CLINAL VARIATION AT MICROSATELLITE LOCI REVEALS HISTORICAL SECONDARY INTERGRADATION BETWEEN GLACIAL RACES OF *COREGONUS ARTEDI* (TELEOSTEI: COREGONINAE)

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Abstract.—Classical models of the spatial structure of population genetics rely on the assumption of migration-drift equilibrium, which is seldom met in natural populations having only recently colonized their current range (e.g., postglacial). Population structure then depicts historical events, and counfounding effects due to recent secondary contact between recently differentiated lineages can further counfound analyses of association between geographic and genetic distances. Mitochondrial polymorphisms have revealed the existence of two closely related lineages of the lake cisco, *Coregonus artedi*, whose significantly different but overlapping geographical distributions provided a weak signal of past range fragmentation blurred by putative subsequent extensive secondary contacts. In this study, we analyzed geographical patterns of genetic variation at seven microsatellite loci among 22 populations of lake cisco located along the axis of an area covered by proglacial lakes 12,000–8000 years ago in North America. The results clearly confirmed the existence of two genetically distinct races characterized by different sets of microsatellite alleles whose frequencies varied clinally across some 3000 km. Equilibrium and nonequilibrium analyses of isolation by distance revealed historical signal of gene flow resulting from the nearly complete admixture of these races following neutral secondary contacts in their historical habitat and indicated that the colonization process occurred by a stepwise expansion of an eastern (Atlantic) race into a previously established Mississippian race. This historical signal of equilibrium contrasted with the current migration-drift disequilibrium within major extant watersheds and was apparently maintained by high effective population sizes and low migration regimes.

Key words.—Cline, *Coregonus*, disequilibrium, historical gene flow, isolation by distance, microsatellites, secondary contacts.

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Wright's (1951) original island model of population genetic structure presents the analytical expectation of balance between gene flow and drift among ideal populations of equal and constant effective size, but stepping-stone models better approximate natural processes in restricting migration to neighboring populations. Impacts of gene flow impediment have been investigated, and the theoretical expectation is that neutral genetic differentiation (or gene flow) among populations should increase (or decrease) with geographical distances if dispersal is geographically restricted (e.g., Slatkin 1993). This pattern of isolation by distance (IBD) is explicitly revealed by a direct relationship between estimates of genetic differentiation (genetic distance, F_{ST}, or gene flow estimators) and geographical distances when populations are at equilibrium under dispersal and genetic drift.

Current population structure, however, is embedded in population history. Following a perturbation in migration and/or demographic regimes, the time to reach migration-drift equilibrium will depend on the effective population size (N_e) or the inverse of migration rate ($1/m$), whichever is greater (Slatkin 1994 and references therein). Although IBD has effectively been observed in nature, there is now growing concern that natural populations of most species have not yet reached equilibrium and that current population structures reveal historical as much as current migration patterns and population sizes (e.g., Lavery et al. 1996; Comes and Abbott

1998; Latta and Mitton 1999). These phenomena may be of special concern in northern temperate areas, where biogeographic shifts caused by past glaciations have often resulted in cycles of range fragmentation and population expansion. When analyzing the genetic structure of such populations, a major challenge is thus to detect nonequilibrium situations, identify the factor(s) responsible, and, ultimately, to partition their relative influence.

Hutchison and Templeton (1999) recently proposed a method to detect nonequilibrium conditions and evaluate the relative importance of gene flow and drift at the scale of regions defined by common historical and ecological features (e.g., age, habitat patchiness, and carrying capacity). The relative importance of each force is revealed by predictable and contrasted patterns of relationship between genetic (F_{ST}) and geographical distances, as well as by patterns of variance among F_{ST}-values over geographical distances. Under equilibrium conditions, the values and variability in pairwise F_{ST} estimates both increase with geographical distance, as gene flow counteracts the effect of drift, but more so over short distances. In contrast, for sets of populations not yet at equilibrium, there is no relationships between F_{ST} estimates and geographical distances. When the recent signal of gene flow still prevails (e.g., just following range expansion), F_{ST}-values should be consistently low over all distances; if drift becomes dominant (e.g., after population fragmentation), F_{ST} should be highly variable. This model accounted for the regional differences in geographical patterns of F_{ST} within ancestral and recently invaded portions of the collared lizard

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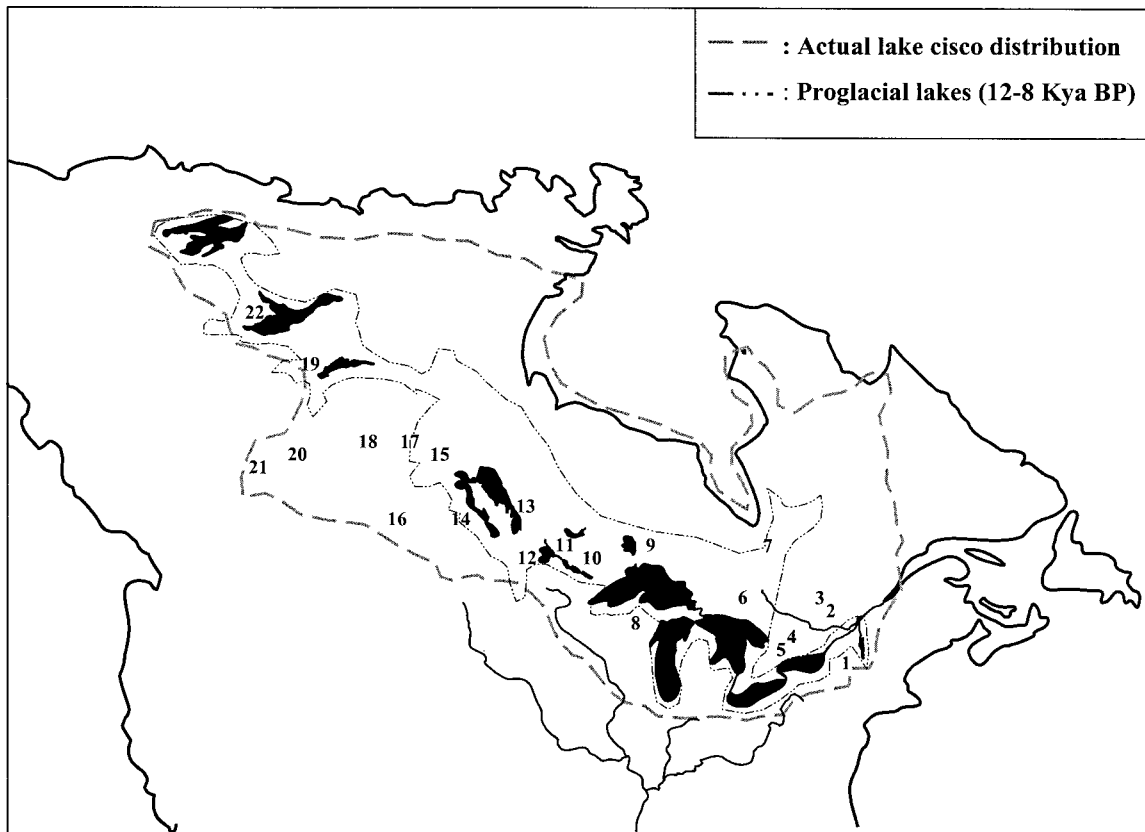
FIG. 1. Sampling locations with numerical codes as in Table 1. The current range of distribution of *Coregonus artedi* distribution and the maximal extent of proglacial lakes (12,000–8000 years ago) are indicated.

(*Crotaphytus collaris*) range (Hutchison and Templeton 1999). It also assumes that the northern regions under study have been recently invaded by a single, homogeneous source population (lineage), a premise that is most probably well founded for the species investigated.

However, for many species now inhabiting areas that were deglaciated only very recently, there is ample evidence that genetically differentiated refugial races have colonized and come into secondary contacts within the extant species range (Bernatchez and Wilson 1998; Hewitt 1999). In most cases, pairwise gene flow estimates based on current allelic frequencies will be significantly biased when population from these contact zones are considered. These past events of introgressive hybridization may affect the spatial pattern of current genetic variation in several different ways depending on the role of selection and dispersal patterns. For example, endogenous selection against hybrids may result in a tension zone (Barton and Hewitt 1985), exogenous selection may generate ecotonal or mosaic patterns of genetic variation (Harrison 1990), and neutral intermixing can result in long-lasting, wide and smooth clines (Endler 1977). Pairwise gene flow estimates based on current allelic frequencies may then reflect the history of intermixing as much as the recent migration regime (Ibrahim et al. 1996; Latta and Mitton 1999), thus impeding the assessment of current migration-drift equilibrial status.

Secondary contacts are particularly common in recently deglaciated regions, and their position is defined by that of refugial areas and available colonization routes (Hewitt 1999). For biota restricted to freshwater habitats of formerly glaciated areas, refugial areas are well identified, and colonization mostly occurred via proglacial lakes at the time of deglaciation. In North America, these lakes were immense ($>2 \times 10^6$ km^2; Teller and Kehew 1994) and provided colonization routes and opportunities for secondary contacts among multiple refugial races of freshwater fish species (Hocutt and Wiley 1986; Bernatchez and Wilson 1998). These lakes were sequentially interconnected over a relatively short period of time (~12,000–8000 years ago) and globally consisted of continuous habitat that can be equated with an interior freshwater sea. The structure of this historical habitat was dramatically modified by isostatic rebound and the emergence of separated extant watersheds (Mackenzie, Hudson, Atlantic) comprising a multitude of interconnected, yet islandlike lacustrine habitats. From an evolutionary standpoint, historical and current demographic/migration regimes are thus highly contrasted. It is very likely that these young populations have not yet reached equilibrium and that their global structure may still reflect a recent history of multiple colonization events and secondary contact/intermixing.

In this study, we use seven microsatellite loci to describe the distribution of genetic variation among 22 populations of a freshwater fish species, *Coregonus artedi*, whose distribution in extant postglacial lakes is intimately tied to the maximal extent of historical proglacial lakes (Fig. 1). Our global objective is to separate patterns of genetic variation

TABLE 1. Collection sites for *Coregonus artedi* showing drainage, geographic location, and distance to Lake Champlain (CHP).

Population (map code)	Water body	Drainage	Latitude (N)/ Longitude (W)	Distance to CHP (km)
1	Lake Champlain, VT	Atlantic	45°03′/73°09′	0
2	Poisson Blanc Reservoir, QC		45°55′/75°45′	218
3	Lac des Écorces, QC		46°32′/75°25′	263
4	White Partridge Lake, ON		45°50′/78°06′	405
5	Lake Ontario, ON		43°45′/78°00′	409
6	Kipawa Reservoir, QC		46°50′/79°05′	514
7	Lake Opasatica, QC		48°05′/79°15′	609
8	Lake Superior, ON		48°00′/88°45′	1239
9	Lake Nipigon, ON		49°45′/88°30′	1274
10	Lake Saganaga, MN	Hudson	48°15′/90°52′	1393
11	Lake Seul, ON		50°30′/92°02′	1556
12	Lake of the Woods, ON		49°18′/94°44′	1697
13	Lake Winnipeg, MB		50°40′/97°15′	1912
14	Lake Winnipegosis, MB		52°45′/100°15′	2163
15	Lake Cormorant, MB		54°30′/100°35′	2231
16	Pasqua Lake, SK		51°47′/103°58′	2375
17	Lake La Ronge, SK		55°14′/104°57′	2517
18	Lake La Biche, AB	Mackenzie	54°45′/112°05′	2975
19	Barrow Lake, AB		59°15′/111°13′	3016
20	Peerless Lake, AB		56°40′/114°30′	3140
21	Utikuma Lake, AB		55°50′/115°30′	3192
22	Great Slave Lake, NWT		61°23′/115°38′	3303

due to extant processes and population history. First, we evaluate the relative influence of historical and current migration and drift processes in shaping the extant pattern of genetic variation. Here, we take advantage of the well-documented hydrological history of the area and use the approach of Hutchison and Templeton (1999) to assess relationships between genetic and geographic distances at scales corresponding to the current and historical landscape structure (extant watershed vs. proglacial lakes, respectively). We then build on results of a previous phylogeographic study showing the existence of two mitochondrial DNA (mtDNA) haplotype groups (Turgeon and Bernatchez 2001) to identify the processes responsible for the origin and maintenance of a historical gene flow signal. We show that the intermixing of the two racial groups composing *C. artedi* results from a stepwise westward expansion of an Atlantic race into a previously established Mississippian race that has generated coincident neutral genetic clines of unprecedented width. The observed population structure is consistent with an historical equilibrium signal that developed in proglacial lake habitat 12,000–8000 years ago and suggests that high effective population sizes and low postglacial dispersal are now delaying migration/drift equilibrium.

MATERIALS AND METHODS

Fish tissue samples were obtained from 22 freshwater interior lakes of Canada and the United States (Table 1). Although unusual phenotypes and/or putative alternative taxonomic status have been reported for some of these populations, all clearly belong to the *Coregonus artedi* species complex (McPhail and Lindsey 1970; Scott and Crossman 1973). Sampling sites are distributed among three major extant watersheds: Mackenzie (Arctic), Hudson Bay, and Atlantic (Great Lakes and Laurentian). These sites are also distributed along an axis running through the area formerly cov-

ered by the proglacial lakes that formed at the margin of the retreating ice sheet at the end of the last (Wisconsinan) glaciation (i.e., from site 1 to 22, Fig. 1). Sites were ordered along this axis according to their distance to the southeasternmost location of the species distribution range (Lake Champlain). For simplicity, this ordination will be hereafter referred to as the ''east–west gradient.''

A total of 593 fish (mean = 27 individuals per population; range = 20–50) were characterized with the six microsatellites markers used in Turgeon et al. (1999) as well as with BWF1 (Patton et al. 1997) following the methodology described in Turgeon et al. (1999). Intrapopulation genetic variability was described (number of alleles, observed and expected heterozygosity) and analyzed by verifying conformance of genotypic frequencies to Hardy-Weinberg expectations. Genotypic linkage disequilibrium was also analyzed at the allelic level using the LINKDIS program of Garnier-Géré and Dillmann (1992) as executed by GENETIX 4.0 (Belkhir et al. 1999). Type I probability error was maintained at $\alpha = 0.05$ by applying the sequential Bonferroni procedure whenever necessary ($p_{adj} = \alpha/k_i$; Rice 1989).

Genetic Affinities among Populations and Geographical Patterns of Allelic Variation

We first attempted to identify population groups exhibiting major genetic discontinuities that could be revealed in the topology of a phenogram. To this effect, PHYLIP 3.5 modules SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE (Felsenstein 1993) were used to calculate D_{ce} distances and build a consensus neighbor-joining phenogram (1000 bootstraps).

We also used the factorial correspondence analysis (FCA) performed by GENETIX version 4.01β (K. Belkir, University of Montpellier, pers. comm.), which can graphically reveal and weigh the influence of specific samples (populations)

and/or variables (alleles) on the global pattern of genetic variance. FCA revealed that specific alleles of four loci were driving the organization of population samples along the east–west gradient (see Results). The apparent clinal pattern of variation of these alleles was first analyzed by regressing allelic frequencies over geographical distances from Lake Champlain, the easternmost sampling site. Second, associations among allele frequencies and distances were appraised by difference matrix correlations using Mantel tests (McColl and McKechnie 1999) performed in GENETIX 4.0.

Genetic Structure versus Contemporary and Historical Landscapes

We used the approach of Hutchison and Templeton (1999) to assess the migration-drift equilibrium revealed by the association between genetic and geographic distances. We evaluated historical and current equilibria by conducting separate analyses for the historical and recent migration domains (i.e., interconnected proglacial lakes with inter- and intrawatershed comparisons and postglacial lakes with intrawatershed comparison only, respectively). These analyses of IBD were performed using $\hat{M}$ (Slatkin 1993) as a pairwise gene flow estimator with all seven loci. $\hat{M}$ was calculated from θ, the estimator of F_{ST} proposed by Weir and Cockerham (1984). Estimates considering allele size (R_{ST}) were not used, because simulations have shown that F_{ST} estimates perform better when difference in average coalescence time is relatively small and mutations have had little time to contribute to the differentiation among population (Slatkin 1995), as well as when a small number of loci (<20) have been used for moderate sample size (Gaggiotti et al. 1999). Moreover, R_{ST} estimates proved to have higher variance and yielded nonsignificant results in the species under study (Turgeon et al. 1999). Geographical distances between sites were calculated as great circle distances using site coordinates provided by samplers or published in provincial/national gazetteers. Within each watershed, riparian distances were also estimated using ArcView 3.0a (Environmental Systems Research Institute, 1997) base maps.

We also tested if and how genetic variation was associated with current watershed configuration by estimating the interpopulation component of pairwise genetic variance and performing an analysis of molecular variance (AMOVA; Arlequin 1.1, Schneider et al. 1997).

Origin and Maintenance of Historical Gene Flow Signal

The clinal genetic variation over the entire species range and the migration-drift equilibrium signal over the historical migration domain (see Results) suggested that historical gene flow resulting from secondary contacts between refugial races was responsible for the observed contemporary structure of genetic variation. To support this scenario and identify how this structure was established and maintained, we further characterized the clinal pattern of variation, conducted nonequilibrium IBD analyses, and evaluated long-term evolutionary population effective size.

The clinal pattern of variation was analyzed in several ways. We first tested the coincidence of clines to distinguish primary from secondary intergradation (Durrett et al. 2000).

For each type of allele (i.e., east and west), the coincidence of clines across loci was appraised by testing the linear relationship between allele frequency at each of the four main clinal loci and the mean frequency over all four loci (Barton and Hewitt 1985). Piecewise regression analyses were also performed for each allele type to identify any significant breakpoint in the gradual pattern of allelic variation. We also confirmed that the intermixing of eastern and western alleles occurred at the genomic (individual) level by examining the pattern of variation of an hybrid index (HI) between the two glacial races. For each individual, this index was calculated by assigning a score of one for the presence of an eastern allele and a score of zero for the presence of a western allele at each of the four loci with dominant alleles exhibiting clinal patterns of frequency along the east–west gradient. The total score was divided by the total number of alleles clearly scored as eastern or western alleles (maximum = 8). This hybrid index is analog to the classic index developed by Barton and Gale (1993), but because there are no fixed differences between the groups being compared, we use only those alleles that are significanlty responsible for defining the clinal pattern of variation (see Results). In each population, the average value of this index thus indicates the degree of admixture of the two races (proportion of eastern alleles). In addition, low HI variances would suggest thorough genomic admixture (interracial gene flow), whereas high variances would point to linkage disequilibrium (and selection; Barton and Gale 1993) or the coexistence of individuals characterized by the different allele types.

We use the approach of Slatkin (1993) to identify historical processes that can be revealed by the relationship between $\hat{M}$ and geographic distances in nonequilibrium systems. Expectations have been derived analytically and/or simulated for the instantaneous expansion model and the unpublished stepwise expansion model of D. Good (reported in Slatkin 1993). Following a rapid and recent expansion, an IBD pattern is not expected, or it may be present only at short distances. This corresponds to the intermediate stage of Hutchison and Templeton's (1999) model, in which the homogenizing action of gene flow has counteracted drift only at short distances. In contrast, a stepwise expansion predicts an IBD pattern, but with large variance in gene flow at short distances. More importantly, pairwise gene flow estimates will depend only on the location of the oldest population (founded at an earlier time during the stepwise expansion) and be independent from the location of any population that has been established from it. Gene flow estimates are therefore expected to increase with distance from the expansion origin, because comparisons will involve more and more recent populations in which drift had less time to create differences. Moreover, comparisons relative to different points of putative expansion origin should reveal the directionality of stepwise expansions, in particular in one-dimension habitats. Proglacial lakes were the main dispersal avenue for lake cisco, and they approximate such a linear habitat. We therefore conducted IBD analyses using the one-dimensional model to examine the relationship between pairwise gene flow estimates and the distance of the population pair closest to potential expansion origins. Here, we performed two separate analyses using Great Slave Lake or Lake Champlain as ex-

pansion origin. Because this model assumes that there have been no severe bottlenecks during the colonization process, we first verified that there was no significant variation in genetic diversity, as estimated by heterozygosity, along the linear spatial gradient ($r^2 = 0.18$, $P = 0.21$).

Estimation methods of long-term population effective sizes in subdivided populations assume equilibrium (Waples 1991), a premise that is obviously violated in the system under study. Here, we computed rough estimates of N_e using the basic equation of $H_t = [1 - (1/2)N_e]^t H_o$ (Hartl and Clark 1989). We set H_o at one, thus providing minimal N_e estimates, and solve for N_e using $t = 2286$ generations, which corresponds to 3.5 years/generation (Scott and Crossman 1973) since the creation of postglacial lakes, approximately 8000 years ago.

RESULTS

Microsatellite Polymorphisms

As expected on the basis of a previous study (Turgeon et al. 1999), all seven microsatellites markers were globally very polymorphic (Table 2). Four loci were moderately polymorphic, with a total of 10, 17, 10, and six alleles per locus (c90, c157, BWF2, and c126, respectively) over all 22 populations, whereas the other three were highly polymorphic with 39, 53, and 41 alleles per locus (c200, c181, and BWF1, respectively). There was a discontinuity in the global allele size distribution of BWF1, with short alleles ranging from 185 to 229 bp clearly separated from longer alleles in the range of 245 to 319 bp.

Within each population, the mean number of alleles per locus ranged from 6.3 to 13.1 (mean = 9.3) and the observed multilocus heterozygosity ranged from 0.465 to 0.736 (mean = 0.637). Sample size was positively correlated with the mean number of alleles per locus ($r^2 = 0.78$, $P < 0.05$) but not significantly correlated with heterozygosity. Genotypic frequencies generally conformed to Hardy-Weinberg expectations, and there was no evidence of linkage disequilibrium among alleles and loci.

Genetic Affinities among Populations and Geographical Patterns of Allelic Variation

The D_{ce}-based neighbor-joining phenogram (not shown) revealed no major discontinuities among populations: All internal branches were short, but the only high bootstrap value (>85%) supported the distinct grouping of eastern (all Atlantic drainage sites, except Lake Nipigon) and western populations (Hudson and Mackenzie drainages and Lake Nipigon).

FCA revealed a geographical pattern of genetic variation in accordance with the results of cluster analysis, and similarly suggested the distinct genetic composition of eastern (1–8) and western (9–22) groups of populations (Fig. 2). It also provided more specific information relative to the alleles most contributing to the observed structure. The six globally most common alleles (c90*102, c90*110, BWF2*153, BWF2*157, c126*167, and c126*202) were those that contributed most to the construction of the first correspondence axis (highest absolute contribution) and whose pattern of var-

iation were best represented by it (highest relative contribution). Seven other alleles also displayed high relative and absolute contributions to FCA axis 1. These alleles (c90*104 and c90*108; BWF2*159; BWF1*199 and BWF1*205; c157*151 and c157*155) were present in several or most populations, and all had global frequencies above 0.05. The expansion and construction of FC2 was mostly associated with the high frequency of a unique allele at locus c126 in population 4 (White Partridge Lake). Altogether, FCA axis 1 explained 23% of the variance in the association between alleles and individuals. However, when rare alleles were excluded (global occurrence less than five, i.e., 145 alleles), this percentage reached 27%, and 63% when only the 13 above-mentioned alleles were considered.

Regression analyses and difference-matrix Mantel tests revealed significant clinal variation at each of these 13 alleles (Table 3). At loci c90, BWF2, and c126, different alleles clearly dominated at the extreme ends of the gradient, and the allele dominating in the east was gradually replaced by an alternative dominant allele in the west. This pattern allowed to easily identified these alleles as being typical of the eastern or western part of the gradient (Table 3). Alleles with lower overall frequencies followed the same pattern at locus c90(*104 and *108) and BWF2(*159) and could also be assigned to the eastern or the western type. At BWF1, the two most common short alleles tended to be more frequent in the eastern part of the gradient. When all alleles of BWF1 were pooled either as short (185–229 bp) or long (245–319 bp) alleles, they were globally of the eastern and western type, respectively. At locus c157, no alleles were globally frequent nor clearly dominating at either end of the gradient, and the clinal pattern of allele *151 was only marginally significant ($P = 0.027$, Table 3). Therefore, our subsequent analyses of clinal variation did not consider locus c157 and rather focused on the other four main clinal loci, that is, c90, BWF2, c126, and BWF1.

Genetic Structure versus Contemporary and Historical Landscapes

All analyses of pairwise genetic estimates indicated that the observed genetic structure reflected the historical rather than the contemporary landscape structure. When all sampled populations were considered, gene flow estimates showed a significant decrease with geographical distance over the entire sampled area, which includes both the historical and contemporary migration domains (Fig. 3a). Striking differences were observed when analyses included populations located in the same or in different extant watersheds. Within each watershed (current migration domain), there were no significant associations between gene flow and either geographic distances (MacKenzie: $R = -0.52$, $P = 0.86$; Hudson: $R = -0.06$, $P = 0.63$; Atlantic: $R = 0.09$, $P = 0.36$; Fig. 3b) or riparian distances (MacKenzie: $R = 0.01$, $P = 0.34$; Hudson: $R = 0.19$, $P = 0.40$; Atlantic: $R = 0.03$, $P = 0.47$). In contrast, in the case of interbasin comparisons (historical migration domain only), this association was clearly significant. Pairwise θ estimates were extremely variable and ranged from 0.003 (populations 5–8) to 0.357 (populations 3–20; $R = -0.65$, $P = 0.005$; Fig. 3a). The AMOVA revealed a sig-

TABLE 2. Genetic diversity at seven microsatellite loci among 22 populations of lake cisco. Allele size range and median size (bp) and observed and expected heterozygosity (H_o/H_e) are given for each locus and mean number of alleles (A) and samples size (N) over all seven loci. Samples are coded as in Table 1 and Figure 1.

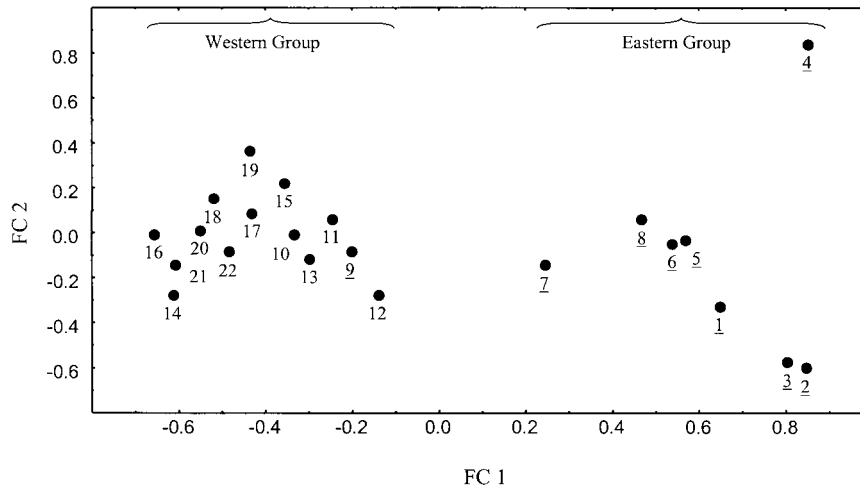| Population (map code)		c90	c157	BWF2	c126	c200	c181	BWF1	A	H_o/H_e	N
					Locus					Multilocus	
1	range	102–120	145–161	153–163	198–202	201–257	176–336	199–265	8.1	0.56/0.68	18.5
	(median)	(110)	(155)	(157)	(202)	(219)	(220)	(205)			
	H_o/H_e	0.36/0.64	0.72/0.77	0.45/0.63	0.10/0.09	0.94/0.91	0.93/0.97	0.42/0.73			
2	range	102–114	145–163	149–165	167–202	209–267	168–364	189–205	9.9	0.47/0.52	27.5
	(median)	(110)	(155)	(157)	(202)	(216)	(222)	(205)			
	H_o/H_e	0.32/0.31	0.57/0.66	0.57/0.59	0.07/0.07	0.71/0.89	0.85/0.96	0.14/0.14			
3	range	102–110	145–161	155–159	202–202	209–233	172–316	203–205	7.0	0.46/0.48	28.2
	(median)	(110)	(155)	(157)	(202)	(217)	(216)	(205)			
	H_o/H_e	0.35/0.42	0.53/0.50	0.70/0.58	0/0	0.62/0.89	1/0.95	0.0303			
4	range	102–112	141–159	153–165	167–202	203–241	180–352	199–205	11.0	0.65/0.66	47.0
	(median)	(110)	(149)	(157)	(202)	(217)	(296)	(200)			
	H_o/H_e	0.65/0.62	0.70/0.69	0.59/0.50	0.46/0.41	0.57/0.78	1/0.97	0.57/0.59			
5	range	96–120	139–161	153–159	167–202	199–233	152–364	199–273	10.4	0.59/0.65	27.7
	(median)	(110)	(155)	(157)	(202)	(215)	(220)	(205)			
	H_o/H_e	0.57/0.65	0.82/0.83	0.46/0.48	0.25/0.22	0.53/0.72	0.78/0.96	0.72/0.68			
6	range	102–110	141–161	153–159	202–202	197–227	180–320	185–215	6.3	0.48/0.52	18.8
	(median)	(102)	(155)	(157)	(202)	(215)	(224)	(205)			
	H_o/H_e	0.60/0.52	0.78/0.67	0.20/0.23	0/0	0.66/0.69	0.81/0.92	0.30/0.55			
7	range	102–110	145–157	153–159	167–202	201–257	172–324	199–275	7.4	0.58/0.64	19.0
	(median)	(108)	(151)	(157)	(202)	(219)	(220)	(255)			
	H_o/H_e	0.50/0.62	0.60/0.74	0.42/0.42	0.25/0.30	0.47/0.61	1/0.95	0.80/0.83			
8	range	102–110	143–157	149–163	167–202	209–231	156–320	199–289	9.9	0.65/0.69	23.7
	(median)	(110)	(151)	(157)	(202)	(215)	(230)	(205)			
	H_o/H_e	0.58/0.59	0.78/0.82	0.68/0.57	0.43/0.36	0.41/0.65	0.95/0.96	0.72/0.80			
9	range	102–110	121–163	147–159	167–202	195–257	168–292	199–279	13.1	0.74/0.76	44.2
	(median)	(104)	(147)	(155)	(200)	(215)	(212)	(256)			
	H_o/H_e	0.60/0.60	0.72/0.81	0.62/0.58	0.57/0.52	0.92/0.92	0.88/0.95	0.80/0.92			
10	range	102–110	145–163	153–157	167–202	199–251	184–340	199–279	10.0	0.69/0.73	27.8
	(median)	(104)	(149)	(153)	(202)	(225)	(216)	(267)			
	H_o/H_e	0.53/0.72	0.79/0.79	0.41/0.43	0.56/0.47	0.82/0.86	0.89/0.92	0.82/0.90			
11	range	102–110	121–163	149–157	167–202	199–245	160–340	197–281	12.9	0.67/0.73	44.2
	(median)	(102)	(153)	(155)	(202)	(215)	(230)	(257)			
	H_o/H_e	0.45/0.51	0.74/0.84	0.62/0.51	0.46/0.50	0.66/0.87	0.95/0.96	0.78/0.91			
12	range	102–110	143–161	153–159	167–202	193–249	180–292	199–271	8.0	0.70/0.67	19.3
	(median)	(102)	(153)	(157)	(202)	(219)	(208)	(205)			
	H_o/H_e	0.40/0.40	0.73/0.71	0.65/0.56	0.57/0.50	0.94/0.90	0.89/0.91	0.66/0.70			
13	range	102–110	143–161	149–157	167–204	197–251	164–300	199–289	9.7	0.72/0.72	18.7
	(median)	(102)	(153)	(157)	(167)	(214)	(210)	(257)			
	H_o/H_e	0.42/0.35	0.78/0.85	0.6/0.51	0.47/0.53	0.88/0.94	1/0.95	0.90/0.91			
14	range	102–104	147–163	153–159	167–202	197–239	196–304	199–285	7.6	0.66/0.66	19.0
	(median)	(102)	(151)	(157)	(167)	(201)	(214)	(267)			
	H_o/H_e	0.36/0.30	0.75/0.70	0.35/0.51	0.45/0.45	0.84/0.80	0.93/0.89	0.94/0.92			
15	range	102–110	131–161	153–161	167–202	199–241	168–288	199–279	9.3	0.60/0.65	26.7
	(median)	(102)	(147)	(153)	(167)	(215)	(196)	(257)			
	H_o/H_e	0.33/0.35	0.96/0.87	0.37/0.56	0.51/0.52	0.34/0.37	0.88/0.95	0.79/0.90			
16	range	102–110	143–165	153–157	167–202	197–229	180–272	199–293	8.3	0.69/0.68	19.5
	(median)	(102)	(151)	(153)	(167)	(203)	(240)	(272)			
	H_o/H_e	0.42/0.42	0.90/0.86	0.40/0.43	0.73/0.47	0.63/0.70	0.90/0.94	0.84/0.93			
17	range	102–110	133–163	149–157	167–202	199–269	172–348	205–289	11.0	0.71/0.72	29.0
	(median)	(102)	(153)	(153)	(167)	(215)	(214)	(265)			
	H_o/H_e	0.43/0.54	0.86/0.85	0.63/0.52	0.51/0.50	0.67/0.75	0.92/0.95	0.92/0.93			
18	range	102–110	145–161	153–157	167–202	203–247	168–308	201–291	8.4	0.63/0.65	18.2
	(median)	(102)	(152)	(153)	(167)	(215)	(200)	(267)			
	H_o/H_e	0.25/0.30	0.80/0.87	0.45/0.46	0.42/0.38	0.58/0.64	1/0.94	0.86/0.95			
19	range	102–110	131–165	153–157	167–202	203–241	168–284	199–281	9.1	0.69/0.73	25.8
	(median)	(104)	(147)	(153)	(167)	(215)	(252)	(266)			
	H_o/H_e	0.69/0.65	0.88/0.83	0.57/0.47	0.42/0.46	0.57/0.84	0.96/0.93	0.75/0.91			
20	range	102–110	147–165	153–163	167–167	207–277	176–276	199–287	9.9	0.66/0.65	20.0
	(median)	(102)	(155)	(153)	(167)	(225)	(208)	(263)			
	H_o/H_e	0.40/0.41	0.70/0.77	0.65/0.53	0/0	0.90/0.91	1/0.95	0.94/0.93			
21	range	102–108	139–157	149–163	167–202	199–265	176–224	205–319	7.7	0.68/0.64	18.2
	(median)	(102)	(153)	(153)	(167)	(217)	(200)	(268)			
	H_o/H_e	0.22/0.20	0.89/0.84	0.25/0.23	0.77/0.48	0.85/0.90	0.78/0.88	0.95/0.92			
22	range	102–110	133–163	153–157	167–204	199–241	168–276	199–271	8.7	0.72/0.72	25.7
	(median)	(104)	(152)	(153)	(167)	(217)	(202)	(215)			
	H_o/H_e	0.57/0.68	0.92/0.85	0.42/0.47	0.61/0.52	0.80/0.87	1/0.93	0.71/0.70			
Total	A	10	17	10	6	39	53	41			

FIG. 2. Position of 22 samples of *Coregonus artedi* in the multivariate space defined by factorial correspondence analysis axes 1 and 2. Populations from the Atlantic drainage are underlined (1–9).

nificant proportion of genetic variance among current watersheds (8.7%, $F_{CT} = 0.088$, $P < 0.001$), but that proportion was almost identical to that accounted for by the significant variation among populations within each watershed (7.2%, $F_{SC} = 0.079$, $P < 0.001$).

Origin and Maintenance of Historical Gene Flow Signal

Analyses of the clinal pattern of variation revealed coincident clines with similar breakpoints, as well as thorough genomic mixing of eastern and western alleles. At *c90, BWF2, c126,* and *BWF1,* eastern and western alleles defined clines that were coincident. For each type of allele (i.e., east and west), the coincidence of clines across loci was revealed by the significant linear relationship between allele frequency at each locus and the mean frequency over all four loci (Fig. 4). Linear regression coefficients were significant for eastern

and western alleles ($P < 0.0001$) and explained 59% and 74% of the variation. However, piecewise regression analyses substantially increased the proportion of explained variance (75% and 85% for eastern and western alleles, respectively). More importantly, the latter analyses identified coincident breakpoints in each allele type at the interface of the Atlantic and Hudson drainages (Fig. 4). Using the same four clinal loci, 544 individuals (92%) possessed seven or eight alleles that could be assigned to either the eastern or western type. The mean hybrid index (HI) of each population varied gradually along the east–west gradient (Fig. 5, $R = -0.916$, $P < 0.0001$). There was no obvious trend of variation in the variance of HI across populations along this gradient, as would be expected in the central part of young tension zone (Barton and Gale 1993) when linkage disequilibrium reveals incomplete genomic admixture (due to selection or insufficient time for genomic recombination).

TABLE 3. Analyses of allele frequency (*p*) clinal variation at five microsatellite loci in 22 populations of *Coregonus artedi* distributed along the axis of proglacial lakes. (Sequential-Bonferroni-adjusted significance levels at $\alpha = 0.05$ and 0.01 are indicated by * and **, respectively). CHP, Lake Champlain.

| Locus | Allele | | | Regression (*p* over distance to CHP) | | Mantel tests Δp vs. geographic distance | |
	name (bp)	p_{tot}	type[1]	R	P-value	R	P-value
c-90	102	0.503	west	0.69	0.0003**	0.44	<0.001**
	104	0.087	west	0.78	<0.0001**	0.55	<0.001**
	108	0.054	east	−0.65	0.001**	0.31	0.0035*
	110	0.339	east	−0.71	0.0002**	0.41	<0.001**
BWF2	153	0.359	west	0.88	<0.0001**	0.70	<0.0003**
	157	0.501	east	−0.71	<0.0003**	0.37	0.002**
	159	0.087	east	−0.71	<0.0003**	0.38	0.002**
c-126	167	0.374	east	0.93	<0.0001**	0.79	<0.0001**
	202	0.593	west	−0.92	<0.0001**	0.77	<0.0001**
BWF1	199	0.155	east	−0.57	0.006**	0.24	0.016*
	205	0.263	east	−0.67	<0.0006**	0.32	<0.004**
	long/short[2]	0.498/0.502	west/east	±0.72	<0.0002**	0.48	0.002*
c-157	151	0.108	east	0.47	0.027*	0.48	0.001**
	155	0.187	west	−0.67	0.0006**	0.37	<0.003*

[1] Allele type is determined on the basis of the area of higher frequency, as revealed by the sign of regression coefficient.
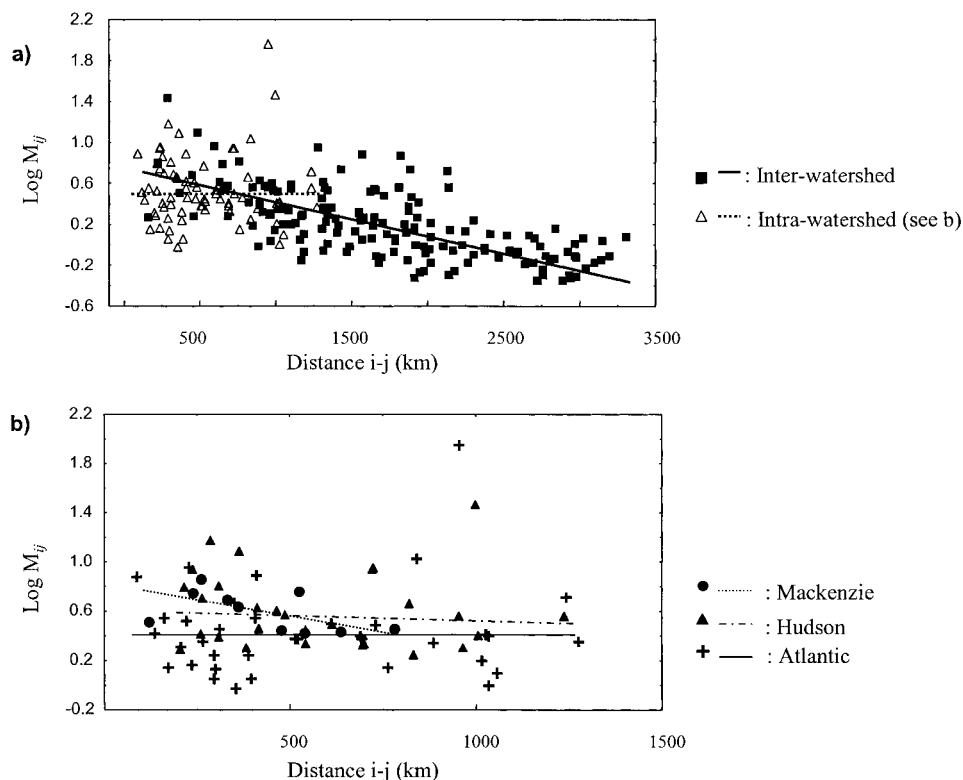[2] Short alleles: 185–229 bp; long alleles: 245–309 bp.

FIG. 3. Relationship between pairwise gene flow estimate and geographical distance among 22 populations of *Coregonus artedi* in (a) the whole range and (b) within watersheds.

Global IBD patterns indicated that a stepwise expansion also contributed to the emergence of the contemporary genetic structure revealed by pairwise gene flow estimates. The global IBD pattern (Fig. 3a) suggested a higher, albeit non-significant, variance of gene flow estimates at shorter geographic distance, an observation that is consistent with nonequilibrium conditions generated by a recent stepwise expansion (Slatkin 1993). The results of Good's model revealed that pairwise gene flow estimates were significantly related to the distance of the population closer to the easternmost population (population 1, Lake Champlain; $R = 0.53$, $P < 0.001$; Fig. 6a) and independent of the distance to the westernmost population (22, Great Slave Lake; $R = -0.11$, $P = 0.32$; Fig. 6b). This suggests an expansion from the eastern end of the sampled species range.

Finally, minimal estimates of effective population sizes yielded a mean value of 2753, with minimum and maximum values of 1540 (Lac des Ecorces, population 3) and 4048 (Lake Nipigon, population 9).

DISCUSSION

Origin of Clinal Genetic Variation

This study clearly demonstrated the clinal pattern of variation in allelic frequencies at four polymorphic microsatellite loci. The replacement of eastern alleles by western ones along a geographical gradient running through the area formerly covered by proglacial lakes is strongly indicative of neutral secondary contacts among previously differentiated races of *C. artedi*. Distinguishing primary versus secondary intergradation cannot easily be achieved by examining the cline properties and is inherently difficult when there is no historical evidence (Endler 1977; Barton and Hewitt 1985, 1989). In the present case, however, both historical and independent genetic information argue for allopatric differentiation and secondary contacts. It is well documented that freshwater fish species now inhabiting postglacial lakes have often been isolated for long periods of time in multiple refuges during the Pleistocene glaciations, and that these refugial races have subsequently come into secondary contacts during interglacial periods (e.g., Billington and Hebert 1988; Bernatchez and Dodson 1991; Bernatchez 1997; Danzmann et al. 1998; Wilson and Hebert 1998). For *C. artedi*, mtDNA haplotypes of populations surveyed in this study belong to two different clades whose level of divergence (0.48%) also indicates divergence during the Pleistocene (Turgeon and Bernatchez 2001). These putative glacial races had significantly different geographical distributions but lived sympatrically over most of the current species range. While the weak mtDNA signal of past range fragmentation was only suggestive of dispersal from distinct refugia followed by extensive intermixing, the evidence of clines at neutral nuclear loci strongly argues for secondary contacts in the proglacial lakes area. Indeed, simulations investigating the effects of spatial origin of hybrid zones in the development of clines clearly revealed that primary intergradation will not produce clines at neutral loci, whereas secondary contacts of genet-
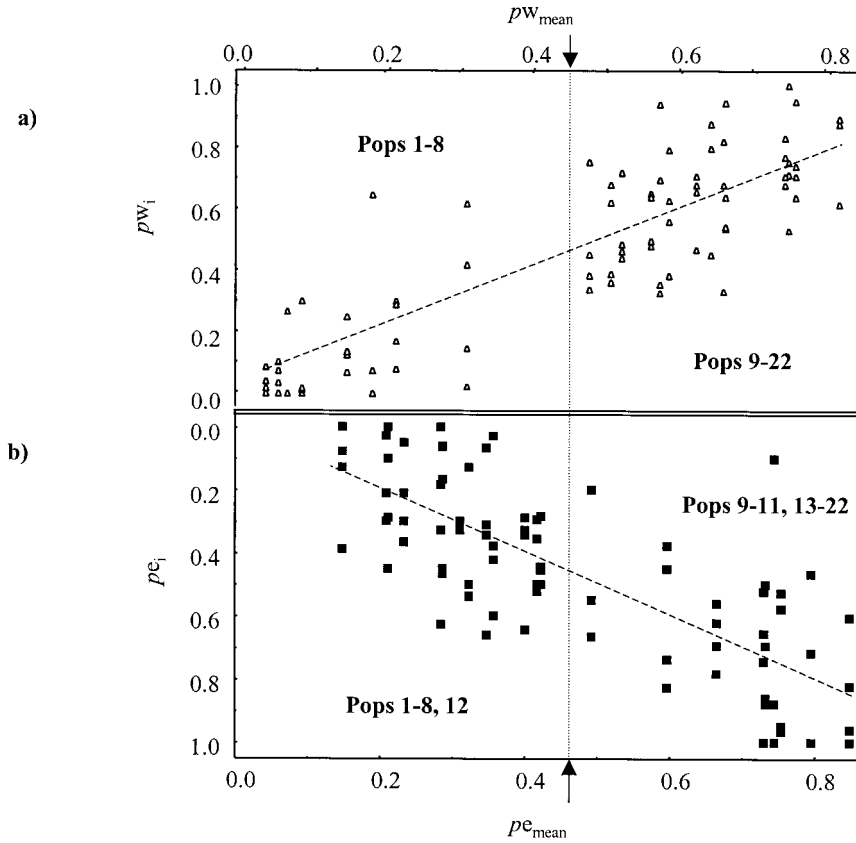
FIG. 4. Coincidence of clines revealed by the linear relationship between the frequency (p) of diagnostic alleles at $i = 4$ loci and the average frequency over four loci, and coincidence of allele frequency breakpoints identified by piecewise regression analysis. (a) western alleles; and (b) eastern alleles. Arrows and dotted line indicate the coincident breakpoints separating populations into eastern and western groups.

ically differentiated groups can produce very wide clines at neutral loci (Nichols and Hewitt 1994; Durett et al. 2000).

Secondary contacts among these divergent races also appear to be selectively neutral for the loci under study. Neutral

FIG. 5. Clinal variation in population mean hybrid index in 22 populations of *Coregonus artedi* along the axis of proglacial lakes. Bars indicate ±1 SE.

secondary contacts initially produce steep clines, but the steep gradients decay with time as intergradation between the two groups proceeds. As a result, cline slopes decrease and cline widths expand, but such clines can persist for several hundreds of generations (Slatkin 1973; Endler 1977). Neutral cline width can be estimated using the diffusion approximation or as the inverse of the maximum slope of allele frequency (Barton and Gale 1993). The first method appears futile due to the lack of a reasonably precise estimate of dispersal rate for lake cisco. However, cluster analysis, FCA, and piecewise regressions identified similar distribution breakpoints of allelic types in the area between Lake Superior (population 8) and Lake of the Woods (population 12). Differences in the frequency of clinal alleles on each side of the breakpoints identified on Figure 5 over the distance separating these two sites provide similar cline width (w) estimates of 2641 and 2704 km. These clines extend across extant watershed boundaries and it can be reasonably inferred that secondary contacts occurred within proglacial lakes (12,000–8000 years ago; Teller and Kehew 1994). Assuming a generation time of 3.5 years (Scott and Crossman 1973), this yields reasonable estimates of dispersal (σ) rate of 31 km/generation (Barton and Gale 1993, p. 16). Although these estimates are only rough approximations, it is clear that the width of these coincident clines greatly exceeds the dispersal
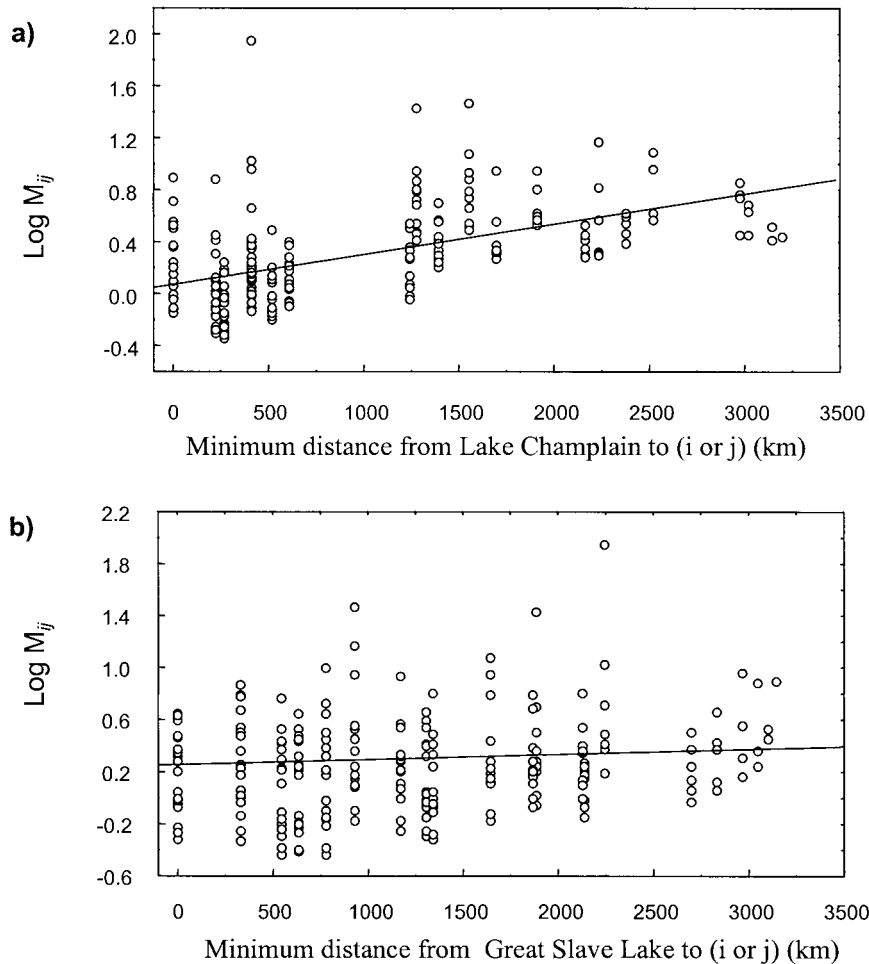
FIG. 6. Relationship between pairwise gene flow estimate and geographical distance of the nearest population of the pair to (a) Lake Champlain and (b) Great Slave Lake.

capacity of lake cisco, a characteristic that is expected for clines resulting from neutral secondary contacts. In addition to their smooth shallow slopes and the extremely large estimated width (relative to dispersal ability), the neutral nature of these clines is also suggested by the apparent absence of any environmental or selective gradient along the sampled area. Finally, although the absence of linkage disequilibrium indicated thorough genotypic intermixing (contacts with gene flow), the small and homogeneous variance of population racial indices along the clinal gradient also supports the idea that negative endogenous selection has not played an important role during the establishment of these clines (Barton and Hewitt 1985). However, it remains plausible that positive selection on hybrid genotypes has played a role during the expansion of the hybrid zone, as is suspected for other wide and recent hybrid zones (Martin and Cruzan 1999).

Population Structure versus Population History

All analyses revealed that the pattern of genetic variation had a strong geographical component. A global pattern of isolation by distance was detected (Fig. 3a) over a very wide range of geographic distances, but the lack of increased variance among gene flow estimates at large distance and the

absence of IBD patterns over all geographic scales (i.e., within watersheds, Fig. 3b) allowed to reject the hypothesis of global migration-drift equilibrium under the Hutchison and Templeton (1999) criteria. Thus, the observed population structure was a historical signal revealing the extent of intermixing between two races rather than one of current balance between migration and drift.

Over the entire species range, pairwise gene flow estimates were related to geographic distance, but this relationship was primarily due to the clinal variation in hybrid index reflecting the racial composition between populations along the east–west gradient. This clinal pattern resulted from secondary contacts that could only take place in the historical habitat defined by the proglacial lakes that existed some 12,000–8000 years ago. Because migration has long ceased across extant watershed boundaries, this clinal pattern of variation is likely maintained by large effective population sizes delaying the erosive action of drift and preserving this historical signal of gene flow.

At shorter geographical scales (within extant watersheds), the extent of variation of racial intermixing was less variable, and the relationship between gene flow estimates and geographic distances probably better reflect the relative impor-

tance of migration and drift. At this spatial scale, the lack of relationship indicated nonequilibrium conditions, with high, although variable, gene flow ($\hat{M} > 1$) over all distances. High gene-flow estimates bearing no relationships with geographical distance are expected in the nonequilibrium radiation model of Slatkin (1993) for species with low dispersal having recently colonized an area and correspond to the expectation of the Hutchison and Templeton (1999) model immediately after an invasion. The persistence of this disequilibrium signal may be due to low migration rate and/or high long-term N_e, but a lack of knowledge of lake cisco dispersal ecology in the study area makes it difficult to identify the critical factor. On the one hand, lake cisco is a truly lacustrine species in the study area, and major interlake (riverine) migrations are not reported, suggesting that the postglacial change in habitat structure has dramatically reduced their previous dispersal capability. The lack of linkage disequilbrium also argues for the absence of major recent migration events. Nevertheless, dispersal potential cleary exists, as suggested by the anadromy of Hudson Bay populations. On the other hand, the very large size of sampled lakes (50% > 1000 km²), their continuous existence as such large water bodies since their formation, and the large sizes of many lake cisco populations (a forage fish) together suggest that these populations have maintained relatively high N_e. Although our estimates of long-term N_e are tentative, their very large values support this hypothesis. Thus, within each watershed, it appears that the scattering of high gene flow estimates over a wide range of geographic distances most likely reflects the initial conditions at time of population establishment (lake formation). Each group of populations was characterized by a similar mixture of divergent races, which inflated the gene flow estimates, and by large population sizes, which slowed down the action of genetic drift despite putatively low migration rates.

Secondary Contacts and Postglacial Colonization Processes

Nonequilibrium analyses of the currently observed pattern of genetic variation allowed us to detect a signal of westward stepwise expansion from the eastern end of the sampled range (population 1, Lake Champlain). In contrast, there was no signal of expansion from the western portion of the range (population 22, Great Slave Lake). These results suggest that an eastern race expanded into habitats occupied by a race having previously and very rapidly colonized most of the distribution range. This colonization scenario corroborates our interpretation of lake cisco mtDNA polymorphisms in populations from the same general area (Turgeon and Bernatchez 2001). First, the absence of an expansion from the west confirms the absence of a Beringian race of lake cisco in North America, an inference that has long been proposed and accepted by traditional biogeography (McPhail and Lindsey 1970; Hocutt and Wiley 1986). Second, the group defined on the basis of microsatellite alleles prevailing in the western reaches of the range most probably dispersed from the Mississippian refuge. Expansion from that refuge into the proglacial lakes giving access to central Hudson and Mackenzie watersheds was possible via its early connection with Lake Agassiz 12,000 years ago (Dyke and Prest 1987) and would

explain the prevalence of this race in the western portion of the sampled area even though it did not disperse *from* it. This interpretation is highly compatible with the current recognition of a single Mississippian refuge for *C. artedi* and with the results of mtDNA molecular phylogeographic revealing that mitochondrial clade A was the only group present in the Mackenzie watershed. Finally, mtDNA polymorphisms had revealed the existence of a distinct group of haplotypes (mitochondrial clade B; Turgeon and Bernatchez 2001), and we suggested that it had a distinct, namely Atlantic, refugial origin. The data from the present study are highly concordant with this hypothesis and bring additional evidence that an Atlantic race combined with the Mississippian race by gradual intermixing and dispersal via the proglacial lakes. The presence of the Mississippian race in this area apparently did not completely restrict the migration of the Atlantic race, possibly because of the immense proglacial lake habitat size and unsaturated carrying capacity. Had these habitats been saturated, the Atlantic race could probably not have invaded the area (Ibrahim et al. 1996). Interestingly, the breakpoints in the distribution of microsatellite alleles typical of each race were located at the current interface of the Atlantic (Great Lake) and Hudson Bay watersheds, a location that corresponds to one of the last points of contact between glacial Lake Ojibway and Agassiz about 9500 years ago via a corridor where Lake Nipigon and Lake of the Woods now stand (Dyke and Prest 1987). This breakpoint may thus correspond to the area (and period) where the western colonization movements of the Atlantic race were slowed down.

Conclusions and Perspectives

Analysis of IBD patterns most often aim at determining if and to what extent gene flow is restricted by dispersal and/or at estimating neighboring size (e.g., Johnson and Black 1995; Pogson et al. 2001). However, there is now analytical (Slatkin 1993) and empirical (Hutchison and Templeton 1999; this paper) evidence that supplementary and qualitatively different information can be retrieved by this approach. As proposed by Hutchison and Templeton (1999), analysis at different geographical scales can reveal distinct evolutionary balance between migration and drift in different geographical taxon components. Moreover, the coalescence approach takes advantage of the historical information contained in population allelic frequencies and allows researchers to define expectations under a variety of realistic colonization scenarios. When combined with independent physiographical information, this theoretical framework can therefore determine the time period over which historical events take precedence over current processes. In cases of organisms having recently invaded an area, this approach can greatly enhance our ability to investigate and disentangle the effects of migration and demographic perturbations on the current patterning of genetic diversity. This type of information should considerably improve our ability to separate ecology from history and appraise the long-suspected role of introgression in the study of the phenotypic diversification process of young, globally sympatric lineages such as North American ciscoes (Svärdson 1970, 1998).

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