack genes conferring metal tolerance die prior to, or shortly after, germination on metal-contaminated soils (Wu et al. 1975; Gartside and McNeilly 1974). Therefore, where colonization of mine sites proceeds via dispersal of propagules from uncontaminated habitats, we would expect survivorship among the founders to be low, depending on the frequency of tolerance genes among the founding members.

Some of the best known genetic studies of metal tolerance in plant populations are the studies of Bradshaw and colleagues on mine-tailing populations of *Agrostis* and *Anthoxanthum* species in the U.K. (reviewed in Antonovics et al. 1971) and *Mimulus guttatus* in California (Macnair 1983). This research largely focused on quantifying the degree to which tolerant populations are genetically differentiated from adjacent nontolerant populations and understanding the genetic basis of heavy metal tolerance. Levels of genetic diversity in populations from contaminated and uncontaminated sites were not measured explicitly in these studies. With the advent of techniques for assaying isozyme variation, opportunities now exist for investigating the genetic consequences of mine invasion by metal tolerant plant populations. There have, however, been surprisingly few studies of population genetic structure in metal-tolerant plants (Farris and Schaal 1983; Verkleij et al. 1985, 1989; Shaw 1991), despite considerable work on isozyme variation in colonizers of other disturbed environments (reviewed in Barrett and Shore 1989).

Deschampsia cespitosa (L.) Beauv. (Poaceae) is a self-incompatible, tussock-forming perennial grass found most commonly in grasslands and woodlands, particularly in sites with poor nutrient conditions and (or) impeded drainage (Davy 1980). Two cytotypes of *D. cespitosa* are recognized: a diploid ($2n = 26$) and a tetraploid ($2n = 52$) (Rothera and Davy 1986). In the U.K. both cytotypes are common; however, the tetraploid is the more weedy of the two. *Deschampsia cespitosa* has an extensive distribution, encompassing mainly circumboreal regions. In Ontario, the species is relatively uncommon, with a scattered distribution along the shores of the Great Lakes and inland along some river valleys. Recently, the spread of *D. cespitosa* to contaminated lands around mine sites has occurred.

Since the discovery of nickel and copper deposits in the Sudbury area of Ontario in the late 1800s, smelting operations have caused large quantities of SO_2 and metal particulates to be emitted into the atmosphere. The deposition of aerial pollutants led to the contamination of soils and ground water with copper, nickel, and cobalt. There are also elevated levels of soil extractable iron and aluminum. In 1972, changes in the mining industry were instrumental in reducing atmospheric levels of SO_2 around Sudbury. A number of smelters were closed, others reduced emissions, and the Coppercliff superstack was built. Shortly thereafter, *D. cespitosa* was first observed colonizing the barren lands around smelters (Cox and Hutchinson 1979, 1980, 1981). In the past 20 years, *D. cespitosa* has spread explosively throughout the Sudbury area; however, no local populations of *D. cespitosa* have yet been found on uncontaminated soils within the Sudbury area despite botanical exploration. The nearest known populations are located 80–100 km to the southwest of Sudbury, at Chutes, on the north shore of Lake Huron and at Little Current on Manitoulin Island.

Current work shows that metal tolerant individuals occur at low frequency (0.1–0.5%) in populations at these and other uncontaminated sites in the Georgian Bay region (C. Schultz and T.C. Hutchinson, unpublished data). Although the distance between these uncontaminated sites and Sudbury is relatively large, a mechanism for dispersal was proposed by T.C. Hutchinson. He suggests that seeds may have been transported to Sudbury as contaminants of coal shipments that were deposited at Little Current, on Manitoulin Island, prior to transport by rail to the Sudbury smelters. Today, two distinct subpopulations of *D. cespitosa* occur at Little Current: one on the limestone pavement typical of the natural habitat, the other growing directly on abandoned coal piles close to the rail line.

The discovery of metal-tolerant populations of *D. cespitosa* at Sudbury led to further exploration of mining regions in Ontario during which populations of *D. cespitosa* were also found at Cobalt, approximately 150 km northeast of Sudbury. Mining started at Cobalt in the early 1900s; however, since smelters were not operated at Cobalt, there was not the widespread destruction of vegetation from SO_2 poisoning so characteristic of Sudbury. Instead, the region around Cobalt is still forested, and populations of *D. cespitosa* are found growing in open patches in the forest on deposits of mine tailings and adjacent to abandoned mines. The tailings are contaminated with cobalt, nickel, and arsenic (C. Schultz, personal communication). To date, no populations of *D. cespitosa* have been located on uncontaminated soils in the vicinity of mine sites, but the possibility of local source populations seems reasonable.

The information presented above illustrates that an excellent opportunity existed for a study of the genetic consequences of mine colonization by *D. cespitosa* in Ontario. An analysis of isozyme variation in contaminated and uncontaminated populations of *D. cespitosa* was therefore used to address the following three major questions that arise from the discussions presented above: (*i*) Has there been a loss of genetic diversity associated with the colonization of mine sites at Cobalt and Sudbury? (*ii*) Do metal-tolerant populations at Cobalt and Sudbury have independent evolutionary origins? Alternatively, has there been dispersal of pre-adapted metal-tolerant propagules between the two mining regions? (*iii*) Is the Little Current population on Manitoulin Island the ancestral source of the populations at Sudbury?

An additional avenue of investigation concerned possible differences in the mating systems of contaminated and uncontaminated populations of *D. cespitosa*. Our interest in this question stemmed from earlier reports in the literature that suggested that the evolution of self-fertility in normally self-incompatible species was associated with colonization of mine sites (Antonovics 1968; Lefèbvre 1970). Whether this is a general phenomenon accompanying the evolution of metal-tolerant populations, either as a mechanism for preserving co-adapted gene complexes in mine environments or for securing reproductive assurance during mine colonization, requires testing. We therefore investigated this problem by estimating the frequency of outcrossing using isozyme markers in a contaminated versus an uncontaminated population.

No previous studies of isozyme variation had been published

on *D. cespitosa* when our study was initiated; therefore, before the above questions could be addressed it was necessary to understand the nature of the banding patterns revealed by electrophoresis. Of particular importance was the ability to interpret the genetic basis of the observed variation and to develop quantitative measures that would enable comparisons to be made among populations. We therefore begin with a short description of our electrophoretic studies of *D. cespitosa*.

Genetics of *Deschampsia cespitosa*

Genetic variation within Ontario populations of *D. cespitosa* was analyzed using horizontal starch gel electrophoresis. Individuals were scored for variability at nine enzyme systems: 6-phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI), peroxidase (PER), phosphoglucomutase (PGM), NAD-dependent malate dehydrogenase (MDH), diaphorase (DIA), shikimic dehydrogenase (SKDH), triosephosphate isomerase (TPI), and aconitase (ACO). Of these nine enzymes, only peroxidase was scored in two distinct and noninteracting zones of the gel.

Interpretation of banding patterns

During preliminary electrophoretic screening of *D. cespitosa* it became apparent that the banding patterns exhibited on the gels were not typical of a diploid species. Rather, the complexity of the multibanded electrophoretic phenotypes indicated that for many enzyme systems multiple isozymes were present. Polyploidy, through duplication of the entire genome, is a major cause of duplicated isozymes. If a species is thought to be diploid and yet shows many examples of duplicated isozymes, then this should lead to a reevaluation of the ploidy level (Ohno 1970; Gottlieb 1982). The occurrence of extensive gene duplication in an organism that behaves cytogenetically as a diploid would suggest that the diploid is actually a diploidized polyploid. We propose that this may be the case for diploid ($2n = 26$) populations of *D. cespitosa* in Ontario. These patterns are in contrast to a recently published study of isozyme variation in alpine populations of *D. cespitosa* from Colorado, where disomic patterns of inheritance for all enzyme systems investigated were reported (Gehring and Linhart 1992). These authors suggest that plants from Colorado, like those from Ontario, are ancient tetraploids but that diploidization has progressed to a point where it was not detectable at the isozyme loci they studied.

The critical outcome of the diploidization process is that the number of gene loci is essentially doubled. For example, in a diploidized tetraploid a single locus that initially shows tetrasomic inheritance would be converted into two independently segregating Mendelian loci. Therefore, a recently diploidized tetraploid should show approximately twice as many isozyme loci as its nearest diploid relative. When species comparisons are not available, the number of enzyme loci observed can be compared with the number expected based upon the theory of conservation of isozyme number in diploid plants (Gottlieb 1982).

Unfortunately, the electrophoretic patterns of diploidized tetraploids can become extremely complex. If an enzyme is oligomeric, heteromeric molecules can be formed between duplicated isozymes located in the same cellular compartment. In fact, in the absence of information on the subcellular location of isozymes, the presence of interlocus heteromeric molecules is accepted as evidence of a gene duplication. If allozymic variants also occur at both of these loci, complicated banding patterns may emerge, as a result of both inter- and intra-locus formation of heteromers. Such patterns are expected in outbreeding diploidized polyploids since high levels of intralocus heterozygosity are expected. This contrasts with the observed fixed heterozygote electrophoretic phenotypes observed in some self-fertilizing diploidized polyploids (Roose and Gottlieb 1976).

Second, duplicated isozyme loci will often carry identical or nearly identical alleles at both loci and this creates problems of overlapping bands on the gel. This is because in diploidized tetraploids, former alleles are incorporated into the genome as separate genes. If the ancestral diploids were closely related, then it is quite possible that they carried identical alleles at some loci. To interpret electrophoretic patterns when some bands are overlapping, it is necessary to rely on the staining intensity of bands to indicate how many doses or copies of a band are present.

Despite the potential for increased biochemical diversity, another factor may function to reduce isozyme number in diploidized polyploids. This is the process of gene silencing (Li 1980). Mutant alleles that render an enzyme inactive are termed null alleles. A locus that is homozygous null will not produce any activity on a gel since the gene has in effect been silenced and no enzyme product is produced. Unfortunately, heterozygous nulls are more difficult to detect since they can only be differentiated from homozygotes for the active allele if staining intensity differences are apparent for the two genotypes.

Our observations and interpretations of electrophoretic patterns in Ontario populations of *D. cespitosa* include examples of all of the complexities described above. Several of the enzymes that are routinely assayed by electrophoretic techniques are oligomeric enzymes. In *D. cespitosa*, 6PGD, PGI, *Per-2*, MDH, and TPI are dimers and *Dia-2* is a tetrameric molecule. Since the subcellular location of isozymes in *D. cespitosa* has not yet been explored, the occurrence of interlocus hybrid molecules was the only means of inferring gene duplication. No inferences about gene duplication could be made for monomeric enzymes.

Isozymes of 6PGD, PGI, MDH, and DIA all appear to have been duplicated, based on the occurrence of interlocus heteromeric molecules. Evidence to support this interpretation is discussed more fully in Bush (1990) in which photographs of gels illustrating complex multibanded phenotypes and genetic models to account for the variation are presented. TPI and anodal PER isozymes (*Per-1* and *Per-2*) do not show evidence of gene duplication, although the cathodal PER bands were not scored because of their multibanded complexity. Two PGM isozymes were present, but because it is a monomeric enzyme, it was not possible to determine whether the isozymes were in the same or separate cellular compartments. And finally, it was not possible to be sure of the number or location of ACO or SKDH isozymes because of uninterpretable phenotypic banding patterns; however, a minimum of two copies of each of these enzymes exist in *D. cespitosa*.

Further evidence of diploidization of the *D. cespitosa* genome is presented by the occurrence of null alleles at the DIA loci. Homozygous null phenotypes were present in both Cobalt and Sudbury populations. Loci heterozygous for null alleles remained undetected, but may have been present in uncontaminated populations also. Whether these alleles were truly silenced through the accumulation of mutations disrupting catalytic function of the enzyme or whether the allozymes

TABLE 1. Protocol for analysis of isozyme variation in populations of *Deschampsia cespitosa* from central Ontario

Enzyme	No. of loci	Subunit structure	Form of analysis	Comments
6PGD	2	Dimer	Alleles, bands, phenotypes	*6pgd-1* is monomorphic in all populations, therefore allele frequencies for *6pgd-2* obtained
PER-1	1	Monomer	Alleles, bands, phenotypes	Allele frequencies for this single monomeric locus obtained
PER-2	1	Dimer	Alleles, bands, phenotypes	Allele frequencies for this single dimeric locus obtained
PGM	2	Monomer	Alleles (Sudbury)	*Pgm-1* is monomorphic at Sudbury therefore allelic frequency data for *Pgm-2* obtained
			Phenotypes Banding pattern and staining intensity differences among bands	Alleles common to both *Pgm* loci in other populations precluding genotypic analyses; staining intensity differences reflect the number of copies of a band present
MDH	3	Dimer	Alleles (11 populations) Phenotypes Banding pattern and staining intensity differences among bands	*Mdh-1* monomorphic in all populations; *Mdh-3* monomorphic in nine populations; common alleles at *Mdh-2* and *Mdh-3* precluded genotypic analyses in seven populations
TPI	2	Dimer	Phenotypes Banding pattern only	*Tpi-1* and *Tpi-2* migrate to adjacent positions on the gel; variant alleles could not be assigned to a locus unambiguously
SKDH	2	Monomer	Phenotypes Banding pattern only	Staining intensity differences among bands could not be consistently scored; some alleles pooled because of migration to adjacent positions and hence could not be reliably distinguished
ACO	2	Monomer	Phenotypes Banding pattern only	Staining intensity differences among bands could not be scored; no genetic model; uninterpretable phenotypes at Sudbury
PGI	2	Dimer	Phenotypes (*i*) Banding pattern and staining intensity differences among bands (*ii*) Blurred phenotypes, record most anodal and cathodal positions	Alleles common to both loci precluded genotypic analysis of the data; blurred phenotypes due to multiple bands migrating to adjacent positions and diffusion of stain across the bands
DIA	2	Tetramer	Phenotypes (*i*) Banding pattern only (*ii*) Blurred phenotypes	Alleles common to both loci and presence of null allele precluded genotypic analysis of data; diffusion of stain across bands in adjacent positions (complex multibanded phenotypes due to tetrameric structure)

remained inactive only under the set of electrophoretic conditions employed remains uncertain.

Two other pieces of evidence support the diploidization of the *D. cespitosa* genome. Rothera and Davy (1986) report that in British populations, regular meiosis is observed and that "Bivalents are produced overwhelmingly with only an occasional quadrivalent suspected in both diploid and tetraploid meiocytes." The latter statement, referring to the occurrence of occasional quadrivalents, provides additional support for a polyploid origin for the $2n = 26$ cytotype of *D. cespitosa*. Furthermore, the presence of common alleles at many of the enzymes assayed in this study (6PGD, PGM, PGI, MDH, DIA) is an additional indication of gene duplication.

Taken together, there are a number of different lines of evidence that lend credence to the theory that populations of *D. cespitosa* in Ontario with 26 chromosomes are diploidized tetraploids. To corroborate this theory it would be necessary to determine the specific subcellular locations of isozymes and also to conduct formal genetic analyses that would confirm the models of inheritance for the isozymes.

Quantitative measures

The complexity of electrophoretic banding patterns arising from polyploidy presents a challenge for quantitative analyses of isozyme variation. The approaches employed in this study can be grouped into two main categories. The most significant difference between these and standard approaches is that variation among loci had to be pooled. The occurrence of multiple isozymes with shared allelic diversity prevented an analysis of variation at individual loci. In some cases though, estimates of allele frequencies were possible and from these, standard population genetic parameters could be obtained.

The first approach involved quantifying the number of electrophoretic phenotypes observed for a particular enzyme zone. This method was used to analyze isozyme variation in the diploidized tetraploid *Avena barbata* (Kahler et al. 1980). Each unique set of bands observed was classified as a unique phenotype. Phenotypes were differentiated by their banding pattern alone, or where possible, by differences in the staining intensity of component bands when such differences were consistently scored.

The second approach focused on the number and frequency of individual bands at an enzyme zone. This approach was used to estimate isozyme variability in populations of *Rumex acetosella* (Farris and Schaal 1983). Each band that is the product of a gene was counted separately. Thus, it was essential that variant enzymes coded by different alleles could be distinguished from secondary isozymes (isozymes formed from post-translational modifications) and from bands representing heteromeric molecules. If these distinctions cannot be made, then estimates of band diversity will be artificially inflated. This method of assessing enzyme diversity is a true measure of the biochemical diversity available to the plant; however, it masks the genetic variability at individual loci by pooling variation among loci.

The protocol followed for analyzing variation at nine poly-

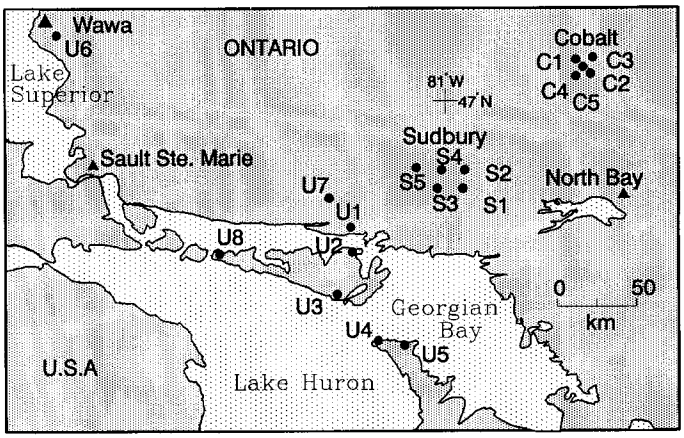

FIG. 1. The location of the 18 central Ontario populations of *Deschampsia cespitosa* investigated in this study. Five populations were sampled from each of the two mining regions of Cobalt (C) and Sudbury (S) and the remaining populations (U) were sampled from uncontaminated sites near Georgian Bay and near Wawa.

morphic enzyme systems representing 10 enzyme zones (peroxidase was scored in two independent zones) is detailed in Table 1.

Materials and methods

Collection of plant material

Eighteen populations of *D. cespitosa* were sampled from three different regions of central Ontario (Fig. 1). The location and sample size of each population is presented in Table 2. Cobalt populations C1, C2, and C3 were growing on tailings that are largely devoid of other vegetation, immediately adjacent to old mines. Population C4 was on an abandoned tailings outwash, and population C5 was growing along a roadside. The populations were separated by 2–10 km and ranged in size from a few hundred to many hundreds of individuals.

Five populations were sampled from the Sudbury area. The populations were growing in the open, sparsely vegetated, barren lands around the copper and nickel refineries and smelters. Populations S1–S4 were all within 20 km of each other, while population S5 was from Levack, approximately 35 km northwest of Sudbury. The Levack population consisted of less than 500 individuals. Population S1 was extremely large (> 10 000), occupying vast expanses of the denuded valley around the disused Coniston smelter. The other Sudbury populations, except S4, were also large (> 1000), and many local populations occurred on the mine properties besides those sampled for this study (see Cox and Hutchinson 1980).

Six uncontaminated populations were sampled from various sites in the Georgian Bay area of Lake Huron. The populations are most often found in shoreline or riparian habitats on calcareous soils that may occasionally be flooded. As mentioned previously, the Little Current population (U2) was subdivided, with plants growing both on limestone pavement and on abandoned coal piles. Two more uncontaminated populations were sampled from granitic Precambrian Shield sites at Chutes (U7) and Wawa (U6). Population sizes ranged from very small (approximately 200 individuals at U7, U1, U3, and U8) to many hundreds of individuals at U5.

For the population surveys, adult individuals were randomly sampled in the field and tillers brought back to the glasshouse for propagation. Since there is very little lateral spread of tussocks, individual genotypes were easy to identify in the field. At least 100 individuals were initially sampled from all populations, although subsequent mortality in the glasshouse reduced sample sizes in several populations (mean sample size = 84.4; range = 44–126; Table 2). For estimates of outcrossing frequency (t), maternal seed families were collected from one uncontaminated (U5) and one mine (S1) population. The seeds were first vernalized, then germinated in Petri dishes, whereupon they were transferred to plastic centrifuge tubes for propagation in solution culture (1/10 Arnon and Hoagland's solution).

Electrophoresis

Horizontal starch gel electrophoresis was carried out on leaf tissue using both young tissue at the base of tillers (where most enzymes showed the greatest activity) and older tissue at the leaf tip (where peroxidase was most active). The leaf tissue was ground in glass spotting plates with a steel-tipped, serrated grinding rod using an extraction buffer that consisted of 1 mg/mL dithiothreitol dissolved in a $NaPO_4$ buffer (0.05 M pH 7), plus a spatula tip of polyvinylpolypyrrolidine (PVPP). The homogenate was absorbed onto filter paper wicks that were then frozen at −80°C for long-term storage. Wicks could be stored for several months without significant loss of enzyme activity.

Twelve percent starch gels were used, to which 10 g of sucrose was added to improve the resolution of bands. Two different buffer systems were used. Buffer system A was a histidine – citric acid buffer, whereas buffer system B was a lithium hydroxide – boric acid buffer (Cardy et al. 1981). The running conditions are listed in Table 3 along with the migration distances of the front that gave the best resolution of bands for the nine enzyme systems scored.

The staining buffers and staining recipes used were modified from those reported in the literature as follows: 6PGD, PGI, PGM, and MDH from Cardy et al. (1981); ACO and TPI from Soltis et al. (1983); SKDH and DIA from Vallejos (1983); and PER from Shaw and Prasad (1970).

Measurements of genetic variation

Unique phenotypes, representing particular patterns of bands, were recorded at each enzyme zone. In some cases, phenotypes were distinguished only on the basis of banding pattern, while in other cases, two phenotypes with identical band positions were differentiated by staining intensity differences of component bands (see Table 1). Two measures of phenotypic diversity were used, the number of phenotypes per enzyme zone and P_j, an estimate of diversity derived from the following formula:

$$[1] \quad P_j = \sum_{i=1}^{n} p_i(1 - p_i)$$

where p_i is the frequency of the ith phenotype in the jth zone, and n is the number of phenotypes in that enzyme zone (Kahler et al. 1980). This calculation yields a measurement of diversity for each enzyme zone, from which estimates of mean population diversities can be derived. This formula is essentially that of the Simpson's index used for estimates of community diversity by ecologists (Simpson 1949).

With reference to Table 1, the next level in the hierarchical interpretation of electrophoretic data involved the identification of different bands within an enzyme zone. Each band counted represented the product of a gene; however, no assumptions were made about genetic relationships among bands. They may be allelic products of the same locus or products of different loci. Two measures of band diversity were used, the number of bands per enzyme zone and H, a measure of band diversity calculated as follows:

$$[2] \quad H = -\sum_{i=1}^{b} p_i(\ln p_i)$$

where p_i is the frequency of band i in the enzyme zone within the population, and b is the number of bands in that enzyme zone (Lewontin 1972; Farris and Schaal 1983). This is the Shannon–Weaver formula also commonly used by ecologists to measure species diversity (Shannon and Weaver 1949). It is different from the Simpson's index in that the sum of the frequencies of p_i are not bounded by 1.0. Mean population diversities were calculated directly by summing the values of H for each enzyme zone within a popula-

TABLE 2. Location and sample size (plants sampled) of the 18 populations of *Deschampsia cespitosa* from central Ontario investigated in this study

Cobalt		Sudbury		Uncontaminated	
Pop.	*N*	Pop.	*N*	Pop.	*N*
C1, Agnico Mine	94	S1, Coniston	126	U1, Whitefish Indian Reserve	108
C2, Diabase	87	S2, Falconbridge	69	U2, Little Current	108
C3, Temiskaming Mine	91	S3, Coppercliff	77	U3, S. Baymouth	98
C4, Cobalt	75	S4, O'Donnel Roast Bed	44	U4, Big Tub (Tobermory)	120
C5, Agnico Mine Roadside	44	S5, Levack	88	U5, Cypress Lake	100
				U6, Wawa	65
				U7, Chutes	70
				U8, Mississagi Lighthouse, W Manitoulin	56

NOTE: Major contaminants at Cobalt are Co, Ni, and As, and at Sudbury are Cu, Ni, Co, Al, and Fe.

tion and dividing by the number of enzyme zones. PGI data was excluded from this analysis since the frequency of individual bands could not be estimated. The estimate of band diversity for DIA was a minimum estimate for two reasons: (*i*) the frequency of the null allele is underestimated since only homozygous null phenotypes could be detected, and (*ii*) it was not possible to identify each band comprising some DIA phenotypes due to diffusion of stain among bands.

Where possible, allele frequencies were also calculated for some enzyme systems. Allele frequencies were thus estimated for all populations at three polymorphic loci, *6pgd-2, Per-1*, and *Per-2*, for 11 populations at the *Mdh-2* locus, and for the 5 Sudbury populations at *Pgm-2*. Standard population genetic parameters could then be estimated using this data, including Nei's diversity statistics (Nei 1973), which analyze subpopulation structure through the partitioning of genetic diversity into components within and among populations.

Statistical analysis of diversity estimates

To determine whether there were significant differences in the levels of genetic variation among populations, the populations were grouped into three categories: Cobalt, Sudbury, and uncontaminated populations. ANOVAs were performed on the data to test for significant group differences. Comparisons of all group pairs were subsequently carried out using Scheffé's test. Data transformations were undertaken if this improved the normality and homogeneity of group variances. Variances within groups were tested for homogeneity using the F_{Max} test. It was not possible to use ANOVA to detect group differences in the band diversity index (H), since neither the assumptions of normality nor homogeneous variances were met on either the untransformed or arcsine square root transformed data. In this case, each of the mine regions was compared to the uncontaminated region by means of a non-parametric Mann–Whitney U-test (planned comparison test).

Relationships among populations

Relationships among populations can be inferred from an analysis of the genetic similarity among populations, based on isozyme data. Because of the difficulties associated with scoring individual genotypes, the similarities among populations was estimated using the simple matching coefficient and data on the presence and absence of bands in each population (SYN-TAX III user's manual; Podani 1988). The presence of unique bands or alleles within regions was particularly useful for investigating the colonization history of mining regions. In theory, if populations in one mining region are descended from populations in another region, then one would expect the derived populations to contain no unique variability, assuming no mutation at the sample of isozyme loci analyzed.

Mating system estimation

The model of Ritland and Jain (1981) was used to obtain multilocus (t_m) estimates of outcrossing frequency. Outcrossing was estimated via the Newton–Raphson method and pollen gene frequency (p) was estimated by the expectation–maximization method. Pollen and ovule frequencies were constrained so that they were equal. Standard errors of the estimates were derived as the standard deviations of 100 bootstrapped values with the progeny array as the unit of resampling. Outcrossing frequencies for one mine (S1) and one uncontaminated population (U5) were calculated. Eight to 10 progeny per maternal parent were scored, in 24 and 21 families from the mine and uncontaminated populations, respectively. The multilocus genotype of the maternal parent was known for 23 families (mine) and 17 families (uncontaminated). The loci *Per-1, 6pgd-2*, and *Pgm-2* were used in both cases, and *Mdh-2* data were also used for estimates in the mine population. These were the only loci for which genotypic data were available and that yielded good activity from young seedling tissue. Outcrossing estimates from both the mine and uncontaminated populations were tested for significant departures from $t = 1.0$ using a Z-test (Steel and Torie 1980).

TABLE 3. Running conditions for buffer systems used in starch gel electrophoresis

Buffer	Gel size (mL)	Amp. (mA)	Voltage (V)	Front (cm)	Slicing order*
A	450	55	250–275	9.8–10.4	MDH, 6PGD, PGI, PGM
A	450	55	250–275	9.8–10.4	ACO, SKDH, DIA, TPI
B	300	60	125–175	5.7–6.5	PER

*MDH, NAD-dependent malate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; PGM, phosphoglucomutase; ACO, aconitase; SKDH, shikimic dehydrogenase; DIA, diaphorase; TPI, triosephosphate isomerase; PER, peroxidase.

Results

Isozyme variation in uncontaminated and mine populations

Variability at the 10 enzyme zones is coded by 19 putative isozyme loci. The proportion of polymorphic loci was high in all three groups of populations, with only a small decrease detected at Sudbury (mean percent polymorphic loci = 82.1, 86.3, and 86.9 for Sudbury, Cobalt, and the uncontaminated populations, respectively). Two of the 19 loci surveyed, *6pgd-1* and *Mdh-1*, were monomorphic in all populations.

There were significant differences among the three groups of populations in the mean number of phenotypes per enzyme zone, ($F = 27.70$, 2 df, $P = 0.0001$). Both mining regions had significantly fewer phenotypes per zone compared with uncon-

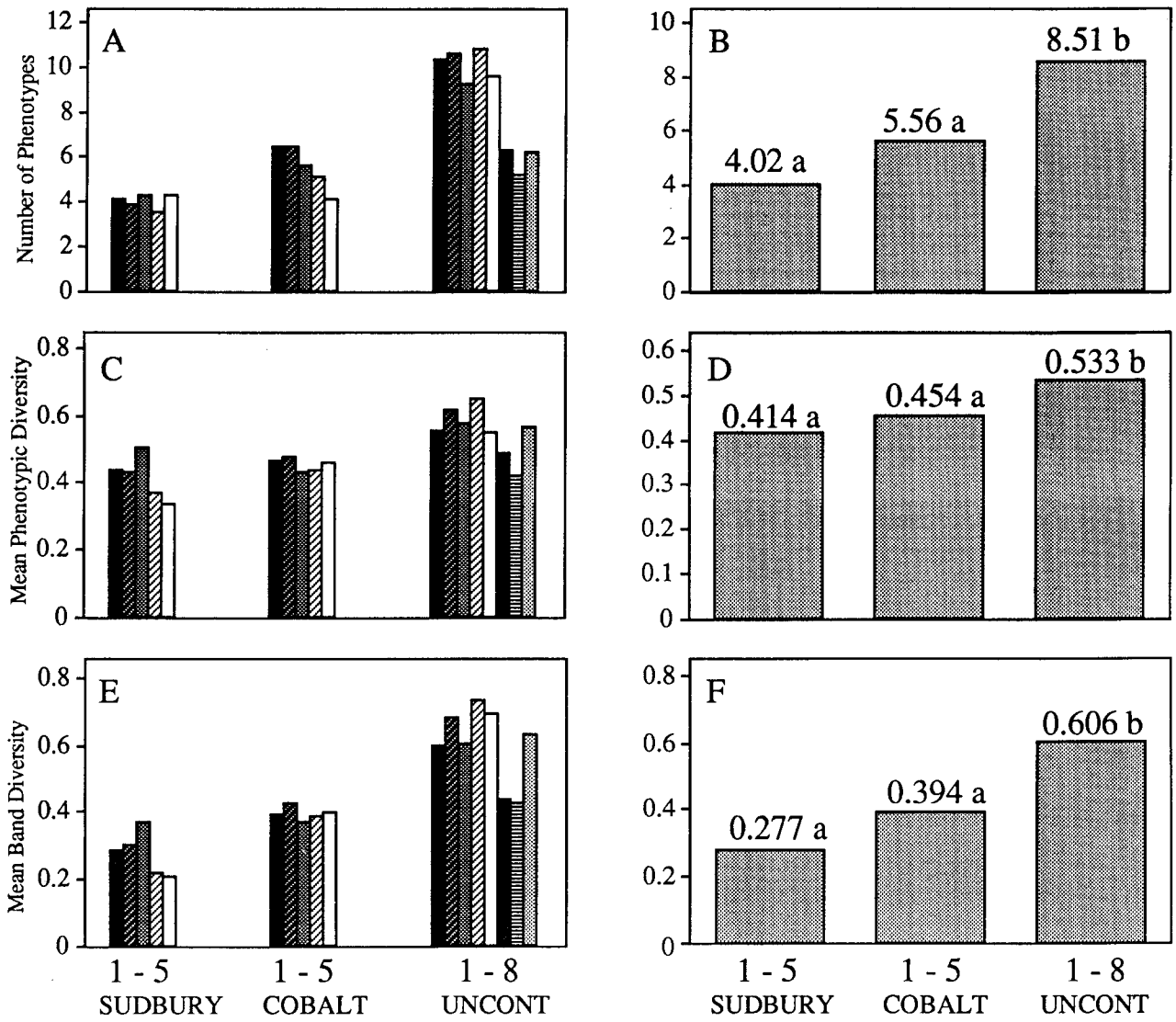

FIG. 2. Comparisons of the mean number of phenotypes (A and B), the phenotypic diversity (C and D) (including PGI data), and the band diversity (E and F) among 18 populations of *Deschampsia cespitosa* sampled from the mining regions of Cobalt and Sudbury and from uncontaminated sites in central Ontario. See Methods for details of how these measures were estimated in each population. (B, D, and F) Group means; means with different letters are significantly different.

taminated populations ($P \leq 0.05$, Figs. 2A, 2B). There were no significant differences between mining regions in the number of phenotypes. When all enzyme zones were included in the analysis, Sudbury and Cobalt had on average 4.02 and 5.56 phenotypes per zone, respectively, whereas the mean number of phenotypes per zone in the uncontaminated populations was 8.51. The total number of isozyme phenotypes in each of the three regions was 70, 110, and 237 for Sudbury, Cobalt, and the uncontaminated populations, respectively. Since there was an unusually high number of PGI phenotypes (ranged from 8 to 44 within populations and 10 out of 18 populations had >20 PGI phenotypes), the data on phenotype number were recalculated excluding PGI. In this case, the total number of isozyme phenotypes was 29, 49, and 136 for Sudbury, Cobalt, and the uncontaminated populations, respectively. The difference in mean number of phenotypes per enzyme zone between mining regions and uncontaminated populations was again significantly different ($P \leq 0.05$).

Phenotypic diversity values calculated with the modified Simpson's index are presented in Figs. 2C, 2D. This is a composite statistic incorporating both phenotypic richness and the evenness of phenotype frequencies. Mean population diversity values were calculated both excluding and including the data for PGI. In both cases, significant differences among groups of populations were evident (excluding PGI: $F = 8.72$, 2 df, $P = 0.003$; including PGI: $F = 9.10$, 2 df, $P = 0.003$). The results of Scheffé's test indicated that there was significantly lower phenotypic diversity within the mining regions relative to uncontaminated populations ($P \leq 0.05$). Phenotypic diversities within Cobalt and Sudbury were not significantly different from one another.

The mean number of bands per enzyme zone (PGM, TPI, DIA, SKDH, ACO, PGI) and the mean number of alleles per locus (*6pgd-1,2, Per-1,2, Mdh-1,2*) were compared among populations. Significant differences were evident among the three groups of populations in both cases. The number of bands per enzyme zone and the number of alleles per locus were both significantly lower in the two mining regions rela-

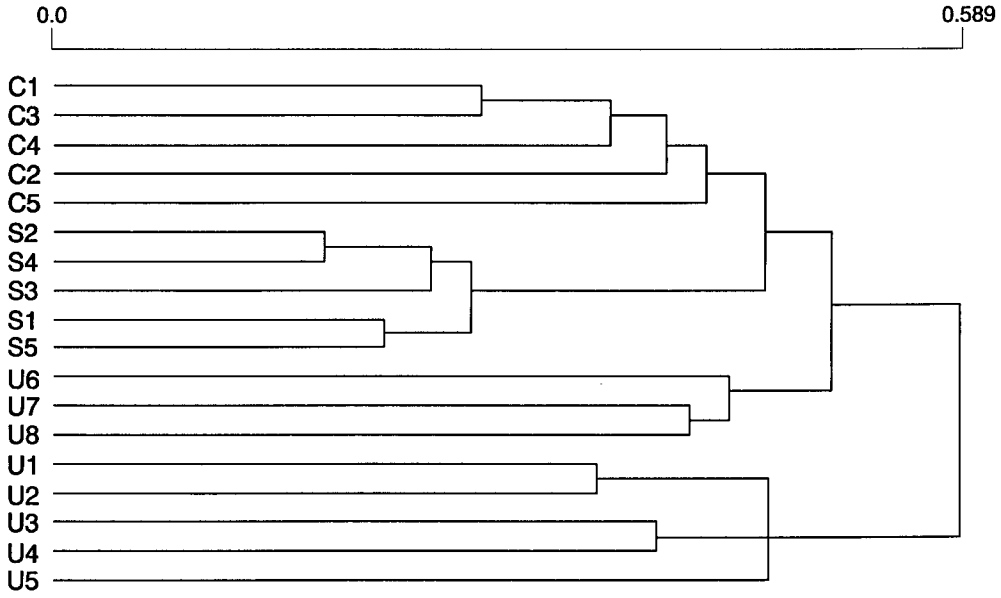

FIG. 3. UPGMA clustering based on simple matching coefficient analysis of binary data on the presence and absence of isozyme bands within 18 populations of *Deschampsia cespitosa* from central Ontario. Populations C1–C5 and S1–S5 are metal-tolerant populations from Cobalt and Sudbury, respectively. Populations U1–U8 are from uncontaminated sites.

tive to the uncontaminated populations ($P < 0.05$), whereas there were no significant differences between the mining regions (data not shown).

The Shannon–Weaver index of diversity is a composite measure that assesses both band richness and the evenness of band frequencies. A nonparametric test was required to test for differences in band diversity among mine and uncontaminated populations, since heterogeneous group variances prevented the use of ANOVA. Mann–Whitney *U*-tests indicated significant differences between Cobalt and the uncontaminated populations ($P = 0.0034$) and also between Sudbury and the uncontaminated populations ($P = 0.0054$; Figs. 2E, 2F).

Table 4 presents the results of an analysis of genetic diversity using Nei's diversity statistics (H_T, H_S, D_{ST}, G_{ST}, R_{ST}) (Nei 1973). The analysis was restricted to a subset of the enzymes assayed. Seven populations were analyzed for variation at three loci, six populations at four loci, and five loci were analyzed in all Sudbury populations. Analysis of variance detected significant group differences in the levels of genetic diversity within populations (H_S, $F = 4.55$, 2 df, $P = 0.028$); however, no significant differences were detected between any pair of groups (Cobalt, Sudbury, and the uncontaminated populations; Scheffé's test). In the group of uncontaminated populations, 87.7% of the total variation is accounted for by variation within populations (H_S/H_T) and 12.3% by variation among populations (G_{ST}). In contrast, both mining regions had a greater proportion of their total diversity accounted for by variation within populations (H_S/H_T = 98.4% for Cobalt and 91.3% for Sudbury).

Relationships among uncontaminated and mine populations

The results of the distance analysis of binary band data revealed that primary clusters coincided with the geographical distribution of populations (Fig. 3). Mining populations within both Cobalt and Sudbury were more closely related to each other than to any other population. According to this analysis, Cobalt and Sudbury populations are also more closely related to each other than to the uncontaminated populations. The groupings are partially explained by differences in the levels of diversity between mine and uncontaminated populations. Populations in both mining regions contain fewer bands than are present in the more diverse uncontaminated populations (Sudbury, 34; Cobalt, 44; uncontaminated, 62). The greater diversity of uncontaminated populations provides more opportunities for differentiation at isozyme loci in comparison with less diverse populations.

Although dendograms can be useful for demonstrating broad relationships among populations, they are not always the most appropriate tool for testing specific hypotheses about historical relationships. In this regard, the presence of unique alleles within populations is of special significance to the delineation of population relationships. Allele frequencies for Cobalt and Sudbury populations are presented in the Appendix. The data provide the most convincing evidence that metal-tolerant populations of *D. cespitosa* evolved at least twice in recent evolutionary history. Populations from the two mining regions are clearly differentiated at a number of enzyme loci. At both DIA and ACO, in particular, there were bands at high frequency in Sudbury that were absent from Cobalt. Similarly, a common DIA band at Cobalt was absent from Sudbury. There were also unique TPI bands at both Sudbury and Cobalt. One of the two

TABLE 4. Genetic diversity (Nei 1973) within and among populations of *Deschampsia cespitosa* from uncontaminated sites and from the two mining regions

Region	N	H_T*	H_S	H_S/H_T	G_{ST}†	R_{ST}‡
Cobalt	5	0.317	0.312	0.984	0.016	0.021
Sudbury	5	0.338	0.312	0.921	0.079	0.107
Uncontaminated	8	0.446	0.391	0.877	0.123	0.162
Total	18	0.404	0.347	0.860	0.140	0.172

NOTE: Values are based on data from three to five polymorphic isozyme loci.
*$H_T = H_S + D_S$.
†$G_{ST} = D_{ST}/H_T$.
‡$R_{ST} = D_M/H_S$.

TABLE 5. Estimates of outcrossing frequency (t) in two populations of *Deschampsia cespitosa* from central Ontario

Isozyme locus	Outcrossing frequency (SE)	
	Population S1 (24 families)	Population U5 (21 families)
Single locus estimates		
Per-2	0.791 (0.133)	1.002 (0.204)
6pgd-2	1.060 (0.112)	0.838 (0.149)
Mdh-2	0.864 (0.121)	not incl.
Mean	0.921 (0.083)	0.943 (0.106)
Multilocus estimate	0.870 (0.077)**	0.970 (0.092)*

NOTE: Population S1 is a mine population and population U5 is from an uncontaminated site. An additional locus (*Pgm-2*) was used to obtain the multilocus estimates for both populations. *, not significantly different from $t = 1.0$ ($Z = 0.33$, $p > 0.37$); **, significantly different from $t = 1.0$ ($Z = 1.63$, $p = 0.052$).

most common *6pgd-2* alleles in Sudbury was virtually absent from Cobalt, detected only rarely in one population, and there was a unique *6pgd-2* allele at Cobalt also. In addition, one of the common Sudbury PGM bands was much less frequent at Cobalt, although this fact could be explained by founder effects and genetic drift in colonizing populations. Finally, Cobalt is also distinguished from Sudbury by the presence of unique MDH alleles.

Mating system estimation

Single locus and multilocus estimates of outcrossing frequency, with their standard errors, are presented in Table 5 for one uncontaminated (U5) and one Sudbury (S1) population. There was no significant difference between the two estimates, based on comparison of the standard errors of the two estimates. However, the outcrossing estimate for the Sudbury population was significantly lower than $t = 1.0$.

Discussion

Metal-tolerant populations of *D. cespitosa* at Cobalt and Sudbury became established in relatively recent times. Although the source of both invasions are unknown, two possibilities can be entertained. Metal tolerance may have evolved once, with subsequent dispersal between the mines, or alternatively, the populations at Cobalt and Sudbury may have had independent evolutionary origins. In the latter case, colonizers may have been derived locally, from uncontaminated populations that are either extant or recently extirpated, or alternatively, colonizers may have originated in distant, uncontaminated source populations. The kinds and amount of genetic variation among populations of *D. cespitosa* will likely differ depending on which colonization scenario is correct. If colonization of the mines involved long-distance dispersal of migrants, then founder effects are expected to be more prominent in influencing population genetic structure than if source populations occurred locally.

Another factor to consider in interpreting the genetic structure of mine populations is the nature of the mine environment. Selection intensities must be very high on the contaminated soils. Concentrations of heavy metals are sufficient to cause rapid mortality in individuals lacking genes conferring tolerance to particular metals (Cox and Hutchinson 1980). Mine environments are likely to differ from natural habitats in other edaphic features as well, such as soil temperatures and moisture availability (Farris and Schaal 1983). Together, these factors indicate that both Cobalt and Sudbury would have presented extremely hostile environments that are certain to have imposed strong selection pressures on colonizers resulting in reductions in genetic variation.

Genetic consequences of colonization

When different measures of isozyme diversity were used to compare the variability of mine populations of *D. cespitosa* with uncontaminated populations, the results consistently supported the prediction that a loss of genetic diversity is associated with the colonization of mines. However, it is important to stress that mine populations were by no means genetically depauperate with respect to isozyme variation. Of the 19 putative loci assayed, the number that were polymorphic in Cobalt, Sudbury, and the uncontaminated populations ranged from 16 to 17, 15 to 16, and 15 to 17, respectively, indicating that mine populations were no less polymorphic than uncontaminated populations. Composite measures of diversity such as the band diversity and phenotypic diversity indices, which encompass both the richness and evenness of diversity, also indicate that despite genetic losses, mine populations retain high levels of variation.

The retention of considerable levels of genetic variation within mine populations can be attributed to a number of reproductive and demographic factors. *Deschampsia cespitosa* is a wind-pollinated outbreeder, and founders from outbreeding populations are likely to be heterozygous at many loci. 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. Furthermore, perenniality, wind-pollination, and at least at Sudbury, the rapid population expansion associated with mine colonization would all contribute to increasing effective population size, thus reducing opportunities for genetic drift (Loveless and Hamrick 1984; Barrett and Husband 1989). In addition, gene flow between local populations within mining regions may also help retain variability within these populations. Finally, the occurrence of duplicated loci arising from polyploidy can increase the potential variability of an enzyme. When both loci remain active, as appears to be the case for most enzymes in *D. cespitosa*, and some unique variation at either locus occurs, this increases the number of electrophoretic phenotypes that can be maintained in a diploid population.

Levels of genetic variation within populations in each of the three regions can provide information regarding the nature of colonization events. Mine populations were not the only ones with lower isozyme variability. Populations U6 and U7, and to some extent U8, are notably less diverse than other uncontaminated populations (Fig. 2; Table 4). This may reflect, in part, the fact that fewer individuals were sampled from these populations (mean sample size for the uncontaminated populations is 91, compared with 65, 70, and 56 for populations U6, U7, and U8, respectively). However, it may also indicate that founder effects and small population sizes will reduce population variability in outbreeders in nonstressful environments also. It is noteworthy that all three populations were small and geographically isolated from other populations in the study.

Although no significant differences were detected between the levels of phenotypic and genotypic variability at Cobalt and Sudbury, a consistent trend was apparent. For each diversity measure used, the variability at Cobalt exceeded that at Sudbury. One of the questions posed at the outset of this study

concerned the possibility that Sudbury and Cobalt populations were related as a source-derivative pair. The higher variability of Cobalt populations casts doubt on the likelihood that Sudbury populations were the source of the Cobalt invasion; ancestral populations are expected to be either more or equally diverse relative to derived populations. The alternative scenario, that Cobalt populations were the source of the Sudbury colonization, may at first seem more probable, based on the genetic variability data alone. However, information on the distribution of unique alleles, discussed below, provide more convincing evidence against the theory that the mine sites are related as a source-derivative pair. Higher levels of isozyme variation at Cobalt compared with Sudbury may be the result of gene flow from local, uncontaminated populations. Although the region surrounding the Cobalt mines has not been extensively explored, the sites of pollution stress at Cobalt are restricted to areas adjacent to the mines and tailing deposits. Suitable natural habitats for *D. cespitosa* are much more likely to occur locally than at Sudbury, where pollution is far more widespread. The lower variability of mine populations at Sudbury suggests that these are more likely to have descended from long-distance colonization event(s).

Reconstruction of colonization history

Inferences about colonization history are readily drawn from studies of isozyme variation in inbreeders, since variability within populations is often restricted to a small number of multilocus genotypes (Brown 1983). When colonizing populations contain different multilocus genotypes, it suggests that populations were founded independently (Moran and Marshall 1978; Golenberg 1989; Husband and Barrett 1991; Novak et al. 1991; J.J. Novak, unpublished data). The tendency for outcrossing species to be more variable within and less differentiated among populations complicates the use of isozyme variability for inferring historical relationships among conspecific populations. One approach that may be used involves a direct comparison of the allelic composition of different populations, in search of unique variability that can be used to infer colonization history.

One of the objectives of this research was to test the hypothesis that metal-tolerant populations at Cobalt and Sudbury have independent evolutionary origins. The critical assumption underlying the analysis of unique variation is that populations in both regions were established recently (no more than 90 years in Cobalt and 20 years in Sudbury). It is unlikely that novel mutations, at the small sample of loci assayed, will have arisen in either region during this short period in evolutionary history. Therefore, if populations in one mining region were descended from populations in the other mining region, the genetic composition of the derivative region should be a subset of variation from the ancestral region.

The results obtained in this study support the hypothesis that metal-tolerant populations of *D. cespitosa* in central Ontario evolved at least twice in recent evolutionary history. Cobalt and Sudbury each have unique bands at DIA loci that are frequent in all populations within each region. Sudbury is differentiated from Cobalt by a unique high frequency ACO allele as well. Two TPI bands present at Cobalt are absent from Sudbury and one TPI band is unique to Sudbury. A common *6pgd-2* allele in Sudbury is extremely rare at Cobalt, occurring at low frequency (0.03) in one population. Furthermore, a high frequency PGM band in Sudbury occurs at low frequency in Cobalt, and three PGM bands in Cobalt are absent from Sudbury. Thus, there is considerable evidence to falsify the hypothesis that dispersal from metal-tolerant populations in one mining region gave rise to populations in the other mining region.

On the other hand, it proved difficult to falsify the hypothesis that the uncontaminated population at Little Current may be the ancestor of metal-tolerant populations at Sudbury. One of two common *6pgd-2* alleles at Sudbury was infrequent in the Little Current population (p_i = 0.014). Although this could be a consequence of founder effects and genetic drift, the probability of a rare allele occurring in a founding population is small, and the probability decreases with decreasing founder size (Nei et al. 1975; Sirkkomaa 1983). There is a unique PGI band at Sudbury that is absent from Little Current; however, given the diversity at PGI (nine different bands in the Little Current population), the failure to detect an additional band may simply be a function of sample size. The third point is that a rare *Per-1* allele occurring in one Sudbury population only was also undetected at Little Current. While these different lines of evidence cast doubt on Little Current as the direct source, they do not enable a clear rejection of the hypothesis.

Although it is difficult to disprove the hypothesis that Little Current was the source of the Sudbury invasions, it is worth considering whether any of the other populations sampled appear to be better candidates as potential source populations. It has been suggested that the population at Chutes (U7) may be the source on the basis of geographical proximity, morphological resemblance, and similar soil conditions (i.e., acidic rather than calcareous) to the Sudbury populations (C. Schultz, personal communication). In light of the electrophoretic comparison of the Chutes population with Sudbury, this hypothesis seems unlikely since there is considerable differentiation between the two areas. Two common *6pgd-2* alleles and a common ACO band in Sudbury are both absent from Chutes, and there are five unique PGI bands at Sudbury.

The origin of the metal-tolerant populations at Sudbury remains unanswered, as does the question of whether there may have been multiple introductions from different source populations. The variability in mine populations makes it difficult to trace putative ancestors, since the common alleles at Sudbury are also present in most of the uncontaminated populations sampled. There is also little differentiation among Sudbury populations. Twenty-six out of 34 bands at Sudbury were common to all five populations sampled. The remaining eight bands are distributed among different populations and occur only rarely, both at Sudbury and in uncontaminated populations. This could indicate multiple origins, or perhaps Sudbury populations are descended from local populations that have since been extirpated. Many alternative colonization hypotheses could account for this pattern. The only anomaly peculiar to Sudbury is a frequent *6pgd-2* allele that occurs only rarely in the uncontaminated populations sampled. The occurrence of this allele favours the theory of a local origin for Sudbury populations.

The Cobalt populations are less likely to have descended from uncontaminated populations sampled in this study. Although much of the variability at Cobalt is a subset of that present in the uncontaminated populations, some unique variability occurs at Cobalt. Given that the distance between Cobalt and the uncontaminated populations is about 240 km and that closer populations are likely to occur along some of the northern rivers of Ontario and Quebec (Dore and McNeill 1980), it

seems more likely that the source of the Cobalt populations is different from those at Sudbury. This implies that genes conferring metal tolerance may not be uncommon throughout populations of *D. cespitosa* in central Ontario.

Mating systems and colonization

Estimates of outcrossing frequency provided no evidence that mine populations had evolved significant levels of self-fertilization. Levels of observed heterozygosity were consistently less than those expected under panmixia; however, there were no differences between mine and uncontaminated populations. Furthermore, the magnitude of heterozygote deficiency is small and thus consistent with the observation that outbreeders have a slight deficit of heterozygotes compared with levels predicted under panmixia (Brown 1979). Gehring and Linhart (1992) also report heterozygote deficiency in *D. cespitosa* and suggest inbreeding as a possible cause. Biparental inbreeding and (or) low levels of self-fertilization could contribute to the observed deficit of heterozygotes. Although *D. cespitosa* is reported to be self-incompatible (Davy 1980), this is the first quantitative study of its reproductive system. The levels of outcrossing recorded here verify that *D. cespitosa* is predominantly outbred. However, the *t*-estimate for the Sudbury population was significantly lower than 1.0 ($t = 0.887$, $Z = 1.625$, $P = 0.052$), providing some indication that self-incompatibility may not be absolute. Indeed, in a preliminary experiment involving the isolation of individuals in the glasshouse and bagging of emerging panicles, small numbers of seed were collected from several individuals.

Two previous investigations of the mating systems of metal-tolerant populations of plants have both found tentative evidence that increased self-fertilization is associated with the colonization of mine sites. However, studies on *Agrostis tenuis*, *Anthoxanthum odoratum* (Antonovics 1968), and *Armeria maritima* (Lefèbvre 1970) were restricted to measuring self-fertility by bagging individual plants in experimental plots or the glasshouse. When Lefèbvre (1976) looked for evidence of self-fertilization in a mine population of *Armeria maritima*, he was unable to detect any. The present study is the first to compare levels of outcrossing using genetic markers in an uncontaminated and mine population. The data are, however, inconclusive, and clearly more populations would need to be examined before any clear generalizations could be made concerning mating-system shifts in mine populations of *D. cespitosa*.

Acknowledgements

We thank Professor T.C. Hutchinson for valuable discussions and assistance in the location of populations. His laboratory's research on metal-tolerant populations of *Deschampsia cespitosa* spawned many of the questions addressed in this study. We also thank Roger Cox and Caroline Schultz for background information on *D. cespitosa*, and Brian Husband, William Cole, and Fanny Strumas for technical assistance and much advice. This research was funded by a Natural Sciences and Engineering Research Council operating grant to S.C.H.B.

Antonovics, J. 1968. Evolution in closely adjacent populations. V. Evolution of self-fertility. Heredity, **23**: 219–238.

Antonovics, J., Bradshaw, A.D., and Turner, R.G. 1971. Heavy metal tolerance in plants. *In* Advances in ecological research. Vol. 7. *Edited by* J.B. Cragg. Academic Press, New York.

Baker, H.G. 1974. The evolution of weeds. Annu. Rev. Ecol. Syst. **5**: 1–24.

Baker, H.G., and Stebbins, G.L. 1965. The genetics of colonizing species. Academic Press, New York.

Barrett, S.C.H. 1988. Genetics and evolution of agricultural weeds. *In* Weed management: ecological approaches. *Edited by* M. Altieri and M. Liebman. CRC Press, Inc., Boca Raton, Fla. pp. 57–75.

Barrett, S.C.H. 1992. Genetics of weed invasions. *In* Applied population biology. *Edited by* S.K. Jain and L.W. Botsford. Kluwer Academic, Dordrecht, The Netherlands. pp. 91–120.

Barrett, S.C.H., and Husband, B.C. 1989. The genetics of plant migration and colonization. *In* Plant population genetics, breeding and genetic resources. *Edited by* A.H.D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir. Sinauer Associates, Inc., Sunderland, Mass. pp. 254–277.

Barrett, S.C.H., and Richardson, B.J. 1986. Genetic attributes of invading species. *In* Ecology of biological invasions. *Edited by* R.H. Groves and J.J. Burdon. Australian Academy of Science, Canberra, Australia. pp. 21–33.

Barrett, S.C.H., and Shore, J.S. 1989. Isozyme variation in colonizing plants. *In* Isozymes in plant biology. *Edited by* D.E. and P. Soltis. Dioscorides Press, Portland, Oreg. pp. 106–126.

Bradshaw, A.D., and McNeilly, T. 1991. Evolution in relation to stress. *In* Ecological genetics and air pollution. *Edited by* G.E. Taylor, Jr., L.F. Pitelka, and M.T. Clegg. Springer-Verlag, New York. pp. 11–32.

Bradshaw, A.D., McNeilly, T.S., and Gregory, R.P.G. 1965. Industrialization, evolution and the development of heavy metal tolerance in plants. *In* Ecology and the industrialized society. *Edited by* G.T. Goodman and R.W. Edwards. British Ecological Society Symposium, Blackwell Scientific Publications, Oxford, U.K. pp. 327–343.

Brown, A.H.D. 1979. Enzyme polymorphisms in plant populations. Theor. Popul. Biol. **15**: 1–42.

Brown, A.H.D. 1983. Multilocus organization in plant populations. *In* Population biology and evolution. *Edited by* K. Wohrmann and V. Loescheke. Springer-Verlag, Berlin. pp. 159–169.

Brown, A.H.D., and Marshall, D.R. 1981. Evolutionary changes accompanying colonization in plants. *In* Evolution today. Proceedings of the 2nd International Congress of Systematic and Evolutionary Biology. *Edited by* G.E. Scudder and J.L. Reveal. Hunt Institute for Botanical Documentation, Carnegie-Mellon, University of Pittsburgh, Pittsburgh, Pa. pp. 351–363.

Bush, E. 1990. Colonization genetics of the invasion of metal-contaminated areas in Ontario by *Deschampsia cespitosa* (Gramineae). M.Sc. thesis, Department of Botany, University of Toronto, Toronto, Ont.

Cardy, B.J., Stuber, C.W., and Goodman, M.M. 1981. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Institute of Statistics Mimeograph Series No. 1317, North Carolina State University, Raleigh, N.C.

Cox, R.M., and Hutchinson, T.C. 1979. Metal co-tolerances in the grass *Deschampsia cespitosa*. Nature (London), **279**: 231–233.

Cox, R.M., and Hutchinson, T.C. 1980. Multiple metal tolerances in the grass *Deschampsia cespitosa*. New Phytol. **84**: 631–647.

Cox, R.M., and Hutchinson, T.C. 1981. Environmental factors influencing the rate of spread of the grass *Deschampsia cespitosa* invading areas around Sudbury nickel-copper smelters. Water Air Soil Pollut. **16**: 83–106.

Davy, A.J. 1980. Biological flora of the British Isles. No. 149. *Deschampsia caespitosa* (L.) Beauv. J. Ecol. **62**: 367–378.

Dore, W.G., and McNeill, J. 1980. Grasses of Ontario. Agric. Can. Monogr. No. 26.

Farris, M.A., and Schaal, B.A. 1983. Morphological and genetic variation in ecologically central and marginal populations of *Rumex acetosella* L. (Polygonaceae). Am. J. Bot. **70**: 246–255.

Gartside, P.W., and McNeilly, T. 1974. The potential for evolution of heavy metal tolerance in plants. II. Copper tolerance in normal populations of different plant species. Heredity, **32**: 335–348.

Gehring, J.L., and Linhart, Y.B. 1992. Population structure and genetic differentiation in native and introduced populations of

Deschampsia caespitosa (Poaceae) in the Colorado alpine Am. J. Bot. **79**: 1337–1343.

Gottlieb, L.D. 1982. Conservation and duplication of isozymes in plants. Science (Washington, D.C.), **216**: 373–380.

Golenberg, E. 1989. Migration patterns and the development of multilocus associations in a selfing annual, *Triticum dicoccoides*. Evolution, **43**: 595–606.

Gray, A.J. 1986. Do invading species have definable genetic characteristics? Philos. Trans. R. Soc. London, B, **314**: 655–674.

Husband, B.C., and Barrett, S.C.H. 1991. Colonization history and population genetic structure of *Eichhornia paniculata* in Jamaica. Heredity, **66**: 287–296.

Kahler, A.L., Allard, R.W., Krzakowa, M., *et al.* 1980. Associations between isozyme phenotypes and environment in the slender wild oat (*Avena barbata*) in Israel. Theor. Appl. Genet. **56**: 31–47.

Lefèbrve, C. 1970. Self-fertility in maritime and zinc mine populations of *Armeria maritima* (Mill.) Willd. Evolution, **24**: 571–577.

Lefèbrve, C. 1976. Breeding system and population structure of *Armeria maritima* (Mill.) Willd. on a zinc-lead mine. New Phytol. **77**: 187–192.

Lewontin, R.C. 1972. The apportionment of human diversity. Evol. Biol. **6**: 381–398.

Li, W. 1980. Rate of gene silencing at duplicate loci: a theoretical study and interpretation of data from tetraploid fishes. Genetics, **95**: 237–258.

Loveless, M.D., and Hamrick, J.L. 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. **15**: 65–95.

Macnair, M.R. 1983. The genetic control of copper tolerance in the yellow monkey flower, *Mimulus guttatus*. Heredity, **50**: 283–293.

Maruyama, T., and Fuerst, P.A. 1984. Population bottlenecks and non-equilibrium models in population genetics. I. Allele numbers when populations evolve from zero variability. Genetics, **108**: 745–763.

Maruyama, T., and Fuerst, P.A. 1985*a*. Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population derived from a large steady-state population by means of a bottleneck. Genetics, **111**: 675–689.

Maruyama, P.A., and Fuerst, P.A. 1985*b*. Population bottlenecks and non-equilibrium models in population genetics. III. Genic homozygosity in populations which experience periodic bottlenecks. Genetics, **111**: 691–703.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, Mass.

Moran, G.F., and Marshall, D.R. 1978. Allozyme uniformity within and variation between races of the colonizing species *Xanthium strumarium* L. (Noogoora Burr). Aust. J. Biol. Sci. **31**: 282–291.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. **70**: 3321–3323.

Nei, M., Maruyama, T., and Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. Evolution, **29**: 1–10.

Novak, S.J., Mack, R.N., and Soltis, D.E. 1991. Genetic variation in *Bromus tectorum* (Poaceae): population differentiation in its North American range. Am. J. Bot. **78**: 1150–1161.

Ohno, S. 1970. Evolution by gene duplication. Springer-Verlag, Berlin.

Parsons, P.A. 1983. The evolutionary biology of colonizing species. Cambridge University Press, Cambridge, U.K.

Podani, J. 1988. SYN-TAX III user's manual. Abstr. Bot. **12**(Suppl. 1).

Ritland, K., and Jain, S.K. 1981. A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci. Heredity, **47**: 35–52.

Roose, M.L., and Gottlieb, L.D. 1976. Genetic and biochemical consequences of polyploidy in *Tragopogon*. Evolution, **30**: 818–830.

Rothera, S.L., and Davy, A.J. 1986. Polyploidy and habitat differentiation in *Deschampsia cespitosa*. New Phytol. **102**: 449–467.

Shannon, C.E., and Weaver, W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, Ill.

Shaw, A.J. 1991. The genetic structure of sporophytic and gametophytic populations of the moss, *Funaria hygrometrica* Hedw. Evolution, **45**: 1260–1274.

Shaw, C.R., and Prasad, R. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet. **4**: 297–320.

Simpson, E.H. 1949. Measurement of diversity. Nature (London), **163**: 688.

Sirkkomaa, S. 1983. Calculations on the decrease of genetic variation due to the founder effect. Hereditas, **99**: 11–20.

Soltis, D.E., Haufler, C.H., Darrow, D.C., and Gastony, G.J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gels and electrode buffers, and staining schedules. Am. Fern. J. **73**: 19–27.

Steel, R.G.D., and Torie, J.H. 1980. Principles and procedures of statistics. A biometrical approach. McGraw-Hill Book Co., New York.

Vallejos, C.E. 1983. Enzyme staining activity. *In* Isoenzymes in plant genetics. Part A. *Edited by* S.D. Tanksley and T.J. Orton. Elsevier Science Publishers, Amsterdam, The Netherlands. pp. 469–516.

Verkleij, J.A.C., Bast-Cramer, W.B., and Levering, H. 1985. Effects of heavy metal stress on the genetic structure of populations of *Silene cucubalus*. *In* Structure and functioning of plant populations. 2. Phenotypic and genotypic variation in plant populations. *Edited by* J. Haeck and J.W. Woldendorp. North Holland Publishing Comp., Amsterdam, The Netherlands. pp. 355–365.

Verkleij, J.A.C., Lugtenborg, T.F., and Ernst, W.H.O. 1989. The effect of geographical isolation on enzyme polymorphism of heavy metal tolerant populations of *Minuartia verna* (L.) Hiern. Genetica (The Hague), **78**: 133–143.

Warwick, S.I. 1990. Genetic variation in weeds—with particular reference to Canadian agricultural weeds. *In* Biological approaches and evolutionary trends in plants. *Edited by* S. Kawano. Academic Press, London. pp. 3–18.

Watterson, G.A. 1984. Allele frequencies after a bottleneck. Theor. Popul. Biol. **26**: 387–407.

Wright, S. 1931. Evolution in Mendelian populations. Genetics, **16**: 97–159.

Wu, L., Bradshaw, A.D., and Thurman, D.A. 1975. The potential for evolution of heavy metal tolerance in plants. III. The rapid evolution of copper tolerance in *Agrostis stolonifera*. Heredity, **34**: 165–187.

Appendix

A comparison of the frequency of alleles and isozyme bands in mine populations of *Deschampsia cespitosa* at Cobalt and Sudbury

	Cobalt					Sudbury				
Zone	C1	C2	C3	C4	C5	S1	S2	S3	S4	S5
6Pgd-2										
1	0.00	0.00	0.00	0.03	0.00	**0.44**	**0.36**	**0.30**	**0.58**	**0.02**
2	0.94	0.94	1.00	0.97	1.00	0.56	0.64	0.70	0.42	0.98
3	**0.06**	**0.06**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PGM										
1	**0.01**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.16	0.17	0.01	0.01	0.14	**0.98**	**1.00**	**0.98**	**0.91**	**0.89**
3	0.00	**0.01**	0.00	**0.13**	0.00	0.00	0.00	0.00	0.00	0.00
4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5	**0.04**	**0.07**	**0.09**	0.00	**0.57**	0.00	0.00	0.00	0.00	0.00
TPI										
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**	0.00	0.00
4	**0.02**	0.00	0.00	**0.07**	0.00	0.00	0.00	0.00	0.00	0.00
5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
9	**0.05**	**0.10**	**0.03**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dia-2										
1	**0.65**	**0.68**	**0.40**	**0.58**	**0.54**	0.00	0.00	0.00	0.00	0.00
N	0.01	0.04	0.01	0.20	0.07	0.02	0.04	0.11	0.07	0.90
4	0.00	0.00	0.00	0.00	0.00	**0.07**	**0.29**	**0.38**	0.00	0.00
5	0.92	0.82	0.93	0.51	0.77	0.98	0.94	0.85	0.93	0.10
ACO										
1	0.00	**0.01**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3	**0.98**	**0.97**	**0.98**	**0.97**	**0.98**	0.29	0.21	0.35	0.16	1.00
4	0.00	0.00	0.00	0.00	0.00	**0.98**	**1.00**	**0.93**	**0.98**	**0.07**
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**	0.00	0.00
Mdh-2										
2	0.00	+	0.00	0.00	−	0.00	0.00	0.00	0.00	0.00
3	0.00	−	0.00	0.00	+	0.00	0.00	0.00	0.00	0.00
4	0.80	+	0.82	0.81	+	0.95	0.96	0.95	1.00	1.00
5	0.62	+	0.49	0.67	+	0.34	0.31	0.47	0.00	0.00
Mdh-3										
1	1.00	+	1.00	1.00	+	1.00	1.00	1.00	1.00	1.00
3	**0.06**	+	**0.10**	0.00	+	0.00	0.00	0.00	0.00	0.00
4	0.00	+	0.00	0.00	−	0.00	0.00	0.00	0.00	0.00

NOTE: Bands that distinguish populations from the two areas are in bold type. Alleles that were not present in either population and enzyme zones for which there was no unique variation in either population were excluded. *6Pgd-2* involves allele frequencies; PGM, TPI, *Dia-2*, ACO, *Mdh-2*, and *Mdh-3* are band frequencies. N is the null allele. Common alleles at the two MDH loci precluded estimation of band diversities for each locus separately. +, presence; −, absence.