Geographic patterns in the genetic diversity of a northern sedge, *Carex rariflora*

Mark Vellend and Marcia J. Waterway

Abstract: We compared the genetic diversity of a northern wetland sedge, *Carex rariflora*, both within and between populations, in different habitat types, and across geographic regions with different glacial histories. Twelve populations were sampled: 5 from each of two regions in northern Quebec, and 2 from northern Yukon. Habitat types were medium to rich fens, wet tidal flat, and alpine stream edge. Allele frequency data revealed a low degree of within-population genetic diversity (H_S = 0.07) and a high degree of genetic differentiation among populations (G_{ST} = 0.47) compared with previously studied northern wetland *Carex* species. Higher genetic variability found in the Yukon than in Quebec could be explained by the presence of a glacial refugium in much of Alaska and the Yukon throughout the Pleistocene. Low genetic diversity in Quebec suggests the occurrence of genetic bottlenecks resulting from founder effects during postglacial colonization. There was a strong positive correlation between geographic distance and genetic distance among populations, and the three geographic regions were genetically distinct from one another. Detrended correspondence analysis of vegetation data revealed clear differences among the three habitat types, but we found no significant correlation between habitat differences and genetic distance.

Key words: allozyme variation, genetic diversity, geographic variation, *Carex rariflora*.

Résumé : Les auteurs ont comparé la diversité génétique d'un carex des terres humides nordiques, le *Carex rariflora*, à la fois dans et entre les populations, dans différents types d'habitats et à travers diverses régions géographiques ayant des histoires galciaires différentes. Ils ont échantillonné 12 populations : 5 de chacune de deux régions du nord du Québec, et 2 du nord du Yukon. Les types d'habitats sont des fens moyens à riches, des estrans humides, et des rives de ruiseaux alpins. Les données sur la fréquence des allèles montrent une faible diversité génétique à l'intérieur des populations (H_S = 0,07) et une importante différentiation génétique entre les populations (G_{ST} = 0,47), comparativement aux études antécédentes conduites chez les espèces de carex des régions nordiques humides. La plus forte variabilité génétique observée au Yukon par rapport au Québec pourrait s'expliquer par la présence d'un refuge glaciaire sur une grande partie du Yukon et de l'Alaska, tout au cours du Pléistocène. La faible diversité génétique observée au Québec suggère la présence d'un goulot d'étranglement génétique résultant d'un effet d'affaissement au cours de la colonisation post-glaciaire. Il y a une forte corrélation positive entre la distance géographique et la distance génétique entre les populations, et les trois régions géographiques sont génétiquement distinctes l'une de l'autre. L'analyse par correspondance avec élimination des tendances, des données de végétation révèle des différences nettes entre les trois types d'habitats, mais les auteurs n'ont pas trouvé de corrélation entre les différences d'habitat et la distance génétique.

Mote clés : variation allozymique, diversité génétique, variation géographique, *Carex rariflora*.

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Introduction

Each plant species has a unique combination of life-history traits, historical factors, and habitat preferences that can influence patterns of genetic variation within and among populations (Nei et al. 1975; Hamrick and Godt 1989, 1996; Mitton 1994). The effects of life-history traits on allozyme diversity in plants were the subject of several review papers (Hamrick et al. 1979; Loveless and Hamrick 1984; Hamrick and Godt 1989, 1996). These reviews included all available allozyme data for plant species and demonstrated some important generalizations about the factors affecting genetic diversity. However, more specific conclusions can be reached by comparing genetic diversity among closely related taxa (Lewis and Crawford 1995). Closely related taxa generally share many common traits, thus reducing the number of factors that must be considered potentially important in determining observed patterns of genetic variability. To date, allozyme data are available for eight northern rhizomatous *Carex* species (Ford et al. 1991; McClintock and Waterway 1993; Jonsson et al. 1996; M.J. Waterway and K.A. McClintock, unpublished data), all of which share a clonal growth form, widespread distribution, wind dispersal, and predominantly outcrossing mating system. Thus, a strong base of comparison exists for allozyme studies in this group of species.

Historical factors may be important in determining patterns of genetic diversity (Nei et al. 1975), particularly in

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Table 1. Sampling locations for *Carex rariflora* populations.

Sampling site	No. of shoots sampled	Location
		James Bay
1. Tidal Flat	38	Near shore of James Bay, at the end of the road from Chisasibi, 53°47′N, 79°03′W
2. Salt Marsh	40	At the W end of Fort George Island, mouth of La Grande Rivière, 53°47′N, 79°04′W
3. Salt Shrubs	38	Shrubby area just E of the Tidal Flat, 53°47′N, 79°02′W
4. Island Fen	40	On the SW side of Fort George Island, 53°49′N, 79°01′W
5. Chisasibi Fen	40	Just W of the junction of the Chisasibi - James Bay road and the Fort George Island road, 53°48′N, 78°58′W
		Schefferville
6. Astray Fen	50	20 km SE of Schefferville, 1 km N of Astray Lake, 54°40′N, 66°36′W
7. Goodream Fen	41	25 km NW of Schefferville, just E of Goodream Lake, 54°56N, 67°07′W
8. Sunny Mountain	40	40 km NW of Schefferville, 55°03′N, 67°13′W
9. Iron Arm Fen	40	16 km NE of Schefferville, just W of Iron Arm of Attikamagen Lake, 54°53′N, 66°38′W
10. Irony Mountain	40	20 km NW of Schefferville, 54°54′N, 67°09′W
		Yukon Territory
11. Tombstone Fen	41	Dempster Highway, km 84, 64°36′N, 138°19′W
12. Ogilvie Fen	26	Dempster Highway, km 124, 64°55′N, 138°17′W

northern regions where continental glaciation resulted in enormous range expansions and contractions (Hewitt 1996). Genetic bottlenecks resulting from founder effects during postglacial colonization are often invoked to explain low genetic variability found in northern plant populations (Fowler and Morris 1977; Schwaegerle and Schaal 1979; Soltis 1982; Bayer et al. 1987). However, reduction of genetic variability due to founder events can be counteracted or reversed by even moderate levels of gene flow from source populations (Nei et al. 1975). The effect of such events on genetic diversity can be examined by comparing populations occurring in regions with different glacial histories.

In this study, we examined the genetic diversity within and among populations of *Carex rariflora* Wahl. among three regions with different glacial histories and different habitat types. *Carex rariflora* is a circumboreal wetland sedge, with both long-spreading and short-clumping rhizomes. It occurs most commonly along the edges of streams and ponds in alpine tundra and around the edges of northern fens and tidal marshes, often on *Sphagnum* hummocks or in newly disturbed areas, but rarely rooted below the water level. The North American distribution of *C. rariflora* extends from Alaska to Greenland, with some more southerly populations in eastern Canada (Porsild and Cody 1980). Our objectives were (*i*) to compare genetic variability of *C. rariflora* with other northern wetland *Carex* species, (*ii*) to determine if genetic diversity is higher in populations occurring in a glacial refugium than in populations from areas colonized since deglaciation, and (*iii*) to determine if allelic differences could be related to habitat differences.

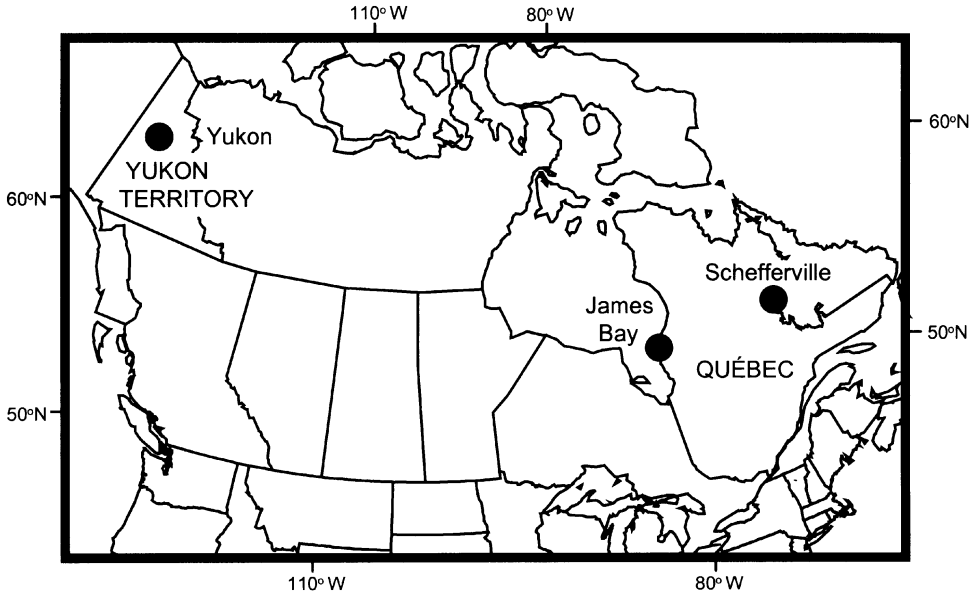
Methods

A total of 474 flowering individuals were randomly sampled from 12 different populations in three distinct geographic regions. We sampled five populations along the northeastern shore of James Bay, which emerged from a glacial sea 3000–4000 years ago (Vincent 1989), five populations in the Schefferville region of Quebec, which became free of ice 5000–6000 years ago (Vincent 1989), and two populations in northern Yukon Territory, much of which was free of ice throughout the Pleistocene (Pielou 1991) (Table 1; Fig. 1). Twenty-six to 50 individuals were sampled at each site, with one ramet collected from each individual for allozyme analysis. To minimize the chance of sampling the same individual more than once, the minimum distance between samples was 5 m. One population (Salt Shrubs) was small enough in area that it was necessary to sample at 2- to 3-m intervals, but in all other populations the intersample distance was greater than 5 m.

Carex rariflora is known to hybridize with the closely related species *C. limosa* and *C. paupercula* (Cayouette and Catling 1992), and these hybrids show the expected heterozygous isozyme banding patterns with diagnostic alleles for each species (M.J. Waterway and K.A. McClintock, unpublished data; M.J. Waterway and N. Djan-Chékar, unpublished data). An inflorescence from each sample was collected so that hybrids revealed by allozyme analysis could be checked for intermediate flower morphology and pollen sterility. Four *C. limosa* × *C. rariflora* hybrids were found, and allozyme data from these plants were not used in the analyses.

All samples were maintained on ice for up to 4 weeks before being planted in the McGill University Phytotron. One newly emerged shoot from each plant was homogenized in about 300 mL of extraction buffer (Gottlieb 1981) with 5–7 g of polyvinylpyrrolidone (PVP-40) per 100 mL buffer solution, absorbed onto filter paper wicks, and stored at –80ºC prior to electrophoresis. Allozyme variation was assayed using standard methods of starch gel electrophoresis and specific enzyme-activity staining (Wendel and Weeden 1989). A lithium-borate – tris-citrate gel system at pH 8.1 (Wendel and Weeden system No. 6) was used for alcohol dehydrogenase (ADH, EC 1.1.1.1), aspartate aminotransferase (AAT, EC 2.6.1.1), triosephosphate isomerase (TPI, EC 5.3.1.1), acid phosphatase (ACP, EC 3.1.3.2), and glucosephosphate isomerase (GPI, EC 5.3.1.9). A histidine – citrate gel system at pH 6.5 (Wendel and Weeden system No. 1) was used for phosphoglucomutase (PGM, EC 5.4.2.2), aldolase (ALDO, EC 4.1.2.13), glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49), and shikimate dehydrogenase (SKDH, EC 1.1.1.25). Aryl esterase (EST, EC 3.1.1.2), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44), peroxidase (PRX, EC 1.11.1.7), malic dehydrogenase (MDH, EC 1.1.1.37), and isocitrate dehydrogenase (IDH, EC 1.1.1.42) were run on a morpholine – citrate gel system at pH 6.1 (Wendel and Weeden system No. 2), while diaphorase (DIA, EC

Fig. 1. Sampling regions for *Carex rariflora*.



1.8.1.4) and menadione reductase (MDR, EC 1.6.99.3) were run on a tris-citrate gel system at pH 7.0 (Wendel and Weeden system No. 3).

BIOSYS-I (Swofford and Selander 1981) was used to calculate allele frequencies for each population–locus combination, percentage of polymorphic loci, mean number of alleles per locus, mean observed heterozygosity over all loci, mean expected heterozygosity under Hardy–Weinberg equilibrium, and Wright's fixation index *F* (Wright 1965). Tests for deviations of genotypic frequencies from Hardy–Weinberg equilibrium (Fisher's exact test) were also calculated using BIOSYS-I. Heterozygotes at the *Mdr-1* locus could not be recognized because this locus was interpreted as monomeric with one null allele; no genotypic data for *Mdr-1* were used in the calculations of heterozygosity. For subsequent analyses, we assumed Hardy–Weinberg equilibrium and calculated allele frequencies for *Mdr-1* using the genotypic frequency of null-allele homozygotes. Nei's (1973) gene diversity statistics, H_T, H_S, and G_{ST}, unbiased for sample size (Nei and Chesser 1983), were calculated for the species, for the three geographic regions separately, and for the two Quebec regions combined using GENESTAT-PC, version 2.1 (Whitkus 1988). Gene flow (*Nm*) was calculated following Wright's (1948) method but using G_{ST} instead of F_{ST} (cf. Slatkin 1985). SIDGEND (Sattler and Hilburn 1985) was used to calculate Nei's (1978) genetic distance and identity statistics for each pair of populations, corrected for small sample size, and incorporating Hillis' (1984) adjustment for unequal rates of amino-acid substitution and Mueller and Ayala's (1982) jackknife method to reduce the bias resulting from a small number of sampled loci. A cluster analysis of the genetic distances using the unweighted pair group means analysis (UPGMA, Sneath and Sokal 1973) was carried out using MVSP (version 1.3, W.L. Kovach, Indiana University).

For all Quebec populations except Island Fen, presence was recorded for all vascular plant taxa in a circular 0.5-m^2 quadrat around each sampled individual. We identified plants to the species level whenever possible, to the genus level for *Juncus* spp., *Salix* spp. (except *S. vestita*), and *Viola* spp., and to the family level for Poaceae (except *Elymus mollis*). For each taxon, frequency at the population level was calculated as the proportion of quadrats in which it was present. The population-level vegetation data were analyzed with detrended correspondence analysis in CANOCO, version 3.12 (ter Braak 1988). We ran the analysis five times in CANOCO, each time with the order of sites randomized, to ensure that this order did not effect the outcome (Oksanen and Minchin 1997). The use of detrended correspondence analysis (DCA) on vegetation data assumes that each taxon is distributed unimodally over environmental gradients (ter Braak 1988); we considered pooling species with different distributions in *Juncus*, *Salix*, *Viola*, and Poaceae to be a violation of this assumption, so these taxa were eliminated from the analysis. As a measure of dissimilarity in vegetation, we calculated the χ^2 distance between each pair of sites using the SIMIL program in the R-Package (Legendre and Vaudor 1991) and termed this the vegetation distance. The χ^2 distance is the implicit dissimilarity measure used in detrended correspondence analysis (Jongman et al. 1995).

To analyze relationships among the geographic distance, genetic distance, and vegetation distance matrices we performed Mantel's (1967) matrix randomization tests, modified by Manly (1997), using the computer program RT, version 1.02C (Manly 1994). In Mantel tests, a regression or partial regression is performed on two or more distance matrices. Because the elements of a distance matrix are not statistically independent, significance levels are determined by iterated randomizations of the distance matrices, and comparison of the observed *F*-ratio to the distribution of *F*-ratios produced by the randomizations. We performed 5000 matrix randomizations for each of our tests. The effects on genetic distance of geographic distance and vegetation distance were first analyzed individually using Mantel tests with two matrices. Partial Mantel tests were then used to analyze the same effects on genetic distance while holding constant the effect of the third matrix.

Proportion of polymorphic loci, number of alleles per locus, and Nei's (1973) gene diversity statistics were collated for all previously studied northern rhizomatous *Carex* species to compare with those calculated for *C. rariflora*. In many of these studies, gene diversity statistics were calculated for ramet and genet data sets separately. Ramet data sets consider each sampled shoot to be a different genetic individual, whereas genet data sets consider all sampled plants in a population that have identical electrophoretic banding patterns to belong to the same genetic individual. We used the genet values in our comparisons for species in which sufficient genetic variability existed to make these calculations meaningful as determined by the authors (*C. lasiocarpa* and *C. pellita*, McClintock and Waterway 1993; *C. bigelowii*, Jonsson et al. 1996; *C. limosa*, M.J. Waterway and K.A. McClintock, unpublished

Table 2. Allele frequencies for polymorphic loci in populations of *Carex rariflora*.

		James Bay					Schefferville					Yukon	
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12
Aat-3	*a*	1	1	1	0.99	1	1	1	1	1	1	1	1
	b	0	0	0	0.01	0	0	0	0	0	0	0	0
Adh-1	*a*	0	0	0	0	0	0	0	0	0	0	0.16	0.12
	b	1	1	1	1	1	1	1	1	1	1	0.79	0.88
	c	0	0	0	0	0	0	0	0	0	0	0.05	0
Gpi-2	*a*	0.03	0.04	0	0.01	0.03	0	0	0	0	0	0.90	0.98
	c	0.97	0.96	1	0.99	0.97	1	1	1	1	1	0	0
	d	0	0	0	0	0	0	0	0	0	0	0.10	0.02
*Mdr-1**	*a*	0.49	0.49	0.46	0.48	0.55	1	1	1	1	1	1	1
	null	0.51	0.51	0.54	0.52	0.45	0	0	0	0	0	0	0
6-Pgd-1	*a*	0.84	0.95	0.92	0.91	0.96	1	0.63	1	1	1	0.97	1
	b	0.16	0.05	0.08	0.09	0.04	0	0.37	0	0	0	0.03	0
Pgm-2	*b*	1	1	1	1	1	1	1	1	0.98	1	1	1
	c	0	0	0	0	0	0	0	0	0.02	0	0	0
Skd-1	*a*	0	0.01	0.03	0	0	0.13	0.03	0.18	0.09	0.05	0	0
	b	1	0.99	0.97	1	1	0.87	0.97	0.82	0.91	0.95	0.39	0.50
	c	0	0	0	0	0	0	0	0	0	0	0.55	0.48
	d	0	0	0	0	0	0	0	0	0	0	0.04	0.02
	e	0	0	0	0	0	0	0	0	0	0	0.01	0
	f	0	0	0	0	0	0	0	0	0	0	0.01	0
Tpi-1	*a*	0	0	0	0	0	0.34	0.06	0.05	0.06	0.03	0.72	0.35
	b	1	1	1	1	1	0.66	0.94	0.95	0.94	0.97	0.28	0.65

Note: Population numbers correspond to those in Table 1.
*Allele frequencies for *Mdr-1* were calculated from the number of null allele homozygotes, assuming Hardy–Weinberg equilibrium.

data); otherwise, we used ramet values (*C. paupercula*, M.J. Waterway and K.A. McClintock, unpublished data). Ford et al. (1991) reported only ramet-level gene diversity statistics for *C. saxatilis*, *C. membranacea*, and *C. rotundata*. Jonsson et al. (1996) calculated H_S and H_T for three populations of *C. bigelowii* but combined two of these populations into one for their calculation of G_{ST}; we recalculated G_{ST} for *C. bigelowii* using the genet level H_S and H_T values. Gene flow (*Nm*) was calculated for each species as for *C. rariflora* (see above). Genet-level statistics were not calculated for *C. rariflora* as very low genetic variability renders these statistics highly inaccurate (Ellstrand and Roose 1987). The accuracy of our ramet-level calculations was maximized by sampling mostly at greater than 5-m intervals.

Results

Ten loci showed clear and consistent banding patterns: *Aat-3*, *Adh-1*, *Ald-1*, *Gpi-2*, *Mdr-1*, *6-Pgd-1*, *Pgm-2*, *Skd-1*, *Tpi-1*, and *Tpi-2*. Banding patterns for the polymorphic loci were interpretable as those expected of diploid plants, with *Mdr-1*, *Pgm-2*, and *Skd-1* being monomeric, and *Aat-3*, *Adh-1*, *Gpi-2*, *6-Pgd-1*, and *Tpi-1* being dimeric. All assayed enzymes showed activity on the gels for one or more loci, but the banding patterns were often too blurry or too faint to score with confidence. All scored enzymes ran anodally, and putative locus numbers and putative allele names are in accordance with M.J. Waterway and K.A. McClintock (unpublished data).

Two loci (*Ald-1* and *Tpi-2*) were monomorphic in all populations, the other eight loci (80%) were polymorphic in at least one population (Table 2), and none were polymorphic in all populations. *Aat-3* and *Mdr-1* were polymorphic only in James Bay, *Pgm-2* was polymorphic only in Schefferville, and *Adh-1* was polymorphic only in the Yukon. Using an uncorrected α-value, genotypic frequencies deviated from Hardy–Weinberg equilibrium in 4 out of 33 (12%) population – polymorphic locus combinations ($p < 0.05$, Fisher's exact test). However, after applying the Dunn–Sidák adjustment for multiple tests, only one deviation from Hardy–Weinberg equilibrium was still considered significant ($p < 0.0015$). This low percentage of deviations from Hardy–Weinberg equilibrium allowed us to assume equilibrium to estimate allele frequencies at the *Mdr-1* locus.

Measures of genetic variability for the Quebec populations were much lower than those for the Yukon populations. At the level of region, we calculated genetic variability measures in two ways (see Table 3): region values consider all individuals in a region as belonging to one larger population, whereas mean values are simply the means over populations within a region. Table 3 gives the number of alleles per locus, percentage of polymorphic loci, and mean observed and expected heterozygosities for each population, for each region, for the two Quebec regions combined, and for the whole species. Nei's (1973) gene diversity statistics, estimates of gene flow among populations, and number of unique alleles are given for each region separately, for the two Quebec regions combined, and for the species as a whole in Table 4. Almost half of the total genetic diversity (46.7%) was found among populations. The coefficient of genetic differentiation (G_{ST}) was lower in each region separately than for the species as a whole, indicating that much of the among-population genetic diversity was due to differences among regions. Gene flow (*Nm*) was greater than 1 in

Table 3. Genetic variability in *Carex rariflora*.

Population	*A*	*P*	H_{obs}	H_{exp}
James Bay				
1. Tidal flat	1.3	30	0.013±0.009	0.036±0.030
2. Salt marsh	1.4	40	0.017±0.009	0.022±0.012
3. Salt shrubs	1.3	30	0.024±0.018	0.023±0.017
4. Island fen	1.4	40	0.025±0.019	0.024±0.018
5. Chisasibi fen	1.3	30	0.014±0.010	0.014±0.009
Region	1.5	50	0.018±0.012	0.023±0.016
Mean	1.3	34	0.019±0.013	0.024±0.017
Schefferville				
6. Astray fen	1.2	20	0.056±0.037	0.076±0.053
7. Goodream fen	1.3	30	0.085±0.069	0.071±0.052
8. Sunny Mountain	1.2	20	0.040±0.029	0.044±0.034
9. Iron arm fen	1.3	30	0.037±0.022	0.035±0.021
10. Irony Mountain	1.2	20	0.017±0.012	0.017±0.012
Region	1.4	40	0.048±0.024	0.058±0.029
Mean	1.2	24	0.047±0.034	0.049±0.034
Quebec				
Region	1.7	70	0.034±0.016	0.043±0.020
Mean	1.3	29	0.033±0.023	0.036±0.026
Yukon				
11. Tombstone fen	1.9	50	0.178±0.081	0.172±0.071
12. Ogilvie fen	1.5	40	0.136±0.073	0.141±0.072
Region	1.9	50	0.162±0.074	0.167±0.074
Mean	1.7	45	0.157±0.077	0.157±0.072
Carex rariflora				
Species	2.4	80	0.053±0.020	0.103±0.039
Mean	1.4	32	0.054±0.032	0.056±0.033

Note: *A* is mean number of alleles per locus; *P* is mean number of polymorphic loci per population; H_{obs} is observed heterozygosity (± SE); H_{exp} is expected heterozygosity (± SE). Population numbers correspond to those in Tables 1 and 2.

both Schefferville and the Yukon, too high to calculate ($G_{ST} = 0$) for James Bay, and less than 1 for the species as a whole (Table 4).

The UPGMA cluster analysis (Fig. 2) showed populations within each of the three regions to be genetically more similar than among regions. In particular, the five James Bay populations were virtually identical, unlike the Schefferville and Yukon populations that showed differences among populations. No evidence of genetic differentiation among habitat types is seen in the cluster diagram (Fig. 2): the salt-influenced populations in James Bay (tidal flat, salt marsh, salt shrubs) and the alpine populations in Schefferville (Sunny Mountain, Irony Mountain) both clustered together with freshwater fen populations from their respective regions. Geographic distance had a strong positive effect on genetic distance both for all 12 populations (Mantel test, $F = 54.8$, $p = 0.005$), as well as after vegetation distances were held constant for the 9 Quebec populations where vegetation data were taken (partial Mantel test, $F = 115.2$, $p = 0.0002$).

We recorded a total of 106 vascular plant taxa in 367 quadrats from the nine sites where vegetation was sampled. In a detrended correspondence analysis of the population-level vegetation data, the marine-influenced populations from the James Bay region were separated from the other populations along the first axis and the alpine sites were distinguished from the fen sites along the second axis (Fig. 3).

Table 4. Nei's gene diversity statistics, gene flow (*Nm*), and number of unique alleles in each region, the two Quebec regions combined, and for the species *Carex rariflora*.

	H_S	H_T	G_{ST}	*Nm*	Unique
James Bay	0.071	0.071	0.000	High*	2
Schefferville	0.044	0.053	0.169	1.229	1
Quebec	0.058	0.077	0.248	0.759	5
Yukon	0.141	0.154	0.088	2.591	7
C. rariflora	0.071	0.134	0.467	0.286	

Note: H_S is genetic diversity within populations; H_T is total genetic diversity; G_{ST} is the proportion of total genetic diversity among populations.

*Calculation of *Nm* requires division by G_{ST},which is 0 in James Bay; no specific calculation can be made.

The first, second, and third axes accounted for 30.2, 12.7, and 6.6% of the total variation, respectively. The input order of sites in CANOCO did not effect the outcome of this analysis. The freshwater fen sampled from James Bay (Chisasibi fen) had vegetation most similar to the three freshwater fens from Schefferville, while being genetically most similar to the marine-influenced populations from James Bay. For the nine populations where vegetation data were taken, no effect of vegetation distance on genetic distance was found either before or after the effect of geographic distance was held constant (before: Mantel test, $F = 0.47$, $p = 0.54$; after: partial Mantel test, $F = 0.63$, $p = 0.47$).

Discussion

The consensus among reviews on allozyme diversity in plants (Loveless and Hamrick 1984; Hamrick and Godt 1989, 1996) is that widespread, long-lived, wind-pollinated, outcrossing species show higher levels of genetic variability than species with other combinations of traits. In *C. rariflora*, 32 of 33 (97%) population – polymorphic locus combinations conformed to Hardy–Weinberg equilibrium, suggesting that outcrossing predominates in this species. Clonal species of *Carex* are potentially very long lived (Steinger et al. 1996), generally wind pollinated (Bernard 1989), and many of the northern species (including *C. rariflora*) are widespread (Porsild and Cody 1980; Hultén and Fries 1986). Thus, relatively high genetic variability was expected for *C. rariflora*. The mean values for proportion of polymorphic loci (*P*) and number of alleles per locus (*A*) were less than, but approached, those calculated by Hamrick and Godt (1989) for wind-pollinated outcrossers, long-lived herbaceous species, and widespread species. However, mean observed heterozygosity (H_{obs}) and genetic diversity both within populations (H_S) and overall (H_T) were much lower than the means for the same groups of species (Hamrick and Godt 1989). This can be explained by the large number of rare alleles in *C. rariflora*: 9 of the 24 total alleles (38%) had frequencies less than 0.05. The high value of G_{ST} indicates that populations of *C. rariflora* are more differentiated compared with species having similar life-history traits.

Allozyme variation in northern *Carex* species

Among all northern rhizomatous *Carex* species for which there are allozyme data (Table 5), *C. rariflora* had the lowest

Fig. 2. Phenogram of 12 populations of *Carex rariflora* derived by UPGMA clustering from a matrix of pairwise comparisons of Nei's (1978) genetic identities corrected for small sample size, incorporating Hillis' (1984) adjustment for unequal rates of amino acid substitution, and Mueller and Ayala's (1982) jackknife method to reduce the bias resulting from a small number of sampled loci.

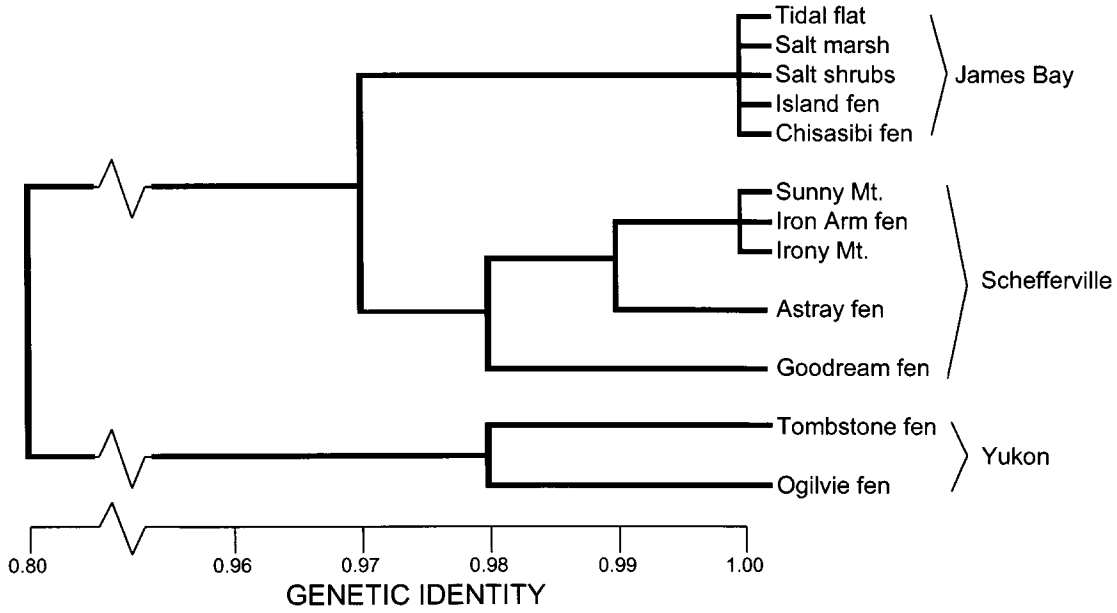
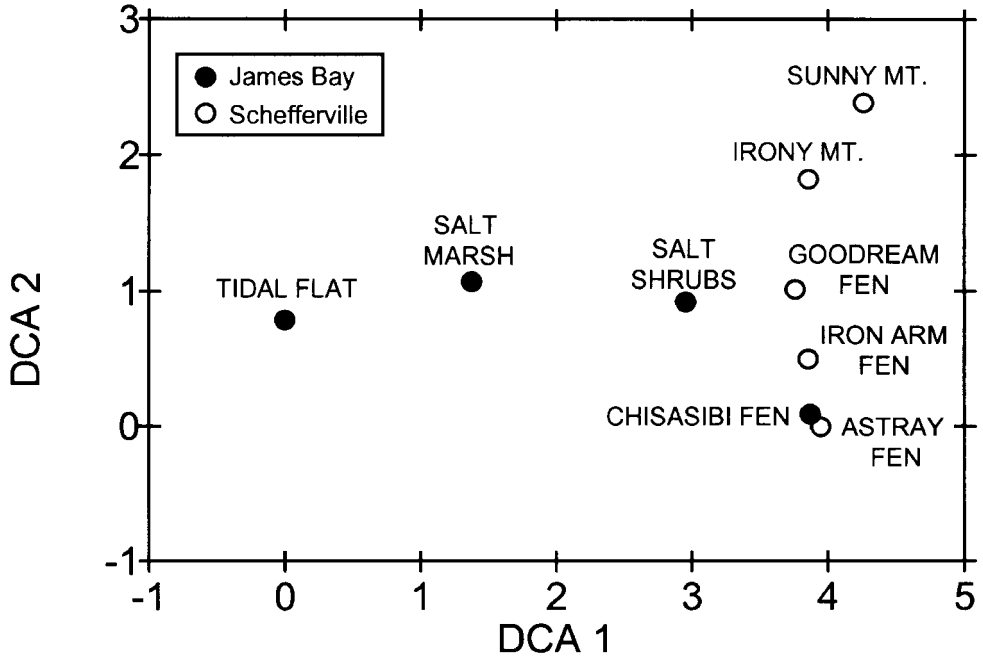


Fig. 3. Detrended correspondence analysis of vegetation data from nine populations of *Carex rariflora* in Quebec.



total genetic diversity (H_T). Among the species in this group, only *C. paupercula* has lower values of *P*, *A*, and H_S, as well as a higher value of G_{ST}, than *C. rariflora*. Remarkably similar patterns of genetic diversity are evident in *C. rariflora* and *C. paupercula*. They both have much lower within-population genetic diversity and much higher genetic differentiation among populations compared with the rest of the group. In both species, two populations from western Canada (Yukon for *C. rariflora*, Northwest Territories for *C. paupercula*) show large differences from the others in Quebec and are therefore largely responsible for the high G_{ST} values. In this study, with more than 1500 km separating the Yukon from Quebec, more than 500 km between James Bay and Schefferville, and a mean of only 17 km between pairs of populations within regions, one would expect greater genetic differences among regions than among populations within regions. However, when the western populations are excluded from the calculations for both *C. rariflora* and *C. paupercula*, G_{ST} values are still larger than for the rest of the group. In contrast, many of the other species, such as *C. limosa*, show little genetic differentiation even among populations from distant regions. Coefficients of

Table 5. Measures of genetic variability for all previously studied northern rhizomatous *Carex* species.

Species	*A*	*P*	H_S	H_T	G_{ST}	*Nm*	Data set	Data source
C. saxatilis	1.6	45	0.146	0.182	0.198	1.01	Ramet	Ford et al. (1991)
C. membranacea	1.6	44	0.162	0.199	0.183	1.12	Ramet	Ford et al. (1991)
C. rotundata	1.6	44	0.120	0.148	0.184	1.11	Ramet	Ford et al. (1991)
C. lasiocarpa	1.6	48	0.226	0.266	0.151	1.41	Genet	McClintock and Waterway (1993)
C. pellita	1.6	44	0.203	0.248	0.181	1.13	Genet	McClintock and Waterway (1993)
C. bigelowii	1.8	49	0.167	0.180	0.072	3.22	Genet	Jonsson et al. (1996)
C. limosa	1.5	42	0.137	0.146	0.063	3.72	Genet	M.J. Waterway and K.A. McClintock (unpublished data)
C. paupercula	1.2	19	0.068	0.151	0.553	0.20	Ramet	M.J. Waterway and K.A. McClintock (unpublished data)
C. rariflora	1.4	32	0.071	0.134	0.467	0.29	Ramet	This study
Mean*	1.6	42	0.154	0.190	0.198	1.61		

Note: *A* is mean number of alleles per locus; *P* is mean number of polymorphic loci per population; H_S is genetic diversity within populations; H_T is total genetic diversity; G_{ST} is proportion of total genetic diversity among populations; *Nm* is gene flow.
*Not including *C. rariflora*.

variation for *P*, *A*, H_S, and H_T calculated for the group of species in Table 5, excluding *C. paupercula* and *C. rariflora*, were all less than 25%, indicating that most of these northern rhizomatous *Carex* species share a similar overall level of genetic variability that is higher than that of *C. rariflora*.

We propose that the low within-population genetic diversity and high genetic differentiation among populations in *C. rariflora* are due at least in part to low abundance. All the species in Table 5 are widespread, and although we did not quantitatively assess the local abundances of these species, there are qualitative descriptions of abundance in the literature and in floras. The following species have been described as either sometimes dominant or common: *C. saxatilis* and *C. membranacea* (Porsild 1943; Ford and Ball 1992), *C. lasiocarpa* (McClintock and Waterway 1993), *C. bigelowii* (Welsh 1974; Callaghan 1976), and *C. limosa* (M.J. Waterway and K.A. McClintock, unpublished data). No such description was found for *C. rariflora*. Throughout most of its range, *C. rariflora* occurs along the edges of streams and ponds in wet, peaty tundra (Scoggan 1978; Porsild and Cody 1980); at bog and fen sites, it usually occurs in the relatively narrow transition zone between wetland and forest or tundra (personal observation). Thus, populations of *C. rariflora* are relatively small. Low gene flow among small, isolated populations is expected to result in inbreeding, loss of heterozygosity due to genetic drift, and genetic differentiation among populations (Barrett and Kohn 1991).

Outcrossing will maintain genetic variability only when accompanied by successful recruitment by seed. Though gene flow might be minimal among scattered populations, new genes that do enter a population via pollen or seed will only become part of the gene pool when accompanied by successful seedling establishment. In many studies of clonal *Carex* species (including this one), very few or no seedlings were observed in the field (e.g., Costello 1936; Bernard 1975; McClintock and Waterway 1993). At Astray fen (Schefferville) in 1995, *C. rariflora* was present in a large portion of the fen where it was absent in 1981 (M.J. Waterway, personal observation). It appeared to have migrated at least 100 m along disturbance tracks, strongly suggesting that substantial seedling recruitment had occurred in this fen over the 14 intervening years. Within Quebec, expected heterozygosity was highest at this site (Table 3). The other population that showed high heterozygosity in Quebec was Goodream fen (Table 3), where *C. rariflora* has also increased in abundance since 1981, again probably due to human disturbance (W. Pollard, personal communication). These observations suggest that limited seedling recruitment contributes to the maintenance of low within-population heterozygosity in *C. rariflora*. This conclusion is consistent with the hypothesis that genetic variability should decrease during succession because of reduced seedling establishment combined with the elimination of genotypes via competition, poor adaptation, or stochastic events (Burdon 1980; Gray 1984; McNeilly and Roose 1984). McClintock and Waterway (1993) observed lower genetic variability and increased clone size in established habitats versus pioneer habitats for *C. lasiocarpa*. Except for the disturbances mentioned above, all habitats in this study were established (sensu McClintock and Waterway 1993). More information is needed on rates of seed production and seedling establishment to determine the effect of recruitment rates on genetic diversity.

Glacial history and genetic variation among regions

It is well established that throughout the Pleistocene, when glaciers covered nearly all of northern North America, most of present day Alaska and part of the Yukon Territory remained free of ice in what is known as the Beringian refugium (Pielou 1991). Approximately 12 000 years ago, as the glaciers began to retreat, the landscape was colonized by organisms from three main sources: (*i*) the zone south of the ice, (*ii*) the Beringian refugium, and (*iii*) areas of unglaciated eastern coastal shelf (Pielou 1991). If the Beringian refugium were the original source of colonizers for Quebec, we would expect Quebec populations to contain a subset of those alleles found in the Yukon. Of the 24 alleles found in *C. rariflora*, only 12 were present in both areas: 7 alleles were unique to the Yukon and 5 were unique to Quebec. This suggests either a southern or eastern origin for *C. rariflora* populations in Quebec, in which case the Yukon and Quebec populations have been genetically isolated for at least 100 000 years (Pielou 1991). This could explain the high genetic differentiation between the two areas. In *C. paupercula*, two populations from the Northwest Territories were strongly differentiated from populations in Quebec (M.J. Waterway and K.A. McClintock, unpublished

data), again suggesting different sources of colonization for eastern and western populations.

Colonization of the two Quebec regions occurred quite recently in geological time: the Schefferville region became free of ice approximately 5000 years ago and the James Bay region emerged from the postglacial Tyrrell Sea only 3000–4000 years ago (Vincent 1989). Population bottlenecks resulting from founder effects during postglacial colonization may explain the much lower genetic diversity in Quebec compared with the Yukon. High genetic diversity in populations within or near glacial refugia and low genetic diversity in populations occupying previously glaciated terrain are often explained by postglacial founder events for both plant and animal species (Fowler and Morris 1977; Schwaegerle and Schaal 1979; Soltis 1982; Bayer et al. 1987; references in Barrett and Kohn 1991; Boileau and Hebert 1991; Lewis and Crawford 1995). Boileau and Hebert (1991) found significantly greater heterozygosity in northern Yukon populations than in populations along the western coast of Hudson Bay for a pond-dwelling copepod (*Heterocope septentrionalis*). This pattern was attributed to population isolation and passive dispersal (Boileau and Hebert 1991), a situation similar to the small populations and passive dispersal in *C. rariflora*.

Genetic variation among habitats

A number of studies attributed allozyme differentiation among populations to local selection in contrasting environments (e.g., Hedrick et al. 1976; Nevo et al. 1986, 1988). Prentice and Cramer (1990) also used vegetation data as a quantitative descriptor of habitat and found an association between habitat and allelic variation at one locus in *Gypsophila fastigiata*. Our data show no evidence of association between habitat types and particular alleles (Table 2; Fig. 3). Furthermore, we found no effect of vegetation distance on genetic distance for the nine populations in Quebec, both before and after holding the effect of geographic distance constant. These results suggest that geographic separation is more important than local selection in determining the pattern of genetic diversity in *C. rariflora*. In northern rhizomatous plant species like *C. rariflora*, response to local selection may be constrained by long generation times in populations of relatively long-lived genets (Steinger et al. 1996). This may be particularly true in northern Quebec where populations of *C. rariflora* have been established for no more than a few thousand years. We have not ruled out the possibility that there may be genetic differences among habitat types in unglaciated areas where populations have presumably been established for many more generations than have those in recently glaciated terrain. However, the majority of *C. rariflora*'s range occupies territory that has been free of ice for less than 10 000 years. Gene flow limitation and genetic drift may be particularly important given the relatively sparse distribution of *C. rariflora* populations across the landscape between the three regions we sampled (Porsild and Cody 1980; Hultén and Fries 1986; Cody 1996).

Conclusions

Geographic separation is more important than habitat differences in determining the pattern of genetic diversity in *C. rariflora* and historical factors can have an overriding effect on levels of genetic diversity. We suggest that low genetic diversity and high genetic differentiation among populations can be explained by small population sizes, limited recruitment by seed, and founder effects in Quebec during postglacial colonization from the south or east. Accumulation of genetic diversity over the past 100 000 years in the Yukon may account for the higher genetic diversity in this region. However, many interrelated factors have effects on genetic diversity and our results indicate a need to consider local abundance in comparisons of genetic diversity among species with otherwise similar combinations of traits. Data on abundance at various scales and on rates of seed production and seedling establishment are needed to examine the mechanisms involved in the maintenance of current patterns of genetic diversity.

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