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MOLECULAR PHYLOGEOGRAPHY OF *DRYAS INTEGRIFOLIA*: GLACIAL REFUGIA AND POSTGLACIAL RECOLONIZATION

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ABSTRACT

This thesis addresses the consequences of the last glaciation on the distribution and genetic diversity of arctic flora. The principal aim is to infer the full-glacial and postglacial migrational history of *Dryas integrifolia* M. Vahl. (Rosaceae) from the intraspecific phylogeny of cpDNA haplotypes, along with pollen and macrofossil distribution data. The results suggest that four refugia existed during the last glaciation and that each served as significant sources of recolonization when the ice retreated. The two most important refugia are located in the northwestern Arctic (Beringia and the High Arctic), with two other refugia located southeast of the ice sheet and along the coastal regions of the eastern Arctic. High genetic substructure among populations is likely attributable to past vicariance and recent recolonization events, whereas high local diversity is probably indicative of recolonization from several sources and high gene flow in recent time.

RÉSUMÉ

Cette thèse évalue les conséquences de la dernière glaciation sur la distribution et la diversité génétique de la flore arctique. Le but principal est de reconstruire l'histoire pléniglaciaire et postglaciaire de la migration de *Dryas integrifolia* M. Vahl. (Rosacée), en utilisant la phylogénie intraspécifique d'haplotypes d'ADN chloroplastique ainsi que les données polliniques et macrofossiles sur la distribution passée de l'espèce. Les résultats suggèrent qu'il y avait quatre refuges durant la dernière glaciation et que chacun a contribué significativement à la recolonisation une fois les glaciers retirés. Les deux refuges les plus importants se trouvent dans le nord-ouest de l'Arctique (Béringie et Haut Arctique); les deux autres se trouvent au sud-est de la calotte glaciaire et dans les régions côtières de l'est de l'Arctique. La distribution hautement structurée de la diversité génétique entre les populations est vraisemblablement attribuable à un épisode de vicariance dans le passé ainsi qu'aux récents épisodes de recolonisation, tandis que la diversité locale élevée suggère une recolonisation à partir de plusieurs sources et un flux génique actuel élevé.

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This thesis is dedicated to my family, who have always been present, always supportive. They contributed immensely to all my acheivements.

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INTRODUCTION

I have tried to prove that most arctic and numerous boreal plants radiated from the Bering Sea area.

Eric Hultén 1937 (p. 141)

Arctic Climate Change and Plant Distribution

Glaciations have had a profound influence on the present distribution and genetic diversity of all living taxa in the northern hemisphere: birds (Bermingham et al. 1992), mammals (Graham et al. 1996; Macpherson 1965), invertebrates (Weider and Hobæk 1997), marine organisms (Dunton 1992), boreal and temperate plants (Hewitt 1996; Soltis et al. 1997), and of course arctic plants (Ritchie 1987) underwent important migrations in reaction to these major climatic changes.

Glaciations have long been suspected of playing a central role in the evolutionary history of arctic flora. Darwin (1859) for instance was struck by the similarity of the alpine and arctic flora of Europe. He suggested that the coming and going of glacial periods offers a simple explanation of these plant distributions. During warmer periods, cold-loving plants occurred near the pole, but during colder periods, they migrated south. As the climate improved, they migrated back north, as well as to higher altitudes in southern regions. Nowadays, there is renewed interest in the biogeography of tundra plants, as the possibility of significant climatic change prompts study of the fragile arctic ecosystem, one that is more susceptible than others to climatic variation (Spicer and Chapman 1990; Walker 1995).

The effect of climate change is thought to be greatest at higher latitudes because minor changes in global temperatures are magnified in the Arctic (Cuffey et al. 1995). Any annual variation, even of a single degree, means important changes in plant phenology, reproductive success, and possibly survival in a region where the mean daily temperature sometimes exceeds 0°C only in July and August (Scott 1995). The imminence of a quick and significant global warming has therefore stimulated a great amount of research effort in the Arctic since the beginning of the decade (e.g., the International Tundra Experiment, Molau and Molgaard 1996; Chapin III et al. 1992; Chapin III and Körner 1995; Environment Canada 1996). Warming may have numerous consequences, both ecological and economic, such as the invasion of new species and the extinction of some northern ecotypes (Crawford and Abbott 1994; Environment Canada 1996; Hewitt 1996; McGraw 1995; Pitelka and the Plant Migration Workshop Group 1997; Spicer and Chapman 1990). Studying the history and evolution of the arctic flora is therefore essential, especially in relation to past glaciations, because it may provide clues about the response of the entire ecosystem to environmental shifts.

This thesis therefore concerns the study of the biogeographic distributional consequences of the last glaciation on arctic flora. In particular, the principal aim is to trace the full-glacial (during the maximum extent of glaciation) and postglacial (after the ice retreated) migrational history of one commonly occurring arctic plant species, *Dryas integrifolia*. The full objectives and results are presented after the following review of what is known about the recent history of arctic climate and flora.

History of Arctic Climate and Flora

History of glaciation

Change rather than stability has been the rule for the Earth's climate throughout geological time. The global climate has been shown to have been generally much warmer in the past than it is today. In fact, during 80% of the last 500 million years (My), the Earth has been in a "greenhouse" mode (Spicer and Chapman 1990), interrupted by a number of cold periods, termed "glacial ages", lasting 10 My or more each (Pielou 1991). Within each glacial age, there were numerous intensely cold periods referred to as "glaciations", each lasting 60,000 to 90,000 years. Glaciations are characterized by the building of ice-caps over temperate regions, like those found in polar latitudes today (e.g., Antarctica). They are separated by short, comparatively mild respites called "interglacials", each lasting 10,000 to 40,000 years (Pielou 1991).

In North America the last glacial age started around 1.8 to 2.4 My ago (Pielou 1991). Traditionally, four glaciations have been recognized in the Pleistocene period in North America, each lasting about 100,000 years and referred to as the Nebraskan, Kansan, Illinoian and Wisconsinan glaciations, from the most ancient to the most recent (Flint 1971). The Wisconsinan glaciation is by far the best known. It occurred from 65,000 to 10,000 years ago and extended over most of Canada and the northern United States (Dyke and Prest 1987a). The remnant of the great ice sheet (called the "Laurentian" ice sheet) covering Northern Québec disintegrated some 7,000 years ago.

Origin of arctic flora

Compared to temperate and tropical taxa, high-latitude flora are extremely recent (Spicer and Chapman 1990). North American arctic flora is of multiple origins, comprising some elements that evolved *in situ* and others that migrated over time from different places.

The first arctic plants evolved at the highest latitudes at the end of the Tertiary period (especially the Pliocene epoch, 5-2.4 My ago). At the time, a coniferous woodland covered these latitudes. The ancient forest included some floristic elements we recognize today as part of the tundra. These forests disappeared progressively as the climate chilled and glaciation started. Certain plants, those pre-adapted to the habitats destined to become more widespread during the Quaternary period, remained after the forests disappeared. To this group of arctic plants were added later immigrants that had evolved during the Tertiary period in the high mountain system of Central Asia and western North America. Finally, some new species evolved *in situ* during the Pleistocene and Holocene epochs from existing arctic species (Murray 1987, 1995).

The flora of the Arctic reacted to the repeated advance and retreat of glaciation by undergoing major migrations and recolonizations. Unlike the boreal elements whose full-glacial and postglacial histories are well documented thanks to countless palynological studies, the exact details of arctic plant glacial history remain largely unknown (Ritchie 1992). In fact, the locations where arctic plants survived the last glaciation and how they recolonized the Arctic when the ice retreated have been poorly investigated. This is because of limitations inherent in the type of information provided by pollen and macrofossil remains and phytogeographical data, on which inferences about the history of arctic taxa have been based so far.

Refugia (or where arctic species survived)

We know that glaciations forced major migrations and redistributions of all plants and animals that survived at the periphery of the ice. Areas where plants and other taxa survived and from which recolonization proceeded are known as "refugia".

In 1937, Eric Hultén was among the first to propose that ice-free regions between Alaska and Siberia—called Beringia— could have been a refugium for arctic plants (Figure 1). In fact, during full-glacial time, sea level was approximatively 80 metres below the present level, thus uncovering a large continental bridge between Asia and America. This land bridge remained largely unglaciated throughout the most recent glaciation. Toward the end of the glaciation, around 11,000 years ago, the sea level rose, and the connection between Asia and America was cut off (Elias et al. 1996). At present, the two continents are split by the Bering Strait (hence the name of the region, Beringia). Initially deduced from the pattern of plant distributions, the existence of Beringia was later supported by fossil and geological evidence (Hopkins et al. 1982; Ritchie 1987).

Other possible refugia

Apart from Beringia, four other areas have been proposed as possible refugia: an area southeast of the ice sheet, the High Arctic, the southern Rockies and the eastern Arctic coastal region (Fig. 1).

There are several types of evidence supporting the existence of these putative refugia. First, since the early 1960s, numerous fossils of plants with arctic affinities have been recovered in sites formerly located south of the ice sheet (e.g., Argus and Davis 1962), and it was concluded that a refugium could have been located in that area (Ritchie 1987). This

refugium would have been restricted to a thin band of tundra immediately south of the ice, extending from Iowa to New Jersey (Fig. 1) (Ritchie 1992). The presence of disjunct populations of arctic plants in the northeastern United States and southeastern Canada supports the existence of this secondary refugium (e.g., Morisset 1979, Roberts 1965). Second, in the High Arctic Archipelago, one hypothesis is that the islands were covered only by small independent ice caps (instead of a massive ice sheet), and that some ice-free areas existed where terrestrial and aquatic life remained (Fig. 1) (Edlund 1994; Gray 1994; Hogson 1991). Third, the high elevations found in the southern Rockies could also have hosted arctic plant during the last glaciation (Fig. 1) (Mooney and Billings 1961). Fourth, all glacial margins were certainly punctuated by ice-free oceanic headlands and mountain peaks (collectively called "nunataks") that could have supported arctic plants (Murray 1987). Such areas extended from Baffin Island southward to the Gulf of St. Lawrence in the east, as well as to both western and eastern Greenland (Fig. 1) (Funder 1979; Holland 1981; Murray 1987). Coastal regions of southeast Alaska have also been proposed as refugia (Soltis et al. 1997), but mostly for boreal plants, which are not of central concern here and will not be discussed in further detail.

The existence of these four other possible refugia is not as well supported as the existence of Beringia. For instance, no fossils were found to support their existence (except in the southeast). At best, the existence of the High Arctic, coastal and southeast refugia is suggested by the present-day distribution of some species of rare mosses. The distributions of these mosses indicate that they could be glacial relics, i.e., they survived the Wisconsinan glaciation at or near the sites where they are found today. This may explain the disjunct distributions of isolated populations found in the Gulf of St. Lawrence region, Newfoundland, Labrador, and the High Arctic and west Greenland (Belland 1987; Belland 1994; Belland and Brassard 1988; Belland and Favreau 1988; Belland et al. 1992;

Hedderson and Brassard 1992a; Hedderson and Brassard 1992b; Mogensen et al. 1997). More evidence is needed to confirm the existence of these four refugia.

Postglacial recolonization

About 18,000 years ago, the Laurentian ice sheet started to melt. As soon as this happened, arctic plants began to recolonize the Arctic from refugial area(s). Note that being a refugium and being a source of massive recolonization are two different things. The first does not necessarely entail the second, as explained below.

Hultén (1937) suggested that Beringia served as the most important source area for restocking arctic and possibly boreal flora. Today, this hypothesis is well accepted, even though it has never been tested (Ritchie 1992). Hence, in the case of Beringia and the other possible refugia, it is not known whether they acted as source areas or whether the populations underwent regional extinction (J.C. Ritchie personal communication). The southeast refugium provides a good illustration. In the southeast, it appears that arctic elements closely followed the retreating ice margin at least some distance inland (Flint 1971). However, the final extent of this migration, and whether it reached the present arctic biome, is not known. Arctic plant populations may have become extinct, either due to competitive exclusion by more agressive temperate and boreal taxa in early postglacial time (Given and Soper 1981), or because significant population reductions may have diminished their genetic variability and thereby also presumably reduced their capacity to colonize new habitats (Hultén 1937).

Means of Investigation

As traditional means of investigating the glacial history of arctic plants provide only incomplete answers, other tools are needed to infer their biogeographical and evolutionary past. Since the 1980s, molecular genetic data have been used to complement data from fossils and biogeography, in particular to infer the demographic history of species (e.g., the geographical origin of modern humans, Cann et al. 1987). The use of molecular markers to reconstruct the phylogenies and to study the genetic structure of the populations provides a novel source of information.

Intraspecific phylogeography

The fusion of population genetics, molecular systematics and biogeography led to the creation a new discipline: phylogeography (Avise 1994; Avise et al. 1987). The discipline involves the study of the principles and processes governing the geographic distribution of genealogical lineages (phylogenies), including those at the intraspecific level. In practical terms, it has been noted that many genetic lineages are geographically localized and that they share a common evolutionary history, such as episodes of vicariance (i.e., the separation of a group of organisms by a geographic barrier), major migration and recent expansion. Phylogeographic studies enable the researcher to recognize differences in various populations of a species that would otherwise seem monomorphic. These tools have been applied successfully to a variety of studies involving plants and animals (e.g., Avise 1992; Bernatchez and Dodson 1991; Demesure et al. 1996; Soltis et al. 1992a).

Phylogeographic principles can be summarized in three main postulates (Avise 1994; Avise et al. 1987; Riddle 1996). First, most species are composed of geographic populations whose members occupy different branch tips of an intraspecific, phylogenetic tree.

Second, species with limited or shallow phylogeographic population structure have life histories conducive to dispersal and have occupied ranges free of long-standing impediments to gene flow (Figure 2a). Third, populations separated for a long time by geographic barriers to gene flow should accumulate distinctive mutations in their genome and therefore should constitute groups distinguished by deep phylogenetic gaps (Fig. 2b). Two important corollaries of this third postulate are (a) if the barrier to gene flow is sufficiently significant, then it should mould the genetic structure of independently evolving species in concordant fashion (across taxa); and (b) phylogenetic gaps within species should tend to be geographically concordant with boundaries between traditionally recognized biogeographic provinces.

Reconstruction of a phylogeny

Using the principles of phylogeography requires the availability of a phylogenetic tree for the species studied. Cytoplasmic DNA (mitochondrial or chloroplast DNA) has become the tool of choice for such analysis. For plant studies, restriction site analysis of chloroplast DNA (cpDNA) has emerged as the most popular technique for phylogenetic reconstruction below the family level (Soltis et al. 1992b). The use of cpDNA has many advantages reviewed in detail by Hillis et al. (1996), Olmstead and Palmer (1994), and Soltis et al. (1992b). One important advantage for phylogenetic studies is the uniparental mode of inheritance of cpDNA. Chloroplasts are maternally inherited, present in ovules but not the pollen. In theory, this allows migration patterns based on seed dispersal to be studied apart from gene flow caused by exchange of pollen (McCauley 1995). Another advantage is the non recombining nature of cpDNA.

Despite its relatively slow rate of silent substitution (compared to the more frequently used mitochondrial DNA in animal studies), the cpDNA genome has provided useful

intraspecific variation in some species investigated (Olmstead and Palmer 1994). Recently, the production of universal PCR (Polymerase Chain Reaction) primers for highly variable non-coding regions of the chloroplast has provided an important additional tool for the study of intraspecific variation. The primers are located in conserved regions flanking regions of relatively high substitution rate.

Analysis of genetic diversity and genetic structure

Phylogeographic principles are not the only tools available to reconstruct the evolutionary history of a species; the distribution and structure of genetic diversity can also be useful. For example, it is expected that a source of recolonization should exhibit more diversity than recently recolonized areas because of the loss of variation accompanying successive founding events in the recolonized areas (Hewitt 1996). It is also thought that expansion occurs by the founding of new, small populations, which in turn expand to become the source of the next dispersal and founding event (Cwynar and MacDonald 1987). This observation is based on several studies of boreal and temperate taxa from North America (Cwynar and MacDonald 1987; Lewis and Crawford 1995) and Europe (Demesure et al. 1996; Ferris et al. 1995). This phenomenon has also been observed in mammals (Sage and Wolff 1986), invertebrates (Boileau and Hebert 1991; Weider and Hobæk 1997), and fish (Bernatchez and Dodson 1991). In the study of the origin of modern humans (where the highest level of mitochondrial DNA polymorphism is found in Africa), it is the most convincing argument supporting the hypothesis of an African origin for humankind (Avise 1994; Bowcock et al. 1994). The same phenomenon may also help to locate arctic plant refugia.

Populations of nearly all species exhibit at least some degree of genetic differentiation among geographic locations (Avise 1994). The extent of geographic differences results

from a balance between forces tending to produce local genetic differentiation and forces tending to produce genetic homogeneity (Slatkin 1987). Mutation, genetic drift due to finite population size, episodes of vicariance, and natural selection favouring adaptation to local environmental conditions will all lead to genetic differentiation of local populations, while the movement of gametes, individuals and even entire populations—collectively called gene flow—will oppose that differentiation. The challenge is to identify the evolutionary forces responsible for the observed geographical association and to tease apart their relative influence (Avise 1994). Furthermore, some factors prominent in the past may not be active today and *vice versa*.

Dryas integrifolia as a model organism

To study the consequences of glaciation (essentially the last one) for arctic flora, *Dryas integrifolia* M. Vahl. (Rosaceae), also known as arctic avens, was selected as a model organism (Figure 3). This was done for several reasons. First, *D. integrifolia* is believed to be representative of many other arctic taxa. For instance, it covers the whole Arctic and extends further south in high altitudes in the Rockies and in specialized habitats in southeastern Canada (Ontario, Québec and New Brunswick, Fig. 4), like many other members of the arctic-alpine flora. It extends from 83°N to 45°N, encompassing the whole range of arctic conditions (temperatures, moisture and photoperiods). Second, it is easily distinguished from related species, and its seeds are readily collected and grown in greenhouse conditions.

In its habitat, *Dryas integrifolia* is a successful low mat-forming shrub; it is a long-lived perennial, distributed widely across the northern half of North America and the coastal areas of Greenland (Hultén 1959; Porsild 1947). It is a ubiquitous pioneer species, distinctly calciphilous and most abundant on barren, rocky or gravelly substrates such as

river flats. It is less common in tundra heath where it cannot withstand competition from more aggressive tundra plants (Porsild and Cody 1980). The species is dependent on insects for maximum seed-set but can develop seeds autogamously (Kevan 1972). The fruits are hard, dry and single seeded, with a feathery style (part of the pistil, see Figure 3) that can assist in wind dispersal (Krannitz 1996). Many subspecies have been described and are located primarily in Alaska, Yukon and the Rockies (Porsild and Cody 1980). The species presents no major taxonomic difficulty. It is known to hybridize freely with other species of the genus *Dryas* (*D. octopetala* L. and *D. drummondii* Richards.) in areas of sympatry, mostly in the Rockies and Alaska (Hultén 1959).

Research objective

The central research objective was to test Hultén's hypothesis that Beringia was the principal refugium and the main source of recolonization for arctic plants. Under this hypothesis, results similar to those presented schematically in Figure 2a would be expected. Alternatively, if present-day arctic populations of *Dryas integrifolia* originated from many refugia, results similar to those presented in Fig. 2b would be expected. In other words, the question is whether there was one refugium or many.

MATERIALS AND METHODS

Fossil Data

Studying the past distribution of the species can provide information about how the present-day distribution came about. The past distribution of *Dryas integrifolia* is revealed by two sources: macrofossils and pollen remains. Macrofossils are especially abundant because the tough leaves of *Dryas* are preserved extremely well in sediments (e.g., Miller and Thompson 1979). The advantage of macrofossils is that they are generally identifiable with certainty to the species level, which is not always the case for fossil remains in general. Macrofossil data (locality and age of deposits) were gathered from the literature. The literature review was completed, in part, with assistance of S.T. Jackson (University of Wyoming), who is responsible for the North American Macrofossil Database (NAMD), an exhaustive review of all existing studies involving macrofossil analysis in North America.

Pollen data are also informative regarding the past distribution of the species, but they are not as precise as macrofossil data for two reasons. First, pollen can be reliably identified only at the generic level. Hence, the pollen grain of *D. integrifolia* is hardly distinguishable from the pollen the two other species of *Dryas*—*D. octopetala* L. and *D. drummondii* Richards (Hebda et al. 1988). Second, most researchers use the identification key from Faegri and Iversen (1975), which identifies *Dryas* pollen under the name "Dryas-type", a category that may include other members of the Rosaceae such as *Prunus*. Fortunately, species like *Prunus*, a temperate taxon, do not occur in the arctic environment, so the possibility of error is generally small. Pollen data (locality and age of deposits) were obtained from the North American Pollen Database (analogous to the NAMD) and can be considered exhaustive up to 1995.

Collection of Live Plant Material

Seeds were collected across the entire range of the species during the summers of 1995 and 1996. Collection was performed by the author and by numerous collaborators in Canada, the United States and Europe (Table 1 and Figure 4). Potential collaborators, most of them involved in arctic research, were contacted during the preceding winter to ask their assistance in collecting *Dryas integrifolia* from localities that were not accessible to the author. Of the 120 potential collaborators contacted, about 80 replied positively. The collaborators were then sent a collecting kit that included envelopes for seeds and a small plant press for preservation of voucher specimens (see Appendix A); 56 kits were returned. Not all samples were analysed owing to time constraints: a total of 42 localities were kept for analyses of molecular variation (Fig. 5). As collecting localities were distant from one another (average distance 1918 kilometres, ± 996 km), each collecting locality can safely be assumed to contain a different population.

Since the genotyping of individuals is labour-intensive, an average of just two individuals per population were used for the molecular analysis. Though small, this sampling strategy is recognized as optimal for detecting genetic substructure in species with a wide geographical range. In other words, for a fixed sample size, it is more efficient to increase the number of populations sampled rather than of number of individuals per population (Pons and Petit 1995; Templeton et al. 1995). With the more widespread spatial coverage that such a collection allows, one may be able to discriminate continuous range expansion from long-distance colonization.

Lab Techniques for Genetic Analysis

The procedure outlined below describes the techniques used to isolate specific regions of the chloroplast genome as well as the tools used to characterize the differences between these regions.

A mini-genomic DNA extraction was performed on 10-15 mg of fresh or frozen leaves, seeds, or dried flowers, using the protocol of Jobes et al. (1995). The protocol was modified in three major ways (detailed procedure in Appendix B). First, it was adapted to process very small amounts of tissue. Second, the DNA extraction medium proposed in the Jobes et al. protocol was replaced by a solution of 2% CTAB (cethyltrimethylammonium). Third, two potassium acetate purifications were performed, instead of just one, in order to separate the DNA extraction from a mucilaginous layer that formed during the first steps of the extraction and interfered with dissolution of the DNA in subsequent stages.

The Polymerase Chain Reaction (PCR) technique was used to isolate specific non-coding regions of chloroplast DNA (cpDNA) (Table 2). Six different pairs of universal primers were used, as described in Demesure et al. (1995), Dumoulin-Lapègue et al. (1997b), and Taberlet et al. (1991). The PCR mix was as follows: 18.25 µL of double distilled water; 0.25 µL (0.875 unit) of Taq polymerase (Expand™ Long Template PCR System from Boehringer Mannheim); 2.5 µL of 10x Buffer #1 (recommended buffer); 0.25 µL (400 mM) of each dNTP; 1 µL (8 nM) of each primer; and 1 µL of extracted DNA (final volume of 25 µL). Amplifications were performed using a Stratagene RoboCycler® Gradient 96. The procedure was as follows: an initial denaturation of 4 minutes at 95°C was followed by 35 cycles of denaturation (1 min. at 95°C), annealing (1 min. at 40-60°C; optimal

temperature varies with the primer used), and extension (2 min. at 68°C). The reaction was completed by 7 minutes of extra extension at 68°C. See Appendix B for full details.

The PCR products (5-10 μL) were digested overnight using 2 units of each of different 4-bp cutter restriction enzymes (Table 2). Resulting fragments were separated using 3.5-8% polyacrylamide gels run for 5-7 hours at 160 V. The various gel concentrations and migration times were adjusted to optimize the resolution of the bands of interest (Appendix B). The gels were stained using ethidium bromide, visualised under UV light and photographed with Polaroid 667ISO 3000/36°, Professional Coatterless B&W Instant Pack Film (8.5 x 10.8 cm). Band size was estimated by regression analysis of migration distances of fragments of known length (Gibco 1Kb Ladder).

Composite haplotypes for the cpDNA of each sample were based on the presence and absence of bands of different sizes and were designated on the basis of the digestion profiles from all polymorphic combinations of PCR primers (Table 3). In other words, each haplotype is defined by a unique combination of length variations when all amplified fragments are compared simultaneously.

Analysis of Data

Phylogenetic relationship among haplotypes and populations

A phylogenetic reconstruction is necessary to infer the evolutionary relationship between individual haplotypes and between populations. There are two main approaches to reconstructing phylogenies: quantitative (phenetic) and qualitative (cladistic) methods (Avise 1994). It is usually recommended that multiple methods of data analysis be

attempted. Congruent results between the two methods reinforce the conclusions (Avise 1994).

The phenetic method chosen was Neighbour-Joining, which is conceptually related to cluster analysis. As applied here, this method requires a 42 x 42 matrix of pairwise population genetic distances on which the analysis is performed. The genetic divergence between any two populations, expressed as the number of net restriction fragment polymorphisms, is calculated using haplotype frequencies together with the haplotype x haplotype divergence matrix. The exact calculation follows Nei (see Nei, 1987, equation 10.21) and was performed with the Restriction Enzyme Analysis Package (McElroy et al. 1991). The resulting pairwise distance matrix was used for the Neighbour-Joining analysis itself, performed using the program PHYLIP version 3.52 (Felsenstein 1993).

The cladistic method used was Wagner parsimony, which minimizes the total number of character state changes in the tree (Avise 1994). Each polymorphic restriction fragment was considered to be a different character. For each character, the different length variants are considered to represent different character states. Length variants were coded by numbers that do not imply any evolutionary direction. The data were scored as unordered multistate characters in the computer algorithm. The analyses were conducted using PAUP* for Macintosh (test version 4.0d57), written by D.L. Swofford (Smithsonian Institution, personal communication). This version is similar to version 3.1.1 (Swofford 1993).

Analysis of population substructure

Genetic diversity was quantified using indices of population diversity and subdivision for haploid loci (since cpDNA is assumed to be uniparentally inherited) (Nei 1987; Pons and

Petit 1995). In this study, strict maternal inheritance is assumed rather than demonstrated. It appears to be a reasonable assumption, as the uniparental, maternal inheritance of the chloroplast is nearly universal among flowering plants, where it has been studied in a reasonably large and diverse number of species (Birky 1995), although there are some exceptions (Reboud and Zeyl 1994).

Two haplotype diversity analyses were conducted. In the first analysis, each haplotype was treated as a separate allele at a single haploid locus. The average intrapopulation diversity (h_S), total diversity (h_t), and degree of substructure (G_{st}) were calculated using procedures appropriate for haploid loci (Pons and Petit 1995). Note that G_{st} depends only on the frequencies of the haplotypes and not on their similarities. In the second analysis, the distances, p_{ij}, between haplotypes (number of mutational steps separating the *i*th and *j*th haplotype, normalized by the average pairwise number of mutational steps in the overall sample of haplotypes) were calculated. This allowed calculation of intrapopulation diversity (v_S), total diversity (v_t), and degree of substructure (N_{st}) taking into account degree of haplotype divergence (Pons and Petit 1996). N_{st} is thus influenced by both haplotype frequencies and the mutational distance between haplotypes. Statistical tests for departure of diversity parameter estimates from zero were performed by bootstrap resampling of populations (Efron 1982).

The diversity measures considered above (G_{st} and N_{st}) deal only with the frequencies and divergences of haplotypes in the populations, but do not integrate the geographical information, namely, the geographical distance between each pair of populations. The Mantel's test procedure outlined below includes this information.

Mantel test: relationship between genetic divergence and geographical distance

A Mantel test was used to compare geographical distance with genetic distance for individual population pairs (Mantel 1967). The test determines whether the genetic distance between populations is related to geographical distance. In other words, the test shows whether the observed geographical distribution of haplotypes departs significantly from random (Sokal and Rohlf 1995).

First, pairwise geographical distances were calculated for each of the 42 populations using a trigonometric formula that assumes the earth is perfectly spherical (P. H. Dana, University of Texas, personal communication). The distance *d* between two points A and B is calculated in kilometres, using the latitude and longitude of each point (degrees converted to radian) and taking the measure of Earth radius (6378.137 km) at the equator. Hence, d = acos [sin (latitude A) * sin (latitude B) + cos (latitude A) * cos (latitude B) * cos (longitude point B - longitude point A)] * 6378.137. Pairwise genetic distances between populations were calculated, as previously described for the Neighbour-Joining method, using equation 10.21 in Nei (1987).

The Mantel test then proceeds by calculating the sum of the products of element by element multiplication for the geographic and genetic distance matrices (Mantel 1967). Significance testing is conducted by Monte Carlo procedures: rows and columns of one of the two matrices are randomly permuted, and the sum of the products of element by element multiplication is recorded. This is done 1,000 times, and the distribution of the randomized cross-product distribution is compared with that obtained from the actual (non-randomized) data matrices (Smouse et al. 1986). The whole Mantel test procedure was performed using an algorithm programmed by D. J. Schoen (McGill University, personal communication).

Correspondence with biogeographical provinces or paleoecological factors

If population diversity is not randomly distributed (revealed by the Mantel test for instance), then one may ask how the distribution is organized; i.e., which factors (past or present) could have influenced the distribution of the diversity? In other words, does the geographical distribution of the genetic diversity correspond to the actual boundaries of predefined ecological or paleoecological regions?

On an ecological basis, the Arctic can be divided in three major biogeographical regions: the high Arctic polar desert, the Low Arctic, and the boreal forest (the region below the northernmost limit of forest). The boundaries between these ecoclimatic provinces were determined based on climatic and major vegetation types following Scott (1995) (Figure 3). On a paleoecological basis, it is interesting to know whether the past positions of the ice sheet had any significant influence on the distribution of the genetic diversity that would still be visible today. It is thought that the age of the terrain (i.e., how long it has been unglaciated) may have had a significant effect, since regions recently recolonized are generally less diversified than those that have been unglaciated for a longer period or were never glaciated (Hewitt 1996). Thus, the study area was divided in two regions—glaciated vs. unglaciated—at three different time periods: 18,000, 12,000 and 8,400 years before the present, following Dyke and Prest (1987a; 1987b). Results from such an analysis should provide insight into the majors factors shaping the genetic differentiation and distribution in the Arctic.

Haplotype frequencies between the different groups were compared using a $\chi 2$ (chi-square) test. Small sample size, however, makes analysis of geographic or temporal variation in cpDNA problematic. This is particularly true if the restriction-enzyme survey reveals many relatively rare variants (Roff and Bentzen 1989). One solution to this problem is to

generate the distribution of $\chi 2$ expected if the null hypothesis were true for the particular data set under study. This can be approximated by a Monte Carlo approach, using a computer method for generating such null distributions (Roff and Bentzen 1989). A DOS program (RXC.EXE), based on the algorithm by Roff and Bentzen (1989), was used for this purpose. It was obtained from G. Carmody (Carleton University).

RESULTS

Fossil Record

A map showing the distribution of macrofossil records is presented in Figure 6. Details of the locality, age of deposits and references for these records are presented in Appendix C. Macrofossils are present in two major groups that are spatially and temporally distinct. All sites reliably dated in the southeast are from the last glaciation, from 25,000 to 2,000 yBP, and are progressively younger from south to north, suggesting that *Dryas integrifolia* closely followed the ice retreat in space and in time. In the Arctic, sites are of various ages, dating from the Pliocene epoch to the present, including some interglacial deposits, comfirming that *D. integrifolia* probably evolved in that region at the end of the Tertiary period.

The pollen distribution record (Figure 7) is not only concordant with the macrofossil record, supporting the existence of two refugia during the full-glacial period, but also provides additional distributional information. The new information added is the presence of the genus in the Rockies, in the continental part of the Northwest Territories, and in Labrador. Only two localities in the Arctic Archipelago have been described, principally because of a lack of investigation in the area. In all cases, the data show a rapid recolonization of *Dryas* sp. following retreat of the ice. The presence of *Dryas* sp. in Labrador around 19,000 yBP suggests the existence of a refugium along the North Atlantic coast during the full-glacial period. Finally, it is possible that the pollen record in the Rockies may reflect the presence of the related species *D. octopetala*, which is much more abundant in that region today than is *D. integrifolia*.

Haplotypes

While no restriction site variation (i.e., point mutation) was observed, many short length variants were present (e.g., Figure 8). Based on the presence or absence of these different length variants, 20 distinct cpDNA haplotypes were detected among the 79 sampled individuals from 42 populations. The description of the haplotypes is summarized in Tables 2 and 3 (and detailed in Appendix D).

The geographic distribution of haplotypes is presented in Figure 9. Nine haplotypes were present at more than one site. Among these, only a few haplotypes are widespread: haplotypes A, D, and K. Others are restricted to a particular region: haplotype E to the western Arctic and the Rockies; haplotype H to the Arctic Archipelago; haplotype L to Greenland; and haplotype P to the eastern Arctic. Finally, other haplotypes exhibit disjunct distributions: haplotype B (Hudson Bay area and the Rockies); and haplotype C (Alaska and Hudson Bay). Eleven haplotypes were restricted to a single population. They are located in three different regions. First, in the eastern Arctic, haplotypes I, Q, S, T, U and V are restricted to coastal regions. Second, in the western Arctic, haplotypes G, M and N are restricted to the high elevations of the Yukon and British Columbia. Finally, two haplotypes, O and R, are found in the Arctic Archipelago.

It is notable that many—nearly two-thirds—of the populations sampled show more than one cpDNA haplotype. The homogenous population samples are mostly from the Low Arctic region.

Phylogenetic Analysis

Cladistic and phenetic analyses were conducted to infer relationships among haplotypes and also among populations. By combining information on the geographic distribution of each clade with information on the fossil record and on the distribution and ages of glaciers, one can infer the geographic origins of the different clades. These results are presented below.

Phylogenetic analysis of haplotypes

A Wagner parsimony analysis produced over 10,000 most parsimonious haplotype trees. The small number (seven) of characters that could be found and on which to reconstruct the phylogeny may account for these results. The 50% majority-rule consensus tree is presented in Figure 10 for the first 5,000 trees. Five clades of haplotypes can be distinguished. Four small clades are composed of two haplotypes each: D, L (eastern Arctic and Greenland); E, N (Rockies and western Arctic); H, R (High Arctic); and I, P (eastern Arctic). Members of each pair are always geographically close to each other. The fifth and largest clade is composed of seven haplotypes: B, C, G, M, S, T and V. The common feature to these haplotypes is that they are all located on the continental part of the range of *D. integrifolia* (i.e., none in the Arctic Archipelago); they are spread out over a vast area, from Alaska to Newfoundland. Within this last clade, two sub-clades can be distinguished: G, M (Rockies) and S, T (northern Quebec). All remaining haplotypes (A, K, O, Q and U) are not closely related to each other.

A Neighbour-Joining analysis of haplotypes was performed using the "distance" option of PAUP* (Figure 11). Distance was expressed in terms of the number of mutations separating haplotypes from one another. Four groups of haplotypes can be clearly distinguished. The first group, containing haplotypes D, K, L, and Q, is found primarily

in the east (haplotypes K, L and Q) and from the centre of the Arctic southeastward to New Brunswick (haplotypes K and D). One noteworthy exception in this distribution is the two disjunct individuals with haplotype K found in the western Arctic (populations 16 and 19). The second major group contains haplotypes E, H, and R. This group is found along the Rocky Mountains (E) and in the northern Archipelago (H and R). A third small group found in the eastern Arctic contains haplotypes I and P. Finally, a more cosmopolitan group is made of haplotypes found over a very large area (B, C, G, M, S, T and V); it is identical to the one revealed by the Wagner parsimony technique and contains the same sub-groupings (G, M; and S, T). The remaining haplotypes form two groups of more distantly related haplotypes (N, U; and A, O)

The Wagner parsimony and Neighbour-Joining analyses of haplotypes generally produced condordant results. This confirms the validity of the phylogenetic reconstruction.

Phylogenetic analysis of populations

Figure 12 presents the results of the Neighbour-Joining analysis of populations. This analysis takes into account not only the number of mutational differences between haplotypes, but also the haplotype frequencies in populations. Five major groups of populations can be distinguished (Fig. 13). Three groups come from Beringia: one is made up of the populations composed of haplotype A only, which has its source in Beringia and reaches the eastern coast of Hudson Bay; a second group is restricted to the continental area, and the third splits in two directions, northward in the archipelago and southward along the Rocky Mountains. A fourth distinct group is composed of populations having their source along the eastern coast and possibly the southeast refugia (as suggested by fossil and pollen data). Finally, the fifth group is formed of the remaining isolated populations (40, 42, 44, 57 and 65). From this latter group, population 42 supports the

existence of the High Arctic refugium; population 44 supports the existence of the eastern coastal refugium; and populations 40, 57 and 65 are composed of haplotypes of different origins, suggesting that they were founded by immigrants from different sources.

Genetic Structure and Diversity

Comparison between all populations

Significant subdivision of population genetic diversity was detected using both G_{St} and N_{St} analyses. This indicates that haplotype diversity among populations is significant (Table 4). However, the difference N_{St} - G_{St}, was not significantly different from zero. This indicates that at the local scale there is no statistically significant tendancy for haplotypes that are less divergent than average to co-occur in the same populations (where divergence is expressed in terms of numbers of mutational steps). In other words, haplotypes found in the same population are not always strongly related. Possible explanations for such a pattern are discussed below.

The Mantel test shows that geographical and genetic distance matrices were significantly correlated. The observed sum of cross-products (=469,972) fell into the top 1% of the randomized cross-product sums (Figure 14). In other words, at the regional scale, there is a significant relationship between the genetic distances observed and geographical distances, so that haplotypes are not randomly distributed but are in fact geographically structured.

Comparison between predefined regions

The chi-square analysis compared the haplotype frequencies between the different ecological and paleoecological regions (Table 5). The haplotype frequencies between ecological provinces depart significantly from a random distribution. The comparaison between glaciated and unglaciated areas was significantly different from random at 12,000 yBP, but not at 8,400 or 18,000 yBP. Thus, both actual and past ecological factors may have influenced haplotype frequencies over the entire range of *Dryas integrifolia* (see discussion).

Genetic diversity

As mentioned above, it was anticipated that unglaciated areas might be more genetically diverse than recently recolonized areas. This assumption could not be tested, however, because of the small sample size.

DISCUSSION

The central objective of this study was to apply molecular population genetic data to the question of whether one or many arctic plant refugia existed during the last glaciation and, if there was more than one refugium, to what extent each contributed to the recolonization of the Arctic after the ice retreated. The present work provides information both from molecular analyses and from the reconsideration of other available evidence (fossil record, taxonomic diversity, and plant distribution). Together, this has allowed the evolutionary and biogeographic history of *Dryas integrifolia* to be examined, starting from the time of maximum extension of the last glaciation and extending to the present.

The results suggest that Beringia was not the only refugium; up to four refugia existed during the last glaciation—Beringia, the High Arctic, the eastern Arctic coast and the southeast—and they all made a significant contribution to present-day arctic populations. Furthermore, the available evidence also indicates that the four refugia can best be seen as two complexes of two refugia each. Beringia and the High Arctic form the first complex; it seems to have been the source of most of the populations studied here, covering almost two-thirds of the Arctic. The eastern Arctic coast and the southeast form the second complex; it seems to have contributed to the populations of about a third of the territory. Finally, it appears that the areas recolonized by each refugium overlap, i.e., many populations contain individuals whose ancestors survived in different refugia. The next two sections discuss the details on which these conclusions are based.

Evidence Concerning Each Possible Refugium

Beringia

With regard to *Dryas integrifolia* in Beringia, the molecular data strongly support Hultén's proposition that this region was the most significant refugium and the most important source for the recolonization of the Arctic at the end of the last glaciation (Hultén 1937).

At least two groups of haplotypes and three groups of populations have their origin in or near Beringia. A group of haplotypes or populations is presumed to have originated from a particular refugium if it extends from a refugium toward the formerly glaciated areas. Another, less parsimonious interpretation would be that it migrated from another refugium where it left no descendants and progressed through glaciated areas to the refugium where populations are found today. Fossil evidence also supports the claim that *Dryas integrifolia* survived glaciation in Beringia (e.g., pollen remains dating from 43,000 yBP have been found in Alaska). Interestingly, all fossil evidence originates from the northern part of Beringia. This provides support for Hultén's suggestion that *D. integrifolia* was part of a group of plants that emigrated from "Northern Beringia" (Hultén 1937).

Molecular data from this study confirm that Beringia was not only a refugium, but also a source of widescale recolonization: populations originating in Beringia now occur over large geographical areas, covering most of the Arctic. These populations are distributed along the Rocky Mountains and in the continental part of the Northwest Territories, across the Arctic to Baffin Island and the eastern side of Hudson Bay. There is also an intermediate level of connection with the High Arctic. Figure 15 presents the possible route of migration deduced from the molecular data.

According to the diversity argument (i.e., the argument that source populations should exhibit higher genetic diversity than sink populations, the latter presumably founded from a few individuals), we would expect Beringian populations of *Dryas integrifolia* to be more diversified than those in other regions. This expectation seems to hold true at the morphological level, where most subspecies of *D. integrifolia* are located near or in Beringia: *D. integrifolia* Vahl. ssp. *crenulata* (Juz.) Scoggan, *D. integrifolia* Vahl. ssp. *chamissonis* (Speng.) Scoggan, and *D. integrifolia* Vahl. ssp. *sylvatica* (Hultén) Hultén (McJannet et al. 1993). It is also true for the congeneric species *D. octopetala* (six subspecies or hybrids are found in Beringia) (Porsild and Cody 1980). However, this was not found at the molecular genetic level: Beringian populations of *D. integrifolia* do not show higher levels of cpDNA diversity than those located elsewhere. This may be because higher variability went undetected in the relatively small number of populations collected from the unglaciated regions of Beringia. It is also possible that extensive gene flow within this large refugium and between Beringia and neighbouring regions in early postglacial time led to the widespread dispersal of diverse Beringian haplotypes. The only case of extreme genetic differentiation near Beringia is found in population 23 from the Rockies, where two unique haplotypes were found (haplotypes G and M). However, the specimens collected in that region correspond to the well differentiated subspecies *D. integrifolia* ssp. *sylvatica*, which may explain the high level of divergence.

The High Arctic refugium

Much evidence suggests that the High Arctic was a refugium during the last glaciation. In particular, the High Arctic region contains just as much taxonomic diversity of *Dryas integrifolia* subspecies and species as Beringia. Three taxa have been described from the region north of Ellesmere Island: one new species, *Dryas incisa* (closely related to *D. integrifolia*); one subspecies, *D. integrifolia* Vahl. ssp. *chamissonis* (Speng.) Scoggan; and

one variety, *D. integrifolia* var. *canescen* (Edlund 1994). Furthermore, one rare haplotype (haplotype R, population 68) is restricted to the same region. Finally, population 42 distinguished itself among all other populations in the Neighbour-Joining analysis.

The claim that the High Arctic was a refugium would not have been entirely surprising to Hultén, who suspected that the High Arctic had remained unglaciated and that some plants might have survived the glaciations there, but he stated that "as the Arctic Archipelago does not possess any endemic plants of its own, it has played hardly any part as a centre [of dispersion]" (Hultén 1937). According to Hultén, survivors in that region became adapted to the very severe conditions north of the ice and had no capacity for spreading southward. However, one distinct group of haplotypes (H, R) originates in the High Arctic. It is found throughout the Arctic Archipelago, as far south as Cambridge Bay and Igloolik (populations 40 and 51). This suggests that some populations may indeed have survived glaciation north of the ice and retained their capacity to spread south (Figure 15).

Unfortunately, no fossil evidence is available to support the claim that *Dryas integrifolia*, or any other arctic plant, survived glaciation north of the ice (Blake 1974). Blake suggested that climatic conditions 20,000 years ago may have been too severe for plants to survive in the High Arctic, as suggested by the very low paleotemperature recorded in neighbouring Greenland—21 to 22°C colder than present (Cuffey et al. 1995; Johnsen et al. 1995). If so, this means that all extant High Arctic populations originate from Beringia and that the observed morphological and molecular variation found only in the High Arctic today would have arisen during postglacial time, in about the last 10,000 years (Nordal 1987). However, this explanation seems quite unlikely because of the very slow rate of cpDNA evolution. Furthermore, if such a rapid rate of evolution occurred, then similar postglacial differentiation would have been observed in other regions, such as the Rockies, which is not the case (see below). Postglacial differentiation therefore seems unlikely. However, it

is admittedly impossible to distinguish it from *in situ* differentiation during the full-glacial period.

In conclusion, if the refugium did in fact exist, why have no fossils been found? There are three possible explainations. First, there is the general lack of investigation in the area. Second, it is also possible that the most likely sites for the refugium were near the coast at the time and that they are not accessible today, having been flooded as a result of sea level rise when the ice melted. Finally, it is possible that thin deposits from the time of the maximum extent of glaciation have been eroded (Blake 1974). Similar reasons may also explain the absence of fossils in other refugia (see below).

The southeast refugium

Unlike the other possible refugia, the area of the putative southeast refugium is not inhabited today by *Dryas integrifolia.* Therefore, it is difficult to say with certainty that any present-day populations of *D. integrifolia* originate from the southeast refugium. The closest populations to the southeast refugium are located further north, in formerly glaciated areas in southern New Brunswick, Québec and Ontario. In theory, these disjunct populations are either long-distance immigrants from the north, or populations left behind during a migration from a southern refugium. The evidence gathered in this study supports the latter explanation.

The wealth of fossil remains found from New Jersey to Québec strongly supports the existence of the southeast refugium during the full-glacial period. Furthermore, the fossil record and the presence of the disjunct populations of *Dryas integrifolia* suggest that the species followed the ice retreat northward. Finally, molecular evidence (two groups of populations and one group of haplotypes linked to the disjunct populations 35 and 41)

suggests that the southeast refugium was the source of many populations found today in the eastern and central Arctic. The migration probably took place either via northern Québec or via the western side of Hudson Bay, as shown by the distribution of fossils in Ontario (Figures 6 and 7) and the presence of haplotype D in population 10 (Fig. 15).

The coastal refugium from eastern Arctic

No biogeographic or fossil evidence supports the existence of a coastal refugium: i.e., there are no fossils nor any evidence of disjunct populations. Molecular evidence, however, supports the claim that the coastal region was a refugium, but the evidence is weaker than it is for the other refugia. Only two small groups of haplotypes and one group of populations are exclusive to that region. However, genetic diversity along the east coast of Canada and west coast of Greenland is much higher than anywhere else: eight rare haplotypes are restricted to that region (haplotypes I, L, P, Q, S, T, U and V). The high local diversity in Greenland suggests that that area could even be considered a distinct refugium.

Following the ice retreat, it seems that survivors from these regions did not spread very far into the Canadian Arctic (Figure 15). There are two possible reasons: first, the prevailing winds likely to assist dispersal blow from the northwest, across the Canadian Arctic toward Greenland (Environment Canada, personal communication). Second, westward dispersal was probably blocked by the Arctic Cordillera, a chain of mountains running north-south and traversing Ellesmere Island, Baffin Island and Labrador (the Torngat Mountains).

The southern Rocky Mountains refugium

There is no evidence in either the molecular or the fossil data to support the existence of a refugium in the Rocky Mountains south of the ice. The disjunct population in Montana is

genetically closely related to populations found in Beringia. Thus, unlike those in eastern Canada, this disjunct population seems not be a southern survivor, but rather the result of dispersal from the north. If some populations did in fact survive in this refugium, they appear to have left no fossils and no descendants; or, their contribution is so small that it went undetected in this study.

Complexes of Refugia

The previous section described in detail the evidence supporting the existence of four of the five putative refugia. These four refugia not only allowed the survival of *Dryas integrifolia* during glaciation, but also served as important sources for restocking the Arctic with this species. Moreover, the refugia were not completely isolated from each other, and it seems more accurate to see them as complexes of refugia rather than as discrete entities. Two complexes can be identified: the Beringia/High Arctic complex and the eastern Arctic coast/southeast complex. The reasons for these groupings are presented below.

Beringia/High Arctic complex

The refugia of Beringia and the High Arctic can be considered part of the same complex for several reasons. First, the outline of the ice sheet 18,000 years ago shows a geographic connection between the two refugia, and this is even more prononced in early postglacial time (Figure 1). Populations would have been isolated enough to develop some unique features, but perhaps not enough to be totally differentiated. Thus, on one hand, populations in these areas share some characteristics: the two refugia share the subspecies *D. integrifolia* ssp. *chamissonis*; and the haplotypes E from Beringia and H, R from the High Arctic are closely related, although their distributions do not overlap. On the other

hand, the High Arctic refugium has some intrinsic features, such as some unique variants of *Dryas* and some haplotypes exclusive to that region. Descendants from individuals that survived in this complex are found today over most of the Arctic (Fig. 9 and 13).

Eastern Arctic coast/southeast complex

As explained in relation to the Beringia/High Arctic complex, populations from the coastal and southeast refugia were probably not totally isolated from each other, even if they developed some unique feature. First, there was a geographic connection between the southeast refugium and the eastern coast, via the exposed region of the continental shelf between Nova Scotia and Newfoundland (Pielou, 1991). This connection became more and more important as the ice started to melt, from 18,000 to 8,000 years BP (Figure 1). The close connection between the two refugia is supported phylogeographically by the intimate relationship between the populations and haplotypes from the areas concerned (Fig. 10, 11 and 12). The contribution to present-day populations from this complex is apparently more modest than the contribution from the Beringia/High Arctic complex (Fig. 9 and 13).

Overlap in the major complexes

There is overlap in the distribution of populations that trace their origin to one of these two major refugial complexes. Even within some populations, there are individuals that trace their origins to different refugia (populations 40, 57 and 65). These populations of mixed origin are usually found in the central Arctic, at the intersection of possible migrational routes (Figure 15).

Persistence of the refugia over successive glaciations

Is it possible that the two complexes existed during previous glaciations? It is likely that Beringia and the High Arctic refugia existed for more than one glaciation. That would explain, for instance, the very high level of morphological differentiation observed there. These differentiations would have been reinforced through successive periods of isolation, from one glaciation to the next. By contrast, the coastal and southeast refugia may not have existed during previous glaciations, since no unique morphological variants were observed there, and the limited evidence available suggests that truly arctic taxa like *Dryas integrifolia* were not found in the south during previous glaciations (Flint 1971).

How Representative is *Dryas Integrifolia*?

It could be argued that *Dryas integrifolia* is not representative of other arctic plants in terms of its biogeographic history because it has been shown that plants react individualistically to climate change (Pielou, 1991). However, as in the case of *Dryas integrifolia*, a review of studies of other arctic plants and animals suggests the presence of up to five refugia during the last glaciation. Even if all these plants or animals do not show the same pattern of distribution, these results suggest that indeed many refugia for plants and animals probably existed around the ice.

First, many studies (see Appendix C) suggest that *Dryas integrifolia* is not the only arctic species to have survived in the Beringian and southeast refugia during the last glaciation. Fossils of arctic plants like *Saxifraga oppositifolia*, *Vaccinium uliginosum* and *Salix herbacea* are found fossilized along with *D. integrifolia* in the deposits from these regions. Second, a study by Mooney and Billings (1961) suggests that Beringia was the source of

most of the recolonization in the Rockies. They concluded that all the populations living today in the formerly glaciated Rockies are composed of photoperiod-tolerant biotypes from Beringia. Their findings are consistent with the results of this study. They also found that some populations survived in the south, but these populations seem not to have migrated very far north (i.e., did not reach Canada); they may have been confined to more southerly mountain ranges by post-Wisconsinan aridity barriers. There is no support for the existence of the eastern Arctic coast and High Arctic refugia simply because these regions were not part of the investigations mentioned above.

Although animal dispersal is different from that of plants, comparison of the results of this study with those of arctic animal species is informative, because the existing studies were conducted over a range similar to that of the present study. These studies also suggest that many refugia existed, with general locations corresponding to those proposed here for *Dryas integrifolia*. First, Bernatchez and Dodson (1991) identified four distinct phylogeographic assemblages for the fish *Coregonus clupeaformis* (one in Beringia and three in the southeast). Second, Macpherson (1965) suggested that much of the current geographic diversity of Canadian arctic mammals resulted from isolation in refugia during the last glaciation. A recent study of collared lemmings (Eger 1991) confirms Macpherson's initial proposition. Eger found that lemmings survived in four refugia: Beringia, the High Arctic and two southern periglacial refugia in eastern and western North America. A study of caribous suggests that populations on Baffin Island could have originated from a different refugium than the mainland populations (Ferguson 1996). Third, a series of studies has identified many possible refugia for the fresh water invertebrate genus *Daphnia* (which is widespread in the Arctic): in Beringia, the southwest, the southeast, the High Arctic, and the coastal regions of Baffin Island and western Greenland (Boileau and Hebert 1991; Van Raay and Crease 1995; Weider and Hobæk 1997; Weider et al. 1996).

Genetic Structure and Evolutionary Forces

The values of G_{St} and N_{St} (Table 4) are indicative of relatively high genetic differentiation among populations. This suggests that factors increasing genetic substructure (vicariance, adaptation to local conditions and founding events) are more important overall than factors decreasing structure (essentially gene flow). Lineage sorting also probably contributed to the structure. This section describes in detail the relative importance of these factors in the evolutionary history of *D. integrifolia*.

Vicariance

Episodes of vicariance, resulting from the presence of the ice sheet for more than 55,000 years in the case of the last glaciation, probably contributed the most to genetic differentiation across the range of *Dryas integrifolia*. Vicariance created in the past by the ice sheet is of course no longer active today, but the strength of this historical factor is likely sufficiently important to have had a lasting effect, even if counterbalancing factors like gene flow (discussed below) may have been more important in recent times (Slatkin 1993).

Selection

Selection favouring different alleles in different locations will contribute to produce differences reflecting genetic adaptations to local conditions (Slatkin 1987). Such a situation is expected from a species occupying such a wide geographic range with strikingly different climatic conditions in different areas. Murray (1987) argued that species that are found in a variety of habitats most likely consist of populations of ecotypes or local races. In this study, it appears that haplotype H, restricted to the High Arctic, might be an example

of such a local ecotype. The significant differences in haplotype frequencies between the three major ecological regions (High Arctic, Low Arctic and boreal forest) may also be indicative of ecotypic adaptation.

Founding events

The cumulative effect of successive founding events can also contribute to genetic differentiation among populations (Le Corre et al. 1997). For plant populations that have passed through recent episodes of range expansion, long-distance dispersal events are probably the most important factors in differentiation, especially for maternally inherited genes studied at small or medium geographic scales (Le Corre et al. 1997). In this regard, it is notable that the Mantel test (Figure 14) shows a highly significant structure at the regional scale. It also appears that populations founded less than 12,000 years ago have significantly different haplotype frequencies than populations that have been established for longer periods (Table 5).

Cumulative founding events may also lead to decreased genetic diversity of individual populations, as has been observed in boreal and temperate taxa, e.g., pine (Cwynar and MacDonald 1987), beech (Demesure et al. 1996), *Polygonella* (Lewis and Crawford 1995), and a variety of taxa in the Pacific northwest (Soltis et al. 1997). However, this phenomenon was not detected in this study. On the contrary, the populations studied are mostly heterogeneous for haplotypes. In addition, the value of $G_{St} - N_{St} = 0$ indicates that within a population, individuals are often not closely related. Similar high local diversity has been observed in populations of another arctic species, *Saxifraga oppositifolia* from Spitsbergen (Norway, 78°N) (Abbott et al. 1995). This higher than expected local diversity may be a sign of recolonization from more than one source (Abbott et al. 1995; Ritchie

1992), in contrast to boreal taxa, which generally derive from a single southern source, as well as an indication of a high level of gene flow between populations (see below).

Gene flow

Gene flow, arising from either seed or pollen dispersal, is the main factor contributing to reduction in genetic differentiation between distant populations (Slatkin 1987). In the present case, pollen dispersal is likely to be fairly limited, because *Dryas* is entomophilous, so the grains travel short distances (Ritchie 1987). On the other hand, *Dryas* may have an immense potential for seed dispersal because its seeds are light and abundant and possess a long plumose style well adapted for wind dispersal (Krannitz 1996). Wind dispersal is especially favoured in the Arctic environment, because barriers to gene flow are limited in this open, windy habitat, especially in the winter, over flat snow-covered landscapes (Bonde 1969; Glaser 1981; Miller and Ambrose 1976). Seeds can be dispersed potentially over thousands of kilometres (Savile 1972), mostly from west to east as a result of major wind patterns (Abbott et al. 1995; Crawford and Abbott 1994). Animal transport is also thought to be important because of the synchronous timing of seed production with bird migration from north to south (Abbott et al. 1995; Crawford and Abbott 1994). Rapid recolonization of deglaciated areas, both in the past and in the present (Matthews 1995; Pielou 1991), confirms the great capacity of *Dryas*, and other arctic plants, for quick and efficient long-distance dispersal.

Given the enormous potential of seed dispersal of the species, the estimates of G_{st} and N_{st} probably underestimate the present-day value of gene flow. This discrepency with reality could be explained by the inherent inertia of genetic substructure, which reflects the past more than it does recent population history (Slatkin 1987). Past episodes of vicariance resulting from glaciation may provide an explanation of why genetic structure is so

pronounced. This inertia may be enhanced because of the long-lived nature of *Dryas integrifolia*, which can live up to one hundred years (e.g., a similar situation has been observed with oak in Europe, Dumoulin-Lapègue et al. 1997a).

Lineage sorting

The uniparental mode of inheritance of cpDNA entails a stochastic extinction of cpDNA lineages within a population at each generation (Avise et al. 1987). This process, called lineage sorting, can modify the genetic structure among populations and can give rise to discordance between the phylogenetic reconstruction based on cpDNA markers and the true historical connection between the individuals (Avise et al. 1987; Neigel and Avise 1986). In practical terms, some populations may seem to be more closely related to one another than they actually are. This is possible if the isolation period was short enough and especially given the slow rate of molecular change in the chloroplast genome.

The close phylogenetic relation between haplotypes B (western Hudson Bay) and V (Newfoundland) may be a case of lineage sorting: the two haplotypes are genetically related but geographically extremely disjunct and therefore unlikely to have survived in the same refugium. Consequently, their close relationship probably does not represent recent historical reality. (It may represent a connection that long predated the last glaciation.) Lineage sorting may also explain why haplotype K is the only haplotype found simultaneously in two disjunct refugia, Beringia and the southeast (Figure 9). If two groups of populations with haplotype K survived in two different refugia independently, and if they did not diverge quickly (or at all) because of lineage sorting, then the geographic pattern observed could be explained. In reality, the two groups would have a common ancestor that dates back to the last interglacial or even preglacial time. The fact that

haplotype K is found at the base of the phenetic tree (Fig. 11) is indicative of such a phenomenon (R. J. Petit, personal communication).

Conclusions

This study is the first to apply molecular genetic methods to the question of postglacial arctic biogeography of plants, which remained largely speculative until now. The initial research objective was to determine whether there was one refugium (Beringia) or many possible refugia and sources of recolonization. This study confirms Hultén's hypothesis that Beringia acted as the major source area for modern arctic flora, but it also provides evidence for the contribution of three other sources. The conclusions are therefore best captured by the schematic representation of Figure 2b, with the additional qualification that there is overlap between the contributions of each refugium.

Evolutionary scenario

From the findings presented in this study, it can be concluded that the last glaciation fragmented the range of *Dryas integrifolia*, whose populations survived in two complexes of refugia around the margins of the ice, from which they recolonized the Arctic when the glaciers retreated. Descendants from different refugia eventually came into contact with one another in various parts of the Arctic. It has been suggested that this cycle (vicariance during glaciation and secondary contact during interglacial periods) repeated itself many times during the last two millions years (Ritchie 1992; Stebbins 1984; Stebbins 1985) but this assertion had not hitherto been supported by concrete evidence. This study supports this model for the last glaciation, but does not provide evidence that this cycle repeated itself at each glaciation.

Figure 15 summarizes the possible routes of recolonization in early postglacial time. The pattern presented here for *Dryas integrifolia* was likely followed by many other plants, but there are certainly many variations around this basic pattern. Hultén himself (1937) recognized ten different patterns of survival and recolonization for arctic and arctic-montane plants. All these patterns have one thing in common: all the plants survived the last glaciation in Beringia. They differed in terms of the precise location of the refugium (northern Beringia or Yukon valley), the extent of postglacial recolonization (indirectly measuring the dispersal capacity), the preferred habitat of the plants (tundra or mountain), and their present range (strictly American, strictly Eurasian, or circumpolar) (Hultén 1937).

Genetic diversity

A broad range of studies on tundra plants suggest a high level of genetic differentiation among populations (McGraw 1995). This study adds to this picture and provides evidence for high genetic variation within populations, a situation rarely documented in arctic species (McGraw 1995).

Many factors may have contributed to creating and maintaining a high level of inter- and intra-population genetic diversity in arctic flora. These include the long survival of the seed banks (up to 200 years) (McGraw 1995); the great potential for seed dispersal (Crawford and Abbott 1994; Savile 1972); the large amount of environmental heterogeneity (Bauert 1996; Murray 1987); and the frequent hybridization within related species (e.g., the genus *Draba*, Crawford and Abbott 1994). Finally, as shown in this study, the various episodes of vicariance and secondary contact would also have promoted high genetic differentiation (Crawford and Abbott 1994; Ritchie 1992; Stebbins 1985).

The present study also suggests that particular environmental conditions in the Arctic facilitate the introduction of new immigrants in arctic populations, contrary to what is observed in temperate and boreal regions. As mentioned earlier, in these latter regions, a series of founding events decreases the genetic diversity of newly established populations. This low diversity maintains itself over time because later migrants can contribute little, since they are entering established populations at or near carrying capacity (Hewitt 1996). The species then becomes a barrier to its own dispersal. This situation may not exist in the Arctic, because new immigrants are more likely to be limited by abiotic conditions (e.g., low temperature, dessication, etc.) than by biotic factors like competition. This is especially the case in the High Arctic polar desert, where vegetation cover does not exceed 20% (Scott 1995).

Because of the combined effect of all the factors mentioned above, the recolonization dynamic (i.e., rate of recolonization, directions, consequences for diversity and community structure) in the Arctic may have been very different from what has been described for temperate and boreal regions. Some of these factors are either unique to the Arctic or greatly enhanced in that region, because of the particular environment and history in which these factors acted. Historically, glaciation may have promoted taxonomic diversity (McGraw 1995; West 1995) because of the vicariance episodes and migration it entailed. The environment, as shown above, also stimulates great genetic exhange in the present-day Arctic (e.g., seed dispersal is greater in the Arctic than elsewhere).

This high diversity may contribute to rapid evolutionary response in arctic plants (Crawford and Abbott 1994). It may have allowed arctic plants to cope with the successive episodes of cooling and warming that have characterized the history of the Arctic. It may also assist their long-term survival in the face of future climatic variations (McGraw 1995).

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LIST OF FIGURES

Figure 6. Past distribution of *Dryas integrifolia* as revealed by the macrofossil record. Dates in radiocarbon years before the present ("na" means that dates are not available). Squares represents fossils of *Dryas integrifolia*, and circles represents fossils of *Dryas* sp. If both have been found at the same site at the same time, only the first is depicted.

Figure 7. Past distribution of *Dryas integrifolia* as revealed by the pollen record. Dates in radiocarbon years before the present.

Figure 8. Typical gel showing length variants of cpDNA among *Dryas integrifolia* samples. "M" lanes indicate molecular weight marker, and arrows show the length of two segments in base pairs.

Figure 9. Geographic distribution of the different cpDNA haplotypes of *Dryas integrifolia*. Each letter represents one haplotype. Localities have either one or two haplotypes present. For representation convenience, haplotypes are presented on four different maps; haplotype grouping on each map does not imply any historical or genetic connection between haplotypes.

Figure 10. Unrooted cladistic network showing the phylogenetic relationship between the 20 different cpDNA haplotypes described, as constructed by the Wagner Parsimony method (50% majority-rule consensus of the first 5000 most parsimonious trees of 20 steps; Rohlf's Consistency Index = 0.132). The numbers along the branches show the percentage of trees, of the 5000, that exhibited the particular branching observed. Percentages below 50% are not shown.

Figure 11. Unrooted tree showing the phenetic distance between the 20 different haplotypes described, as constructed by the Neighbour-Joining method. Branch length is proportional to phenetic distance.

Figure 12. Unrooted tree showing the phenetic relationship between the 42 different populations studied, as constructed by the Neighbour-Joining method. Blocks of populations outlined in rectangles refer to the major grouping outlined in the discussion and presented in Fig. 13.

Figure 13. Geographic distribution of the various groups of populations described in Fig. 12. At least two different groups are represented on each map, where each group is represented by a different symbol.

Figure 14. Mantel test results (1000 random permutations) showing the frequencies of the sum of the element x element product for the randomized genetic and geographic distance matrices, compared with the actual data (arrow).

Figure 15. Main routes of recolonization following ice retreat. Note the suggested route along both sides of Hudson Bay for populations that survived southeast of the ice sheet.

Figure 1

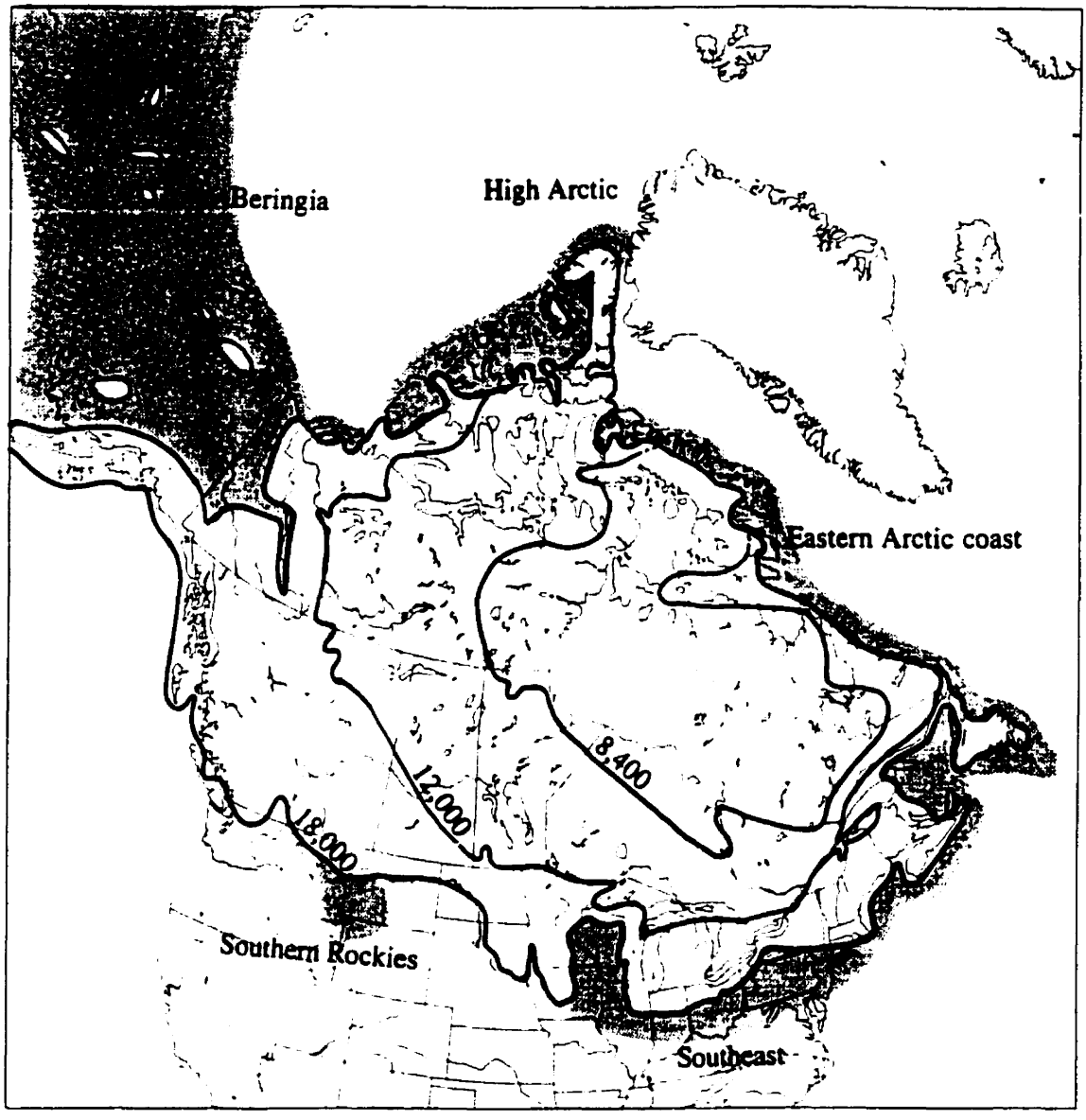
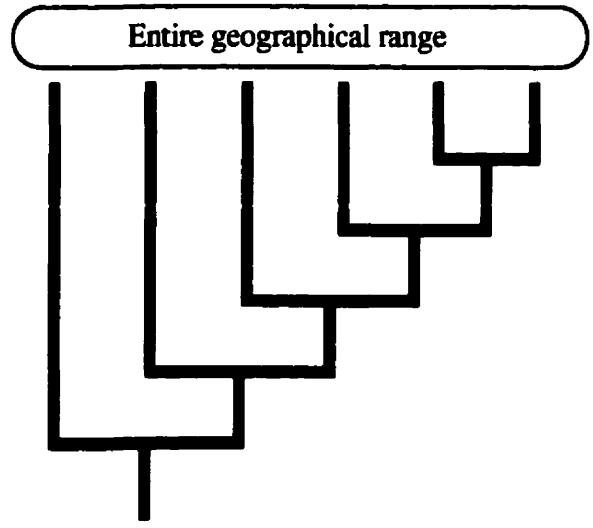

Beringia
High Arctic
Eastern Arctic coast
8,400
12,000
18,000
Southern Rockies
Southeast

Figure 2

a)


Entire geographical range

b)

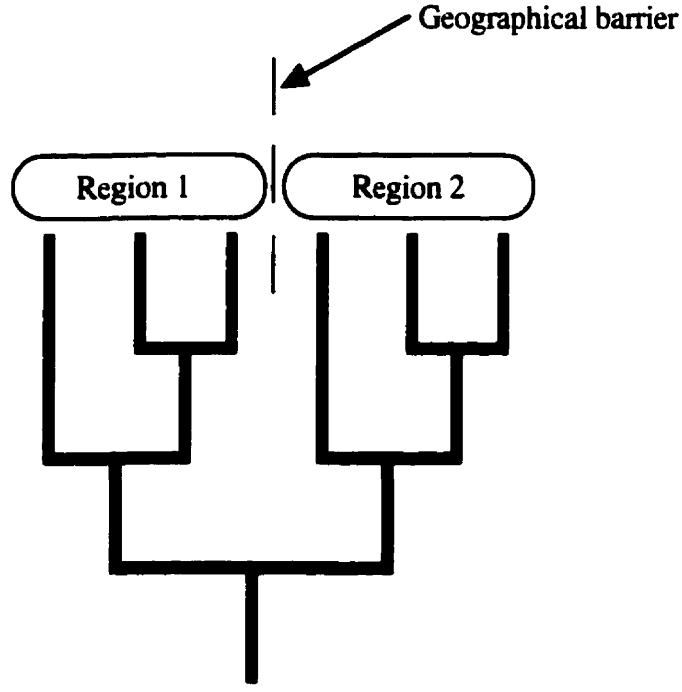

Geographical barrier
Region 1
Region 2

Figure 4

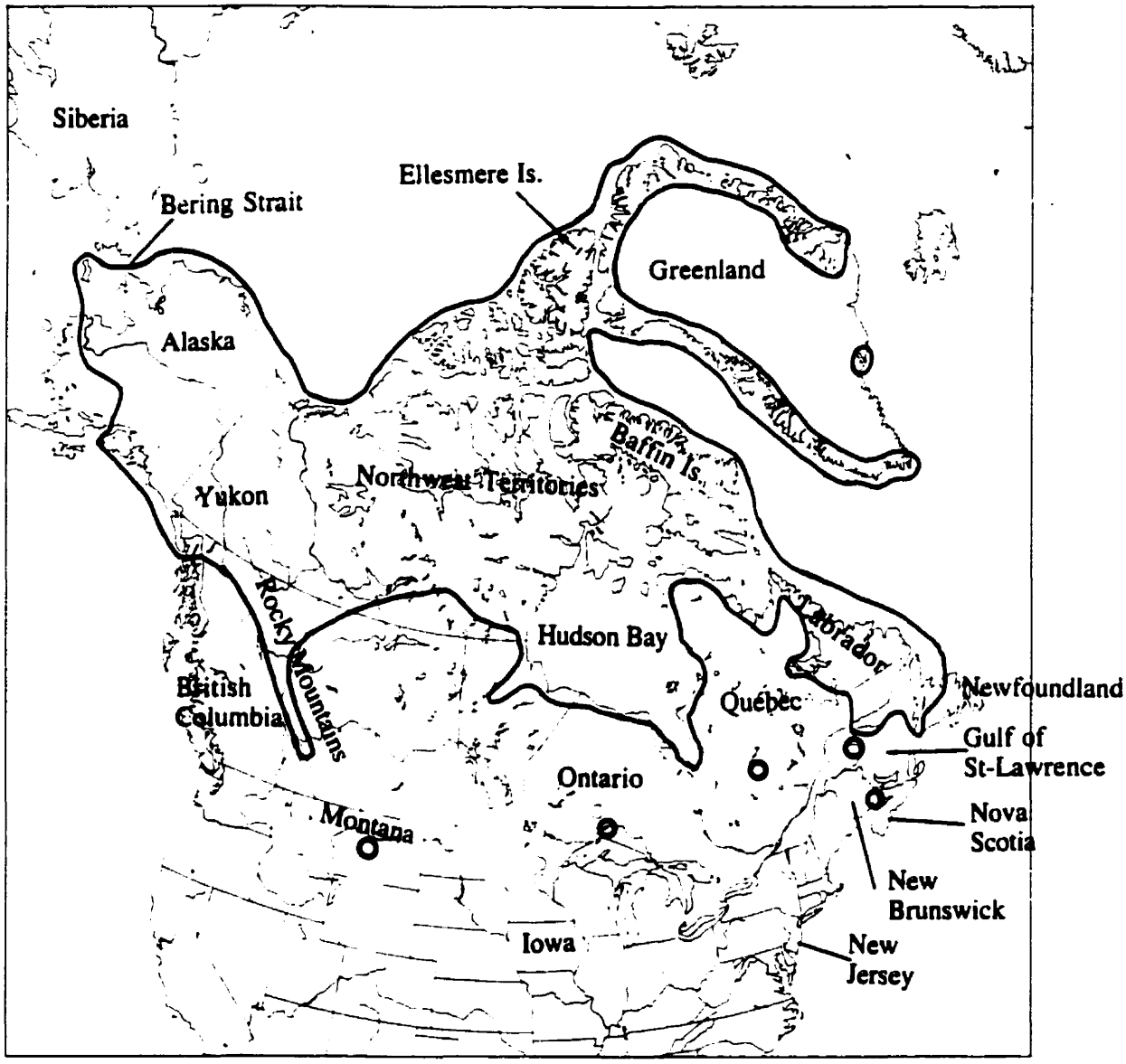

Siberia
Bering Strait
Ellesmere Is.
Greenland
Alaska
Baffin Is.
Yukon
Northwest Territories
Rocky Mountains
Hudson Bay
Labrador
British Columbia
Québec
Newfoundland
Gulf of St-Lawrence
Ontario
Montana
Nova Scotia
New Brunswick
Iowa
New Jersey

Figure 5

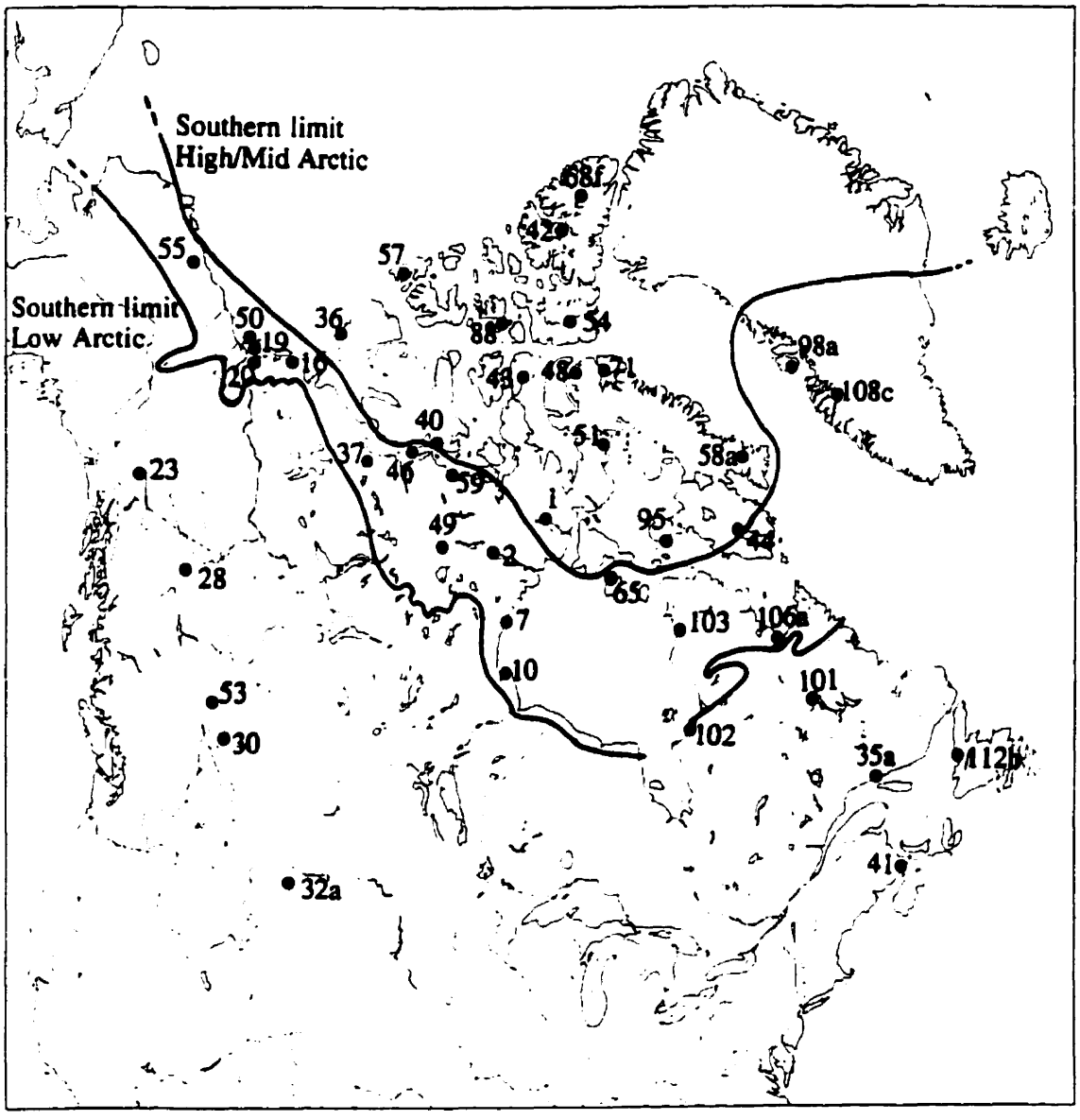

Southern limit
High/Mid Arctic
Southern limit
Low Arctic
55
57
50
36
19
16
40
37
46
59
23
49
28
2
7
10
53
30
32a
68f
42
88
54
48
51
58a
1
95
44
65
103
106a
101
102
35a
112b
41
98a
108c

Figure 6

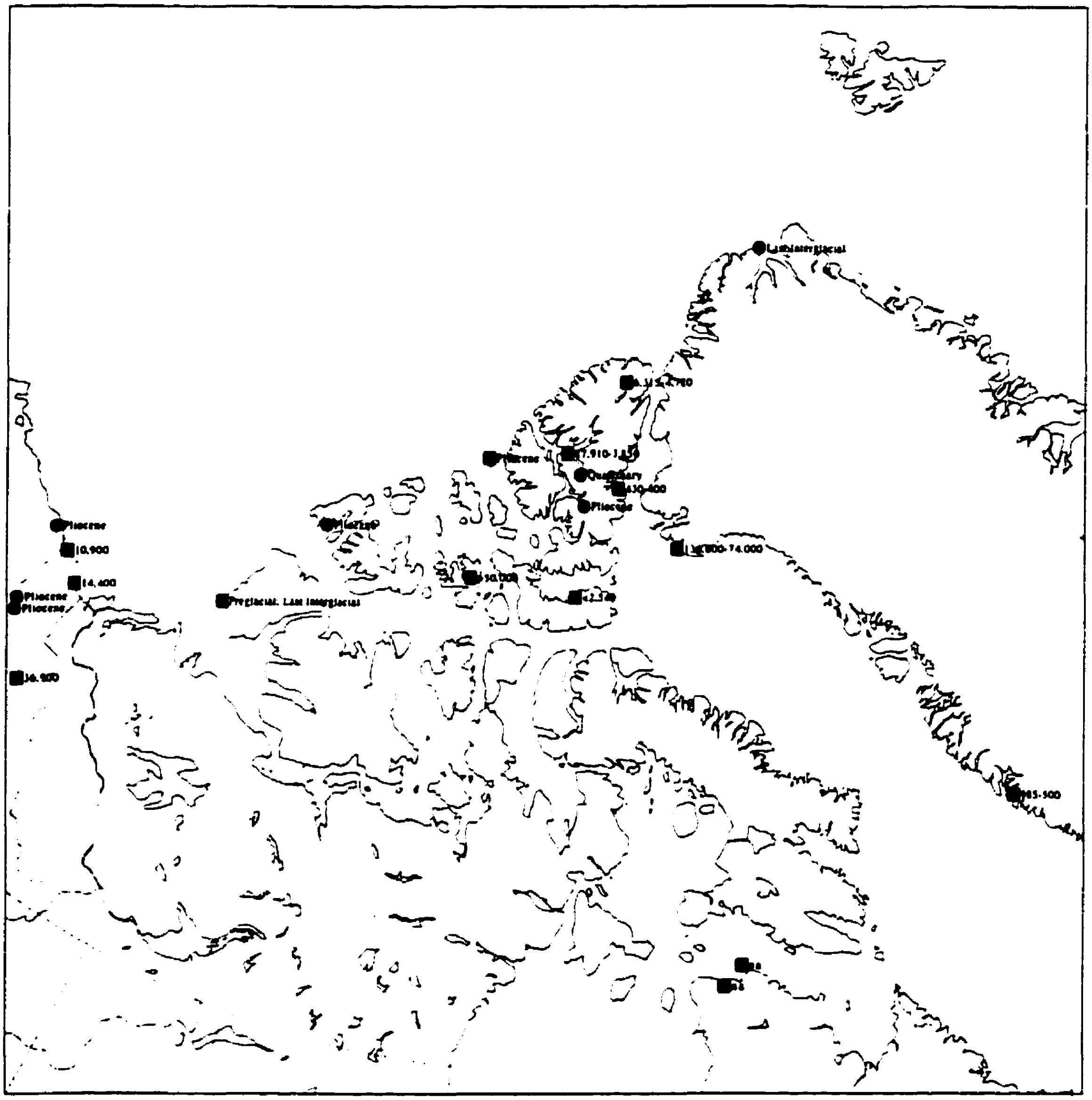

Pliocene
10,900
14,400
Pliocene
Pliocene
36,900
Preglacial, Last Interglacial

Figure 6 (concluded)

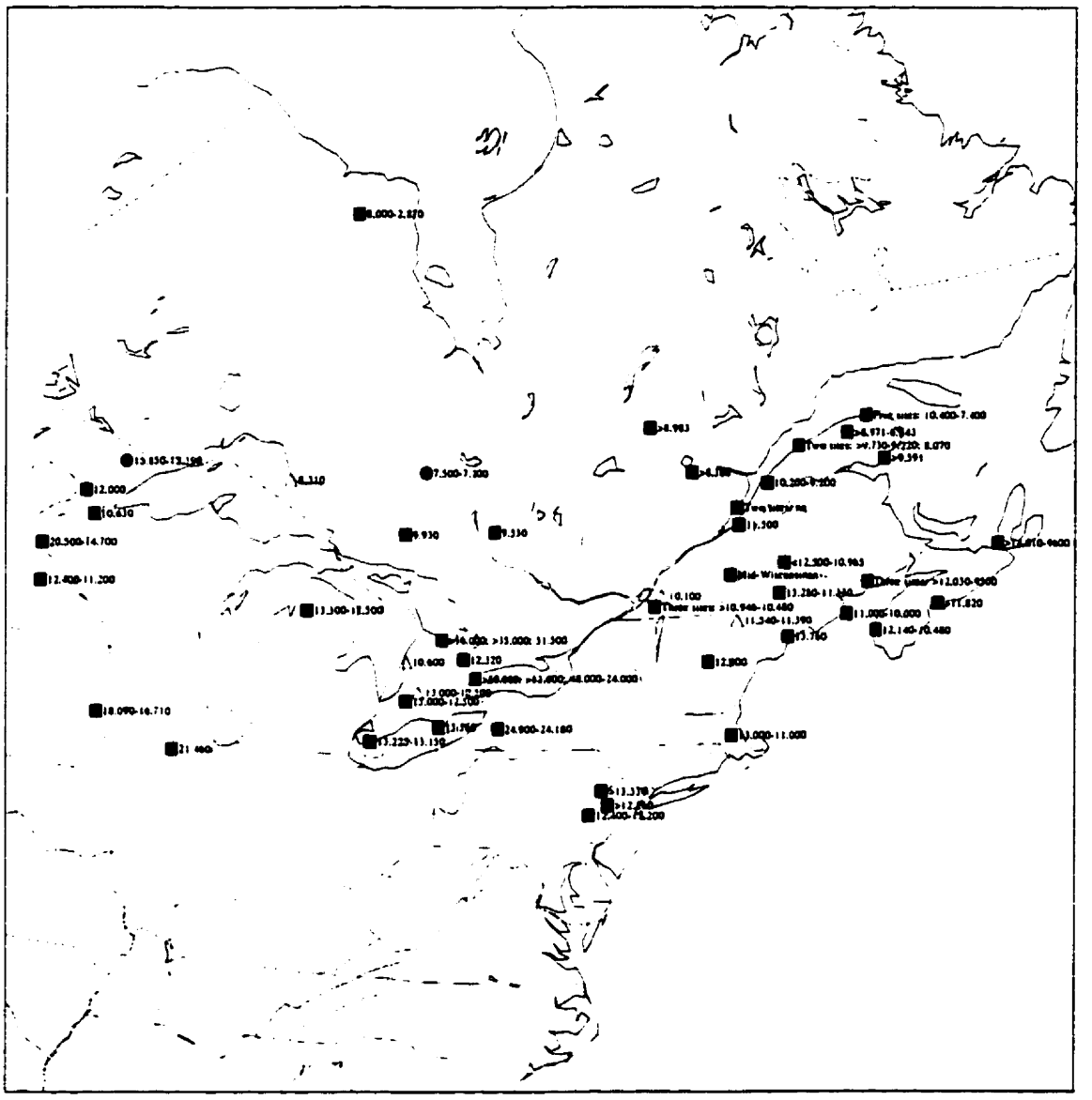


Figure 7

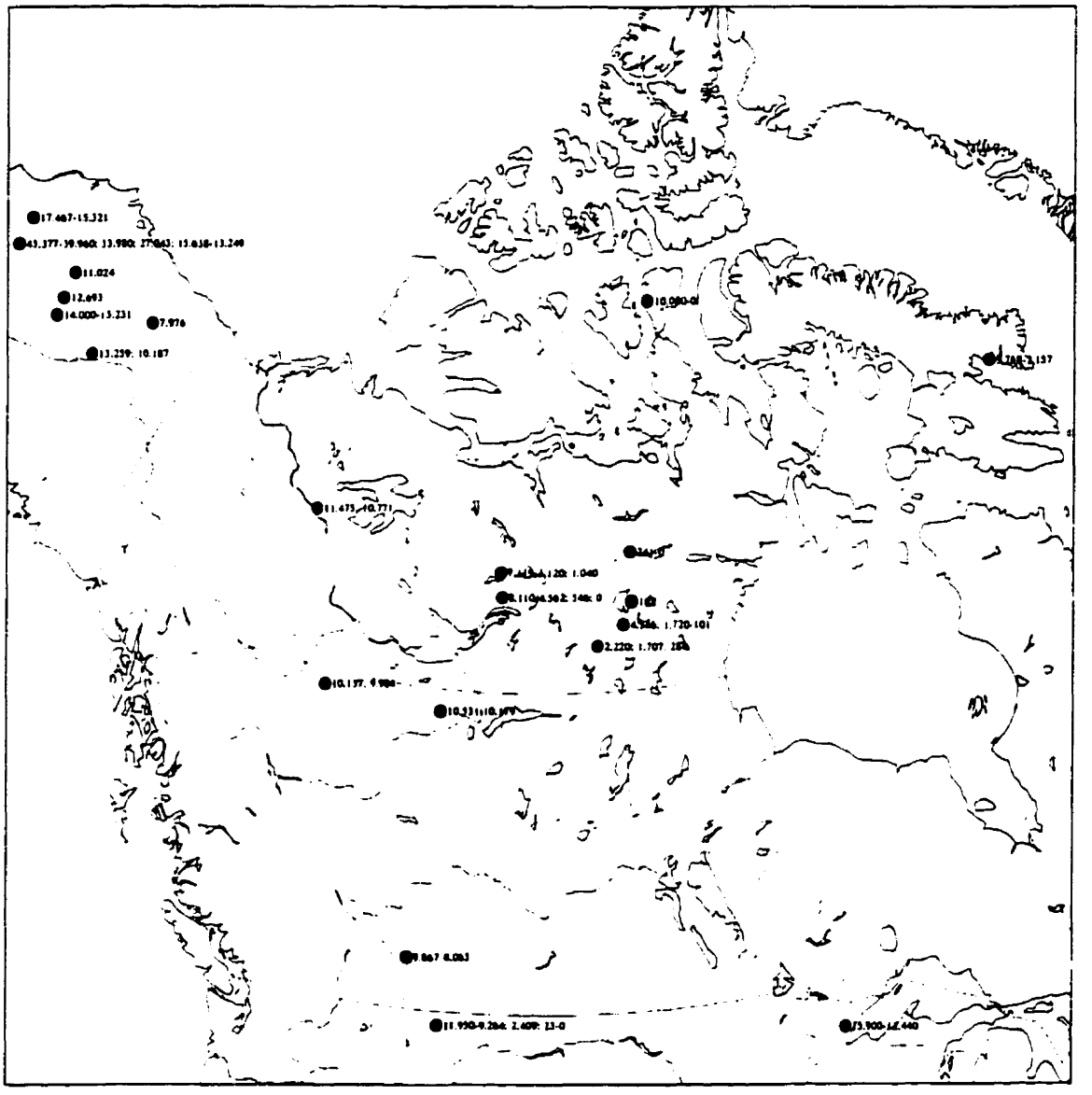


Figure 7 (concluded)

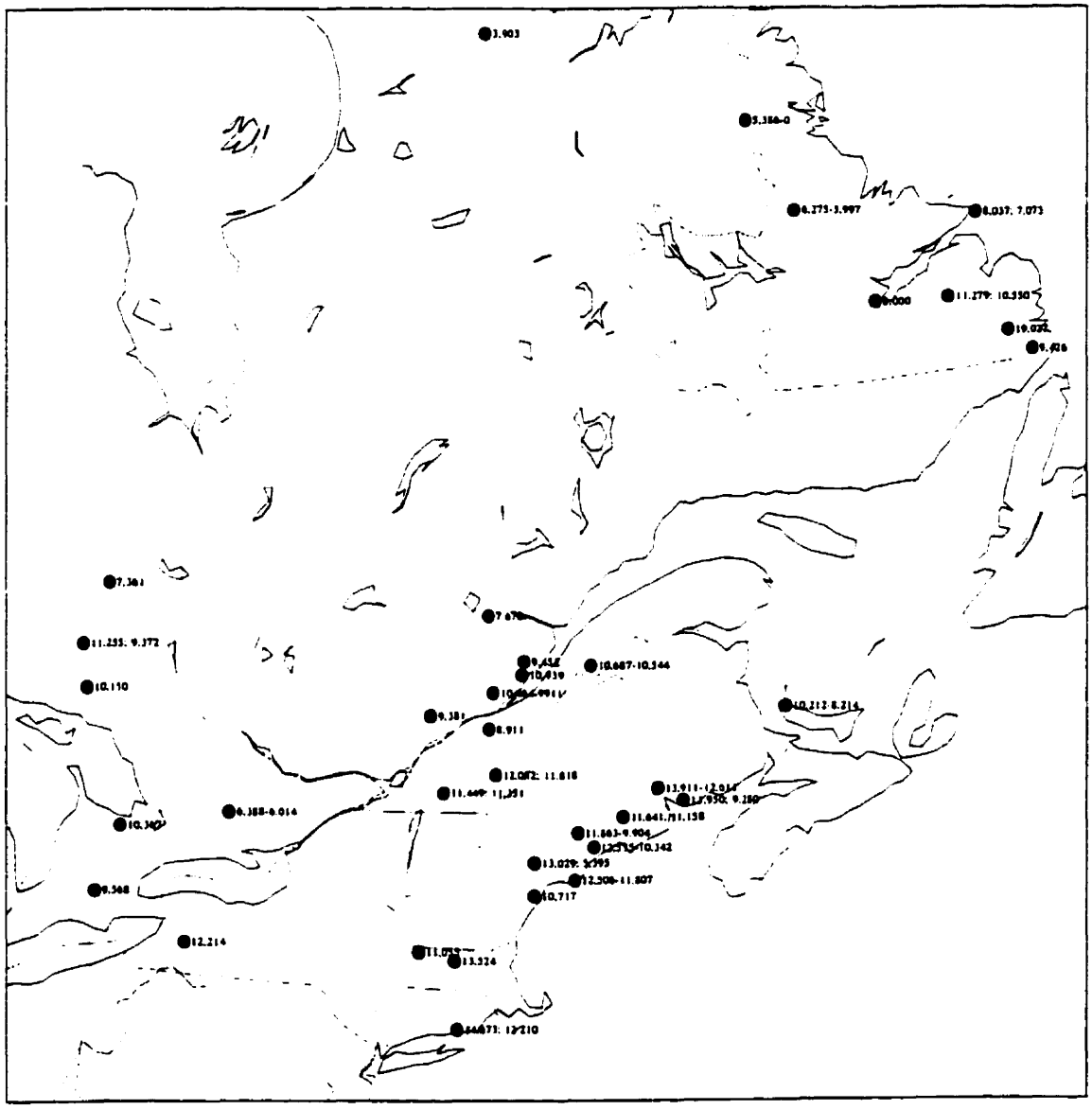


Figure 8

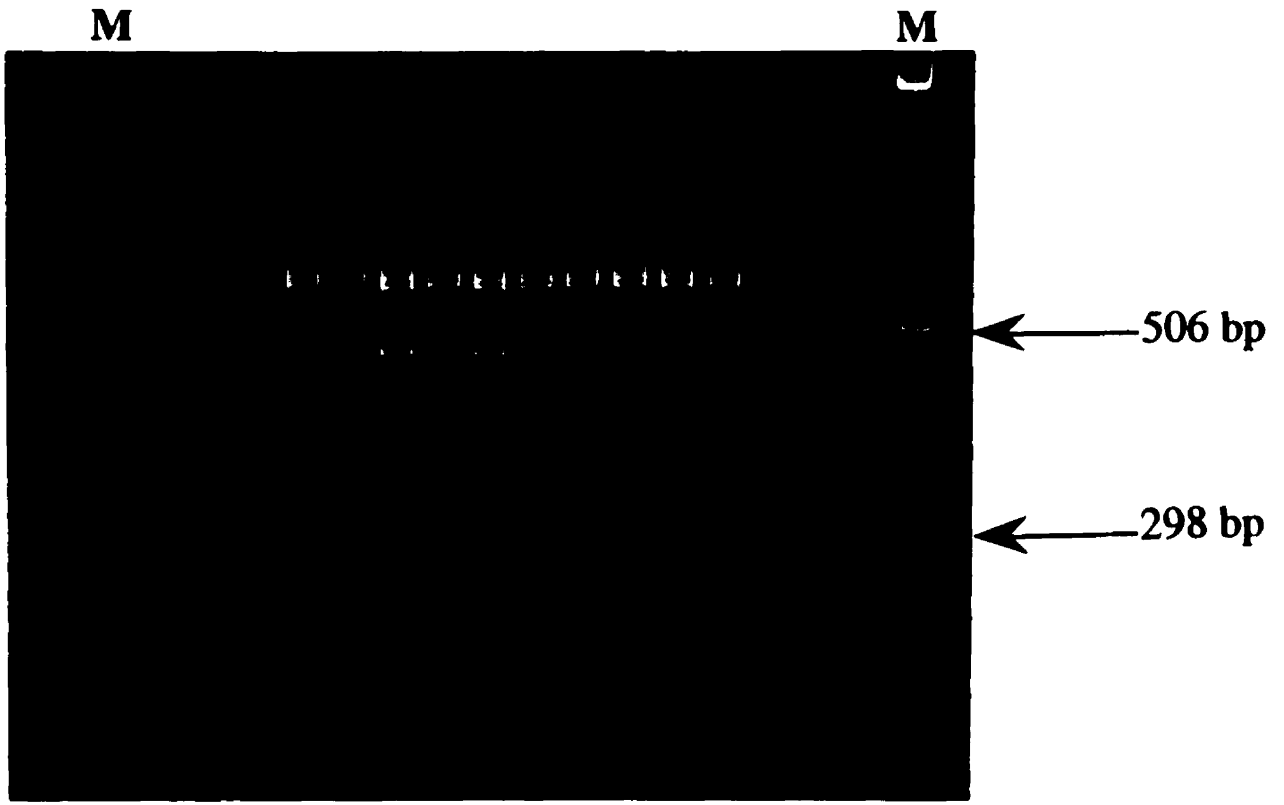
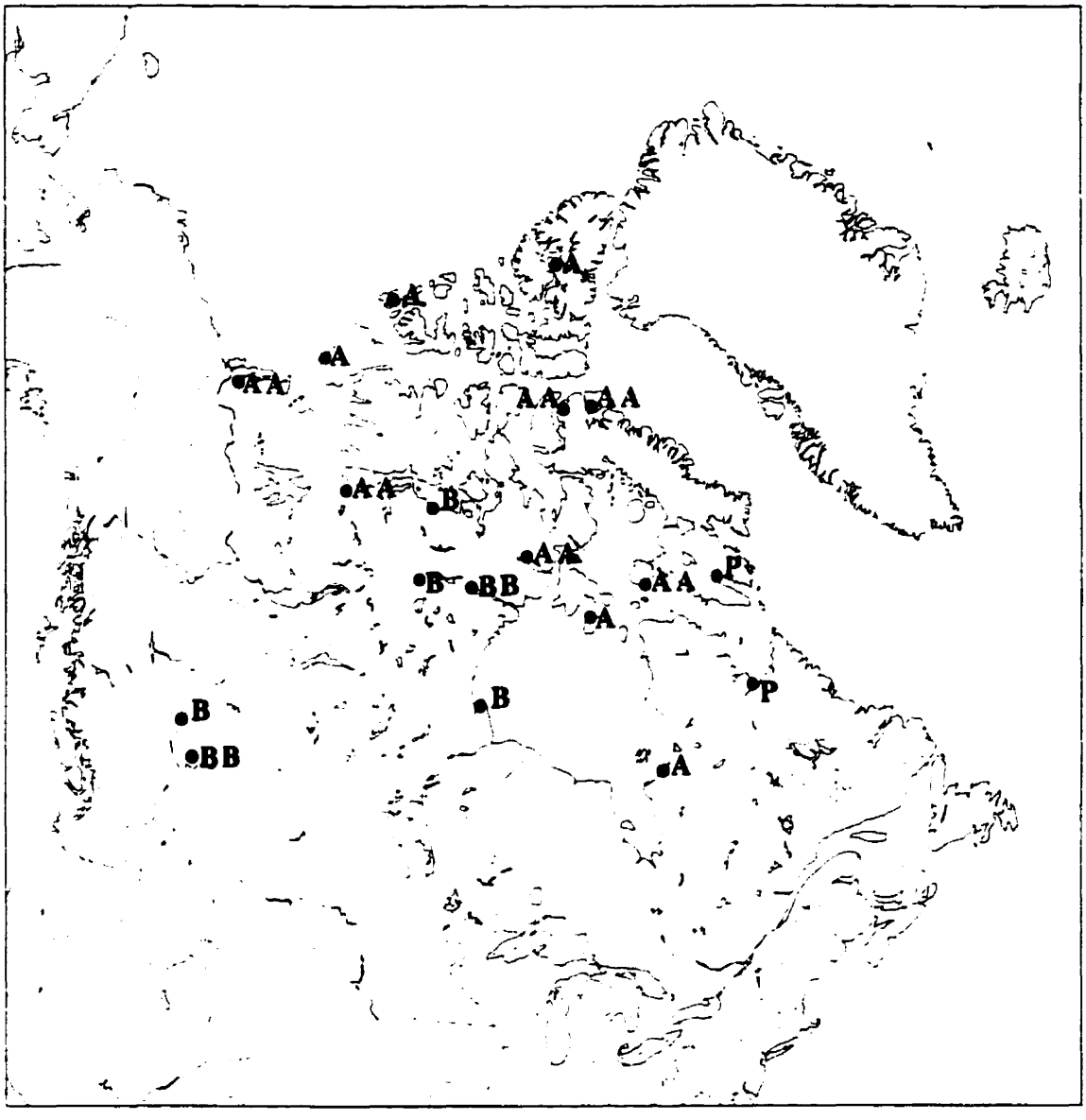


Figure 9



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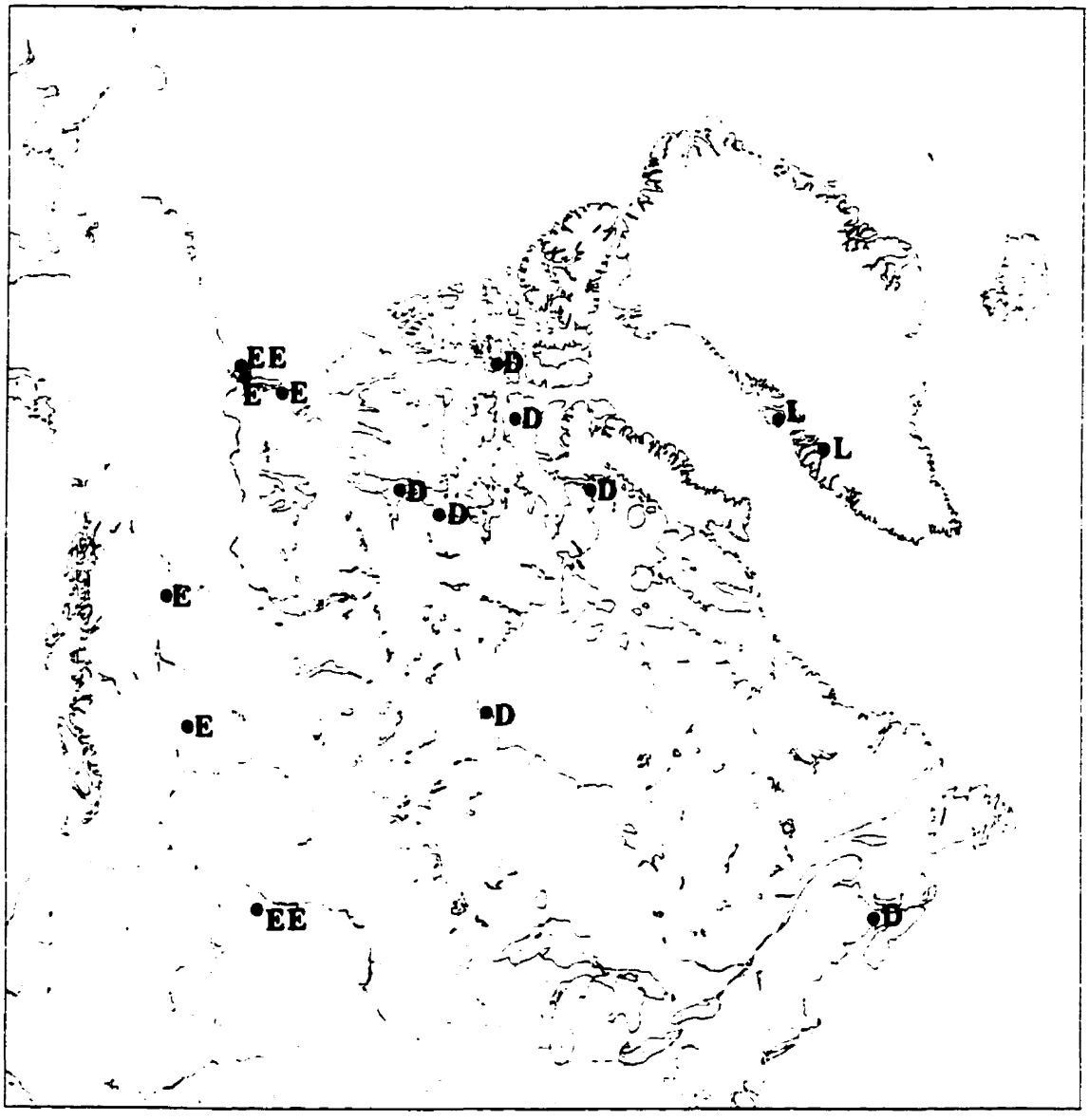


Figure 9 (continued)

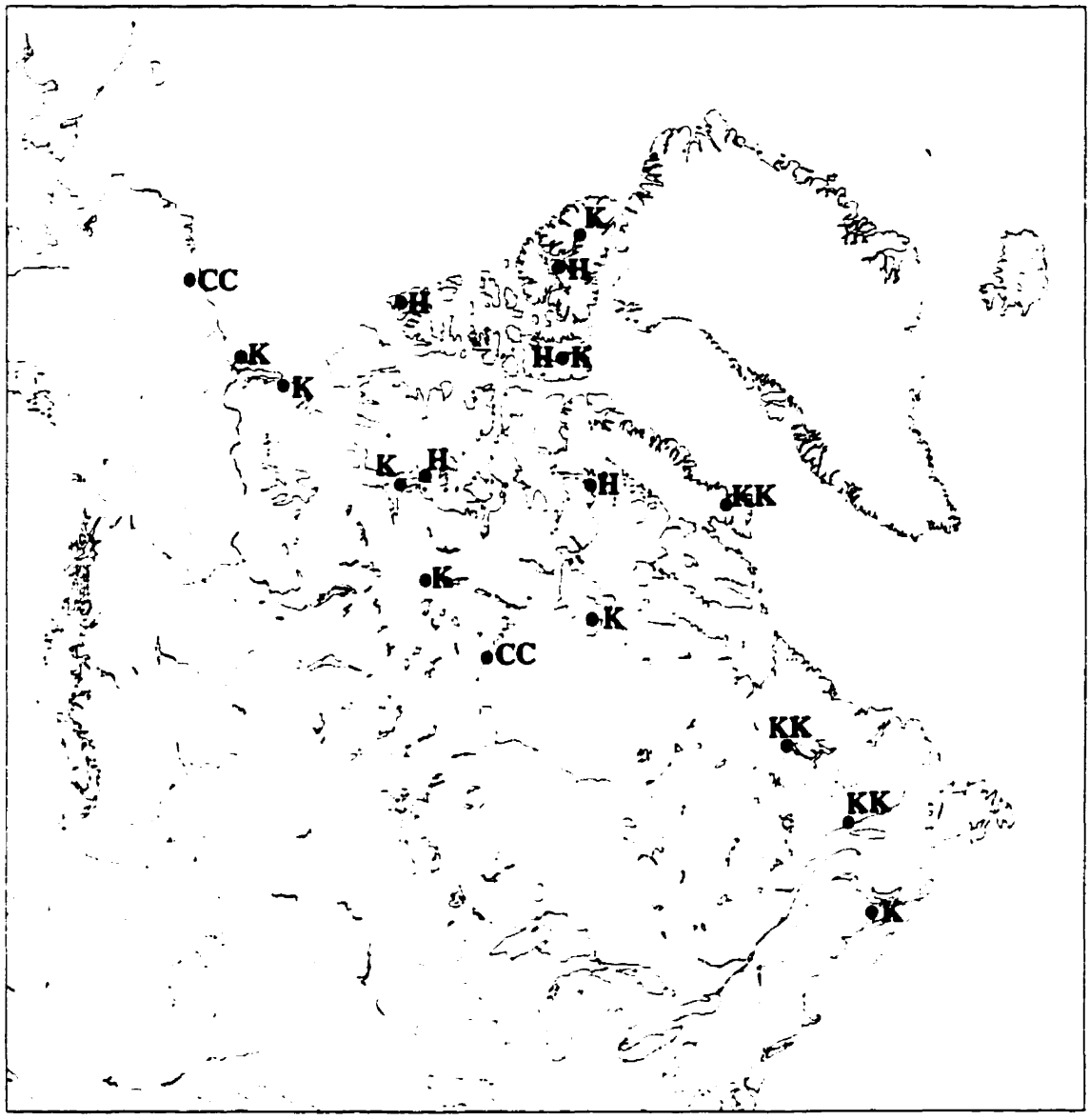


Figure 9 (concluded)

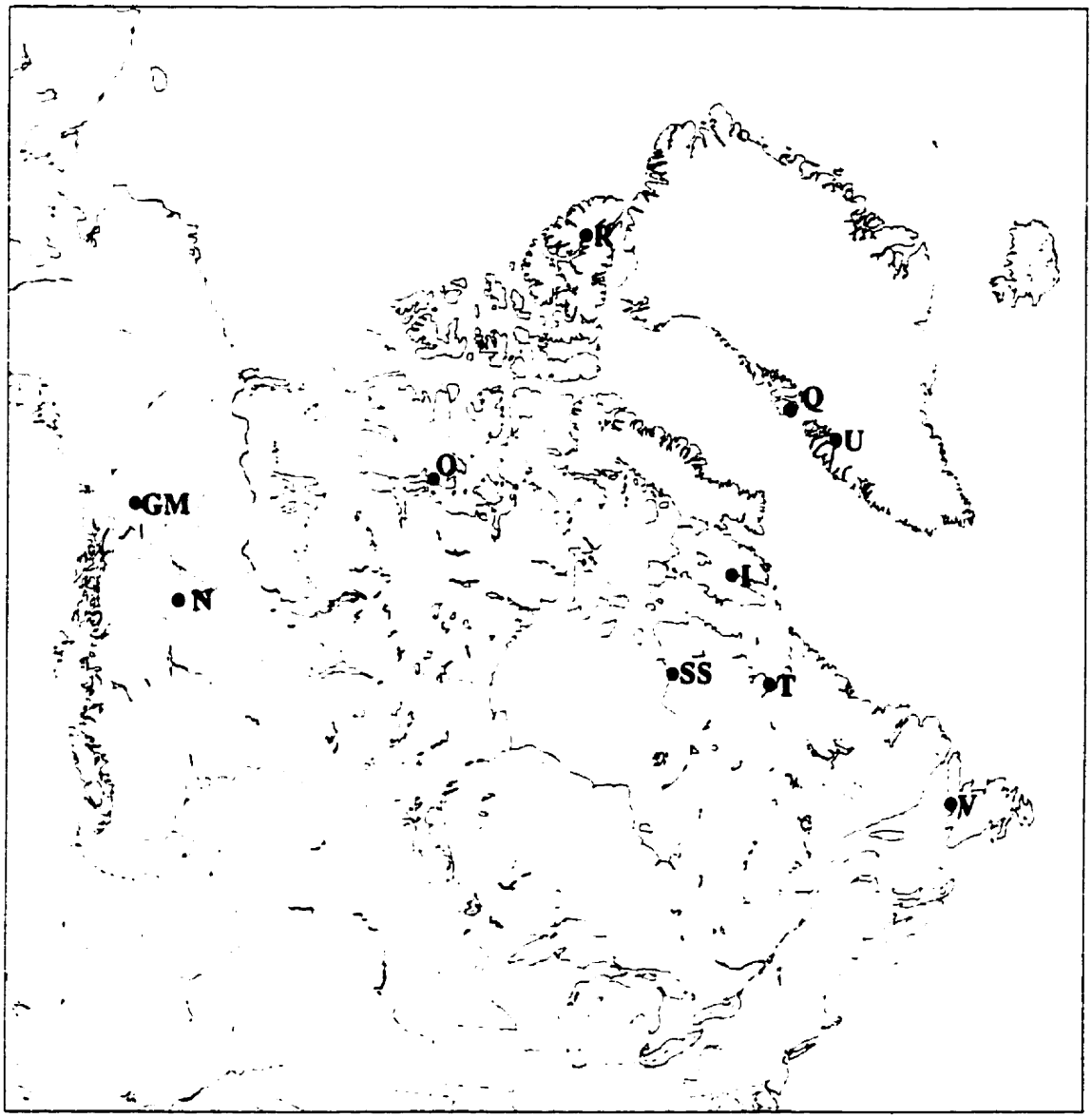


Figure 10

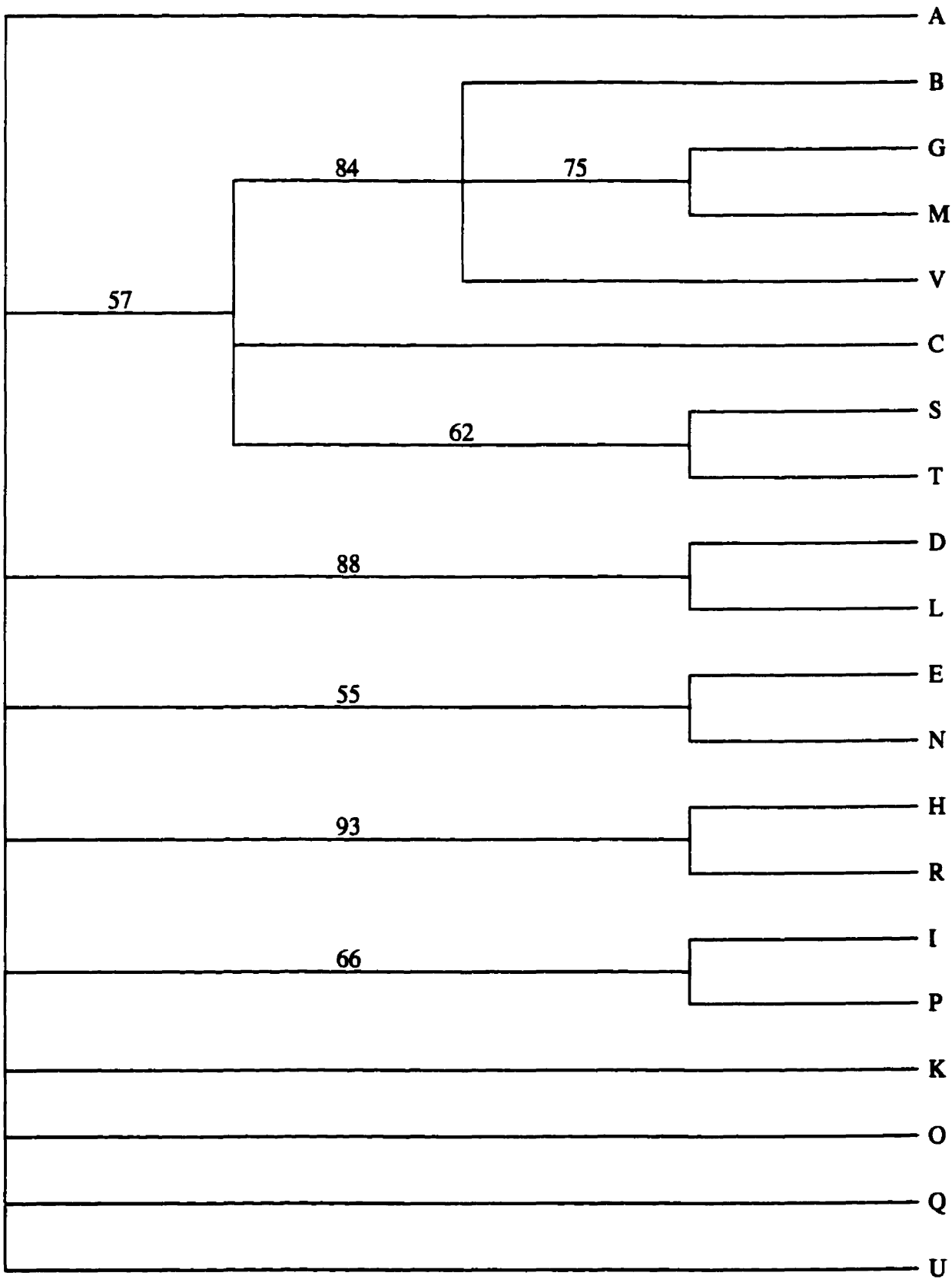


Figure 11

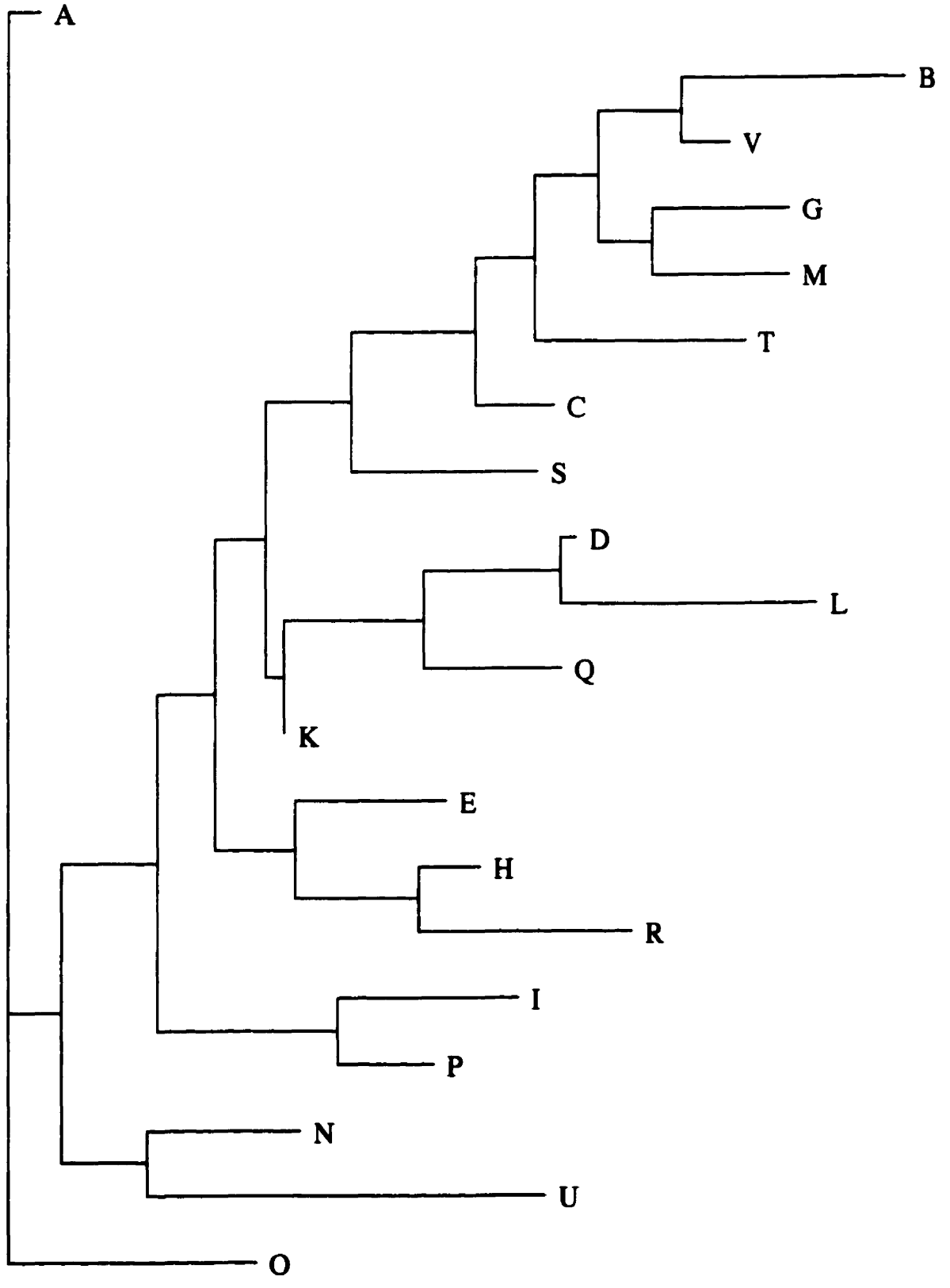


Figure 12

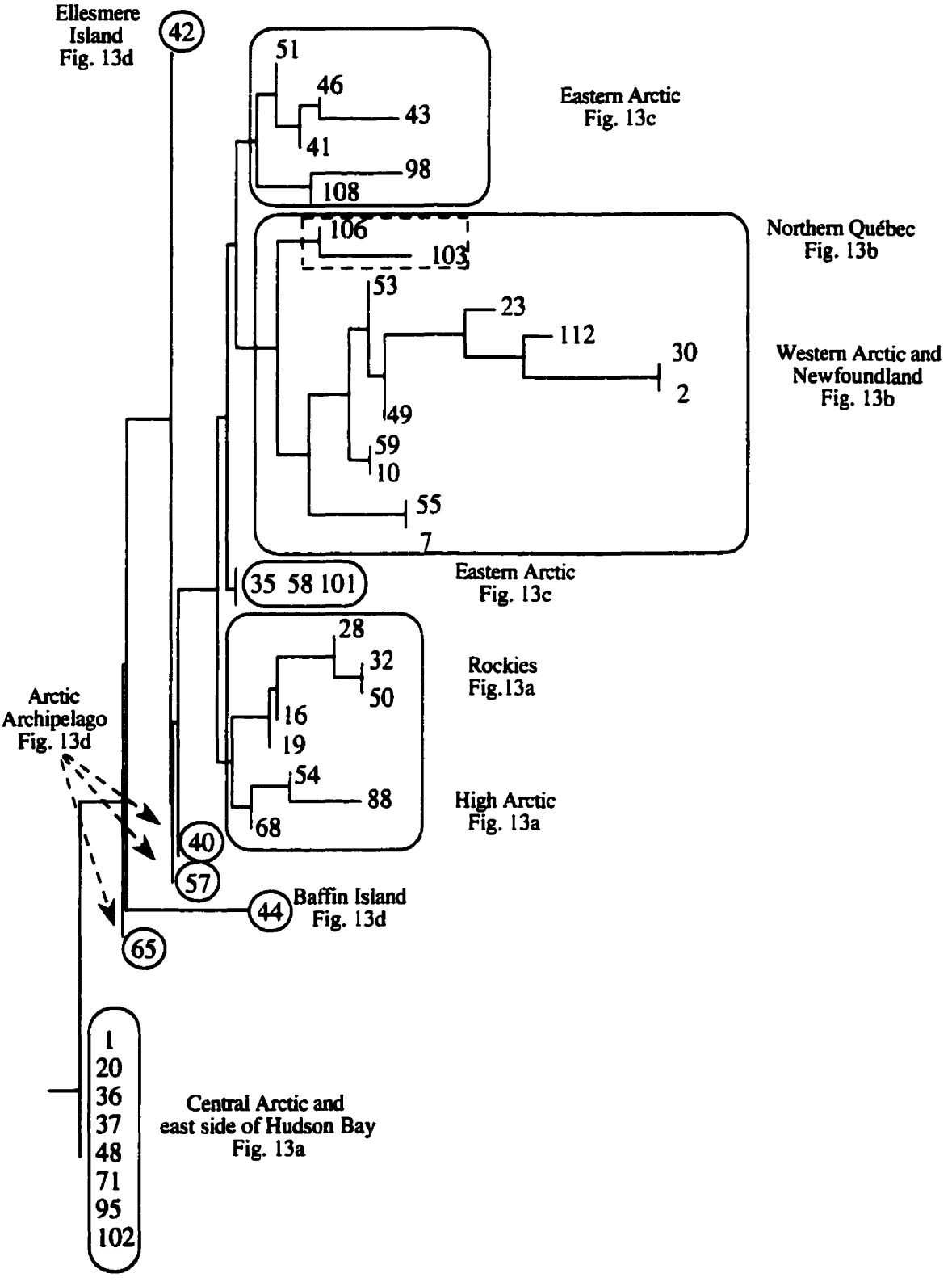

Ellesmere Island Fig. 13d
42
51
46
43
41
98
108
Eastern Arctic Fig. 13c
106
103
Northern Québec Fig. 13b
53
23
112
30
2
49
59
10
55
7
Western Arctic and Newfoundland Fig. 13b
35 58 101
Eastern Arctic Fig. 13c
28
32
50
16
19
54
88
68
Rockies Fig.13a
High Arctic Fig. 13a
Arctic Archipelago Fig. 13d
40
57
44
Baffin Island Fig. 13d
65
1
20
36
37
48
71
95
102
Central Arctic and east side of Hudson Bay Fig. 13a

Figure 13a

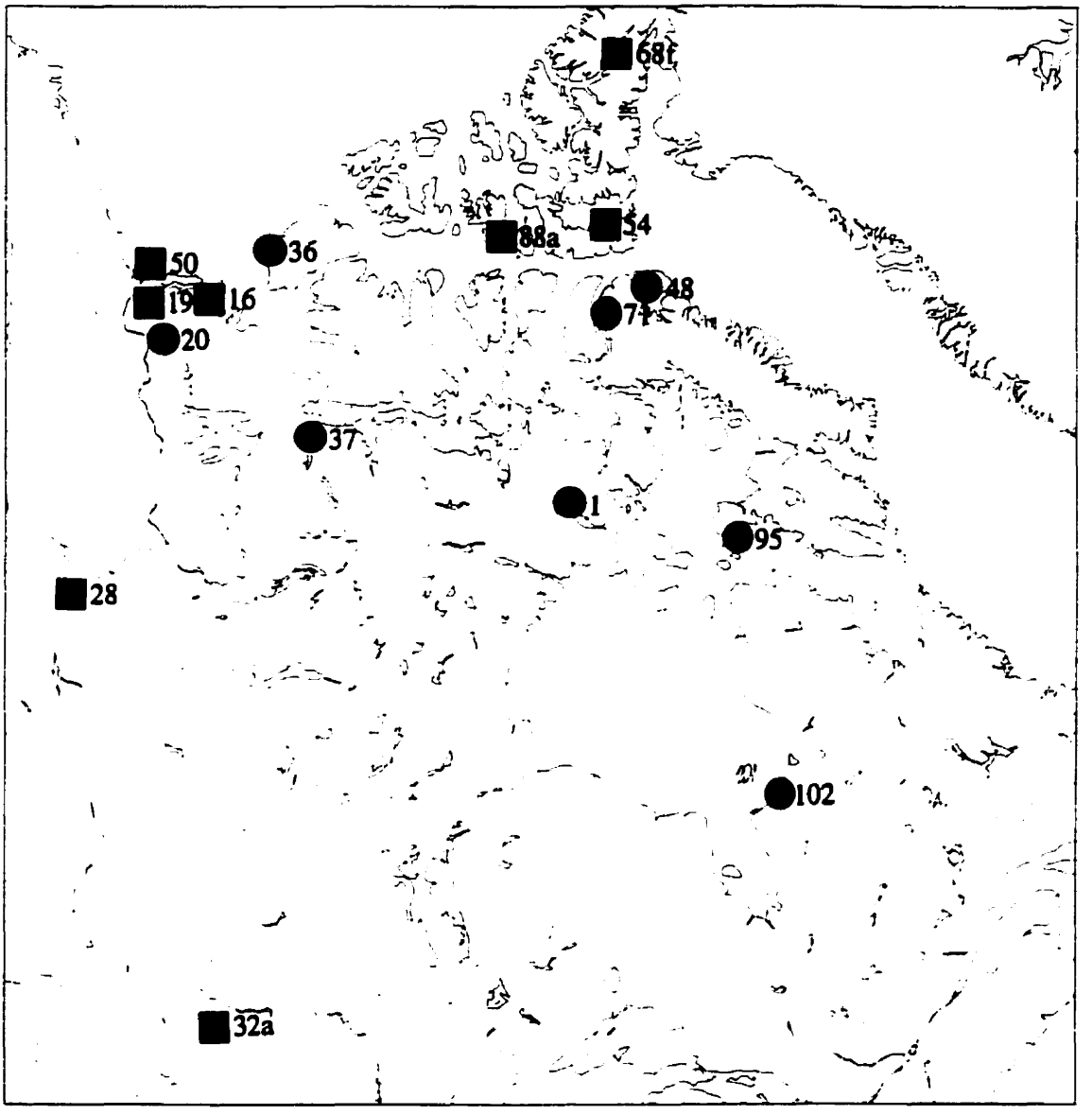


Figure 13b

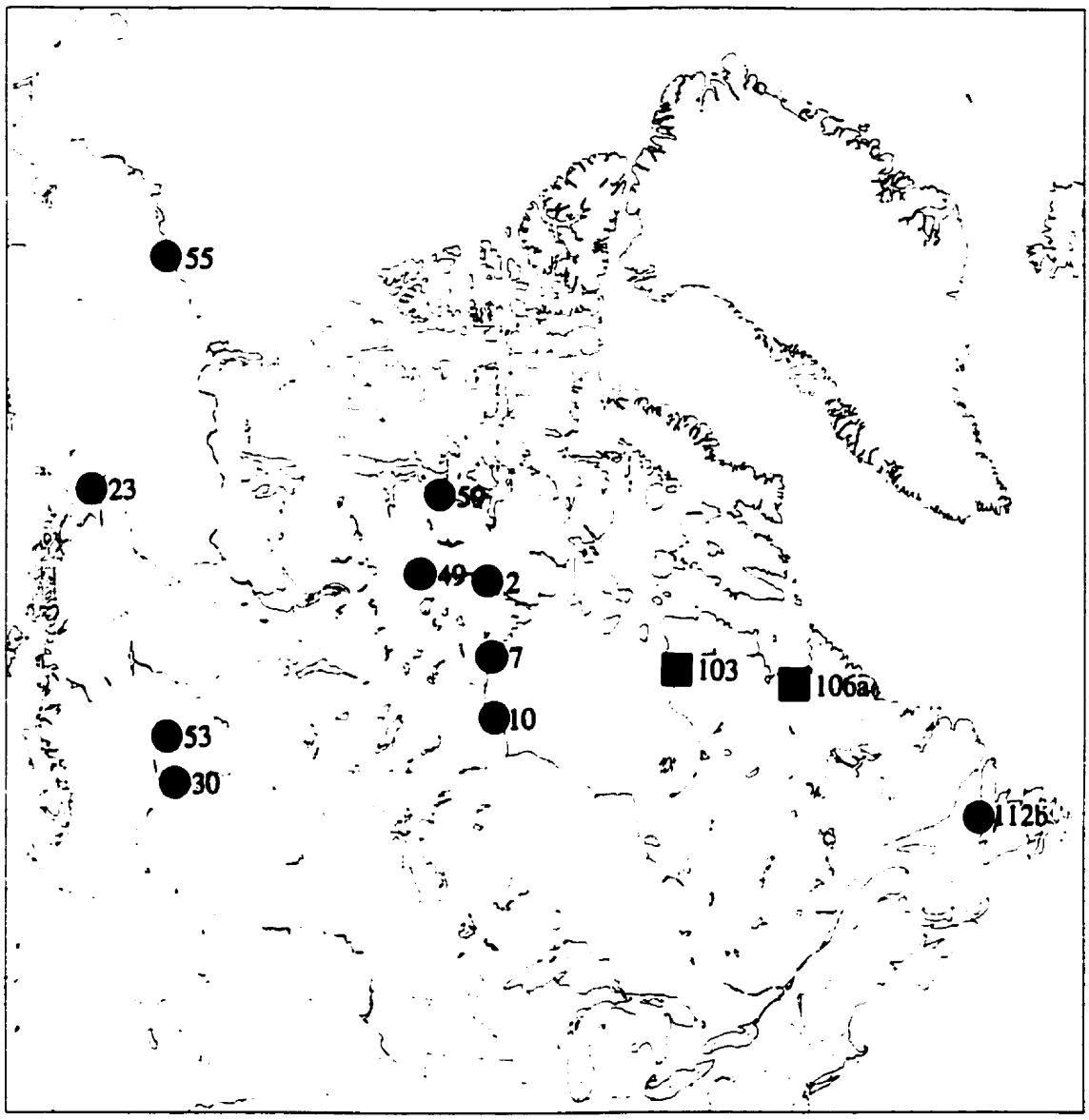


Figure 13c

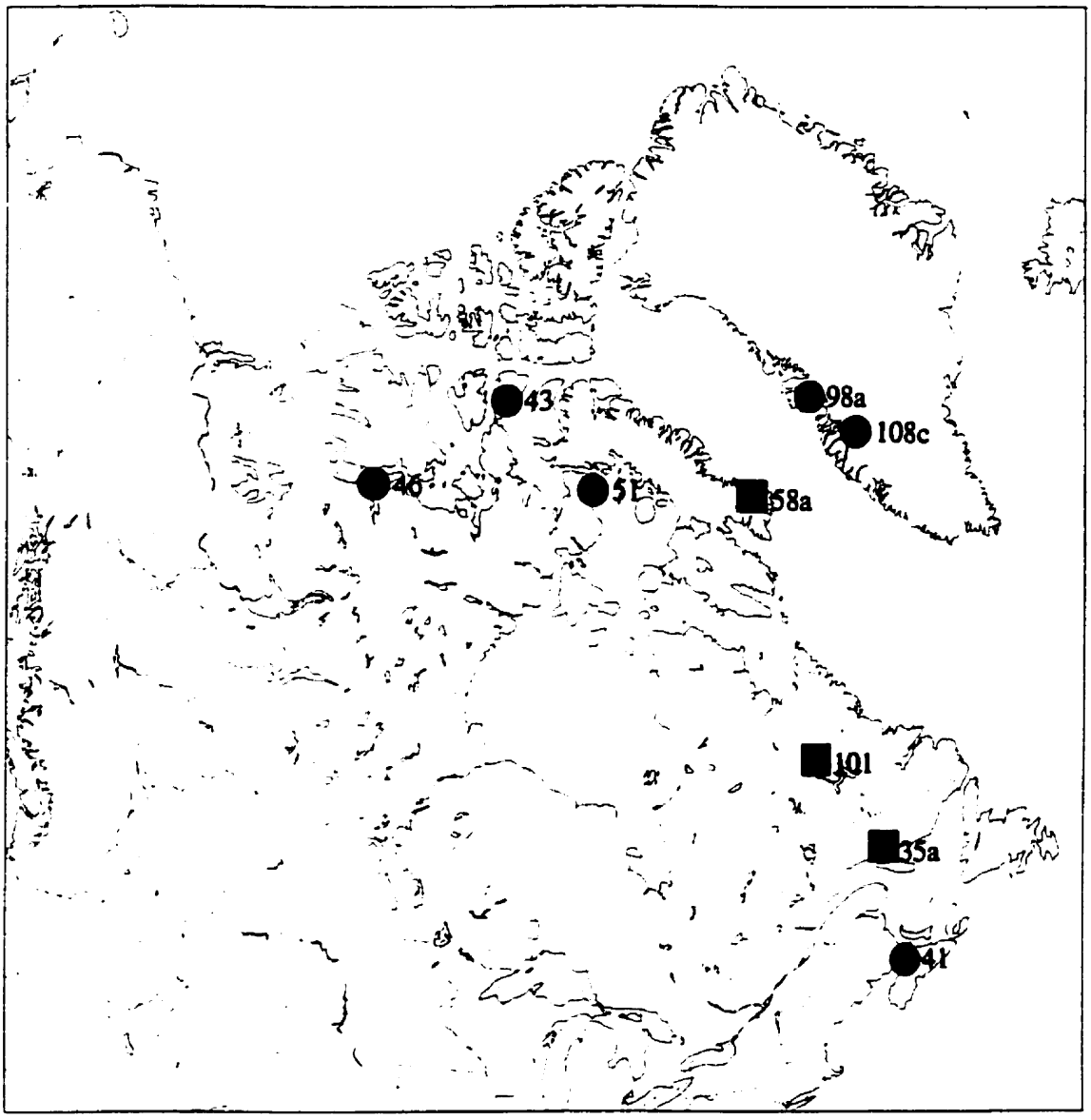


Figure 13d

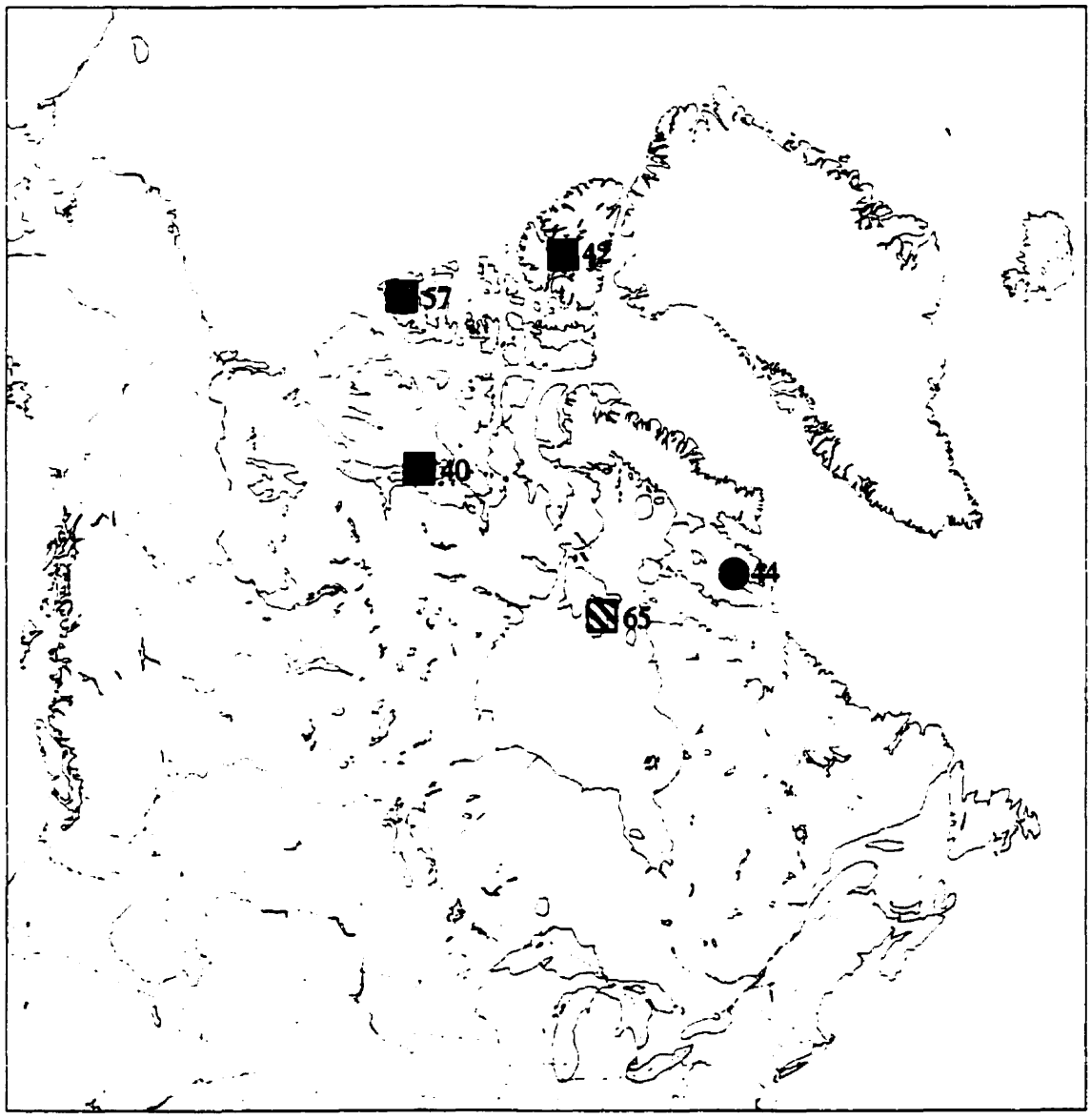


Figure 14

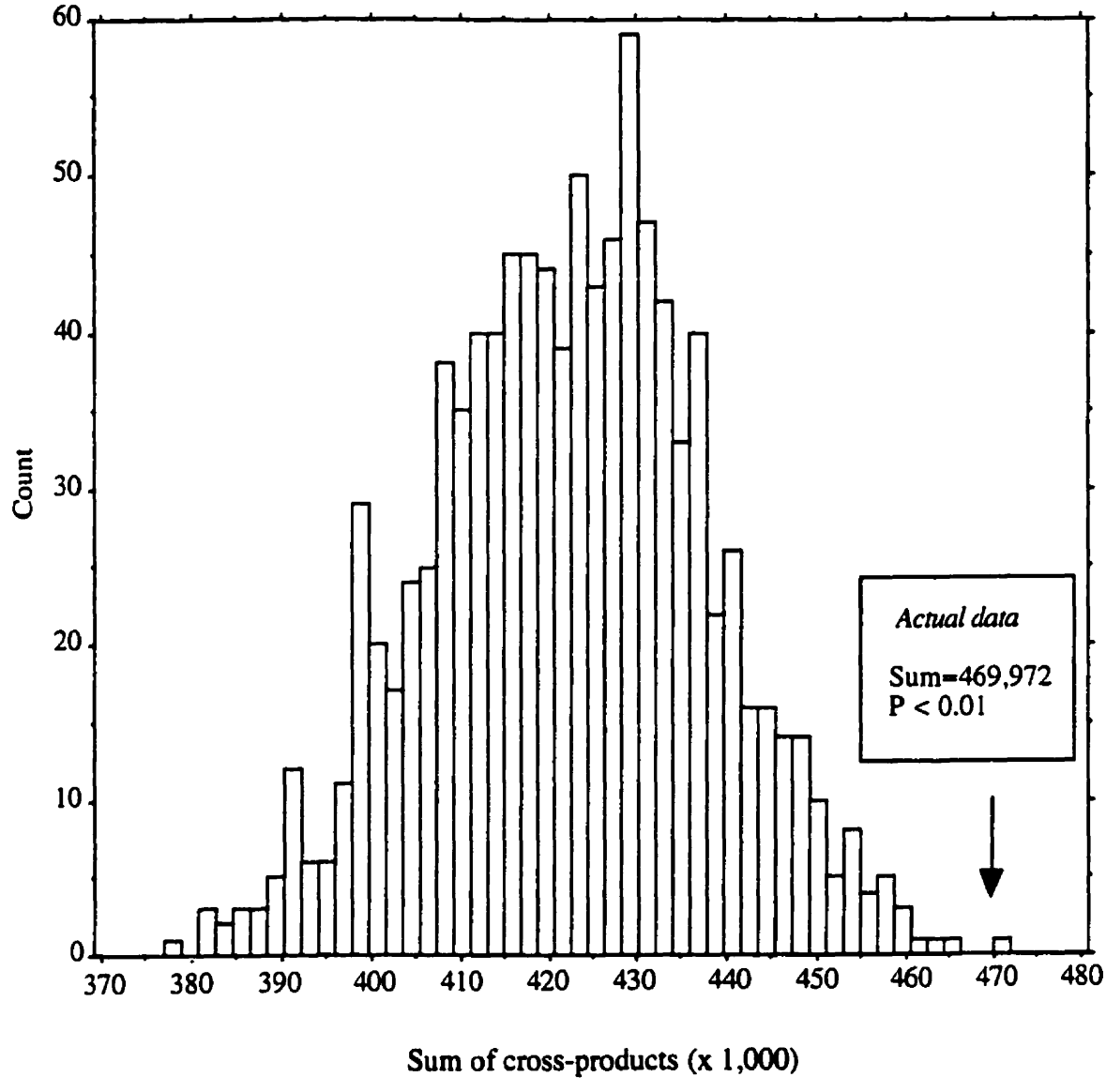
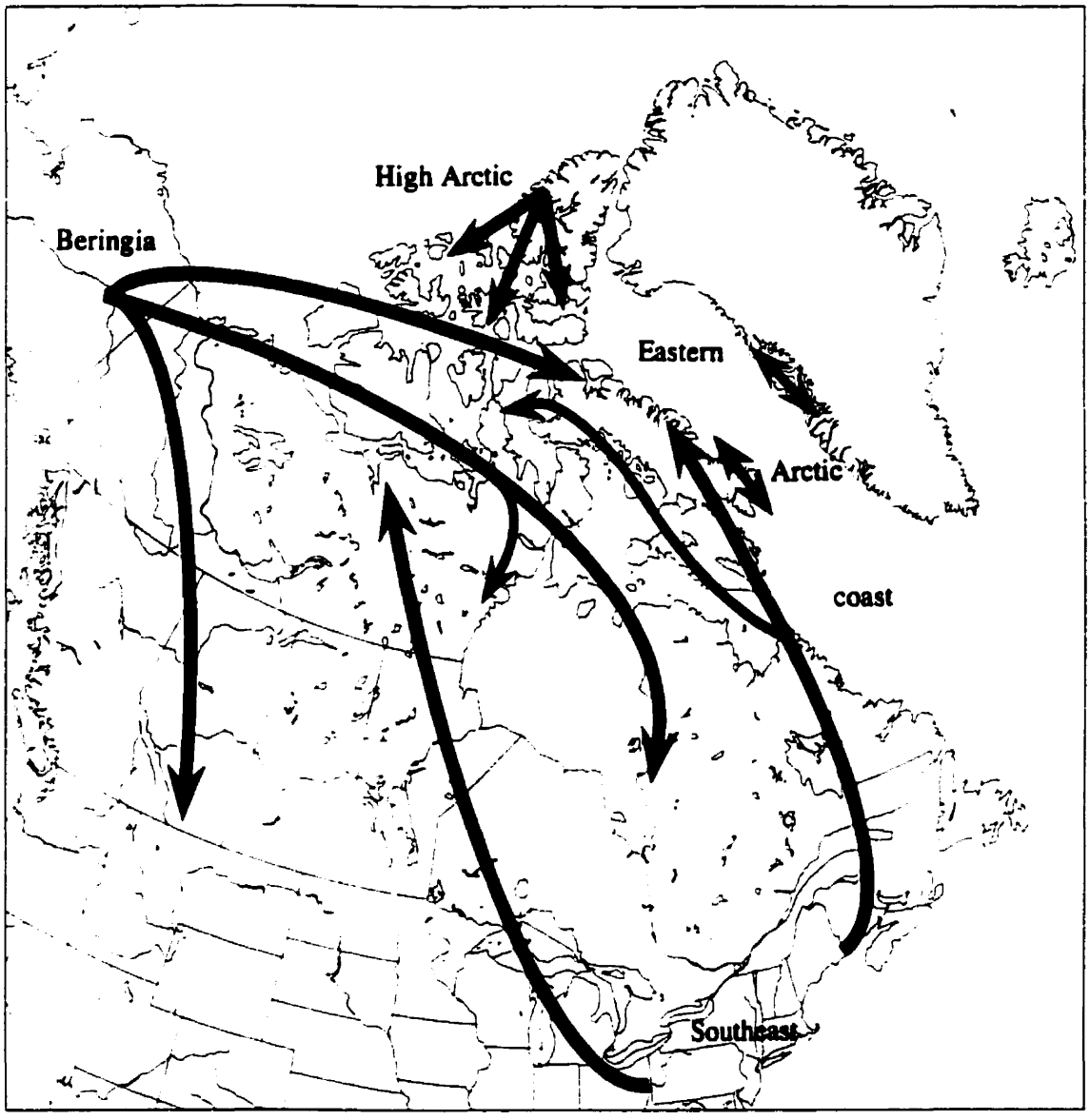

60
50
40
30
20
10
0
Count
370
380
390
400
410
420
430
440
450
460
470
480
Actual data
Sum=469,972
P < 0.01
Sum of cross-products (x 1,000)

Figure 15



TABLES 1 TO 5

Table 1. Collection data for *Dryas integrifolia* specimens used in this study.

Site number	Description	Collector(s)	Number of samples[a]
1	Wager Bay, NWT 66°N; 90°W	Bruno Lelièvre Pfastatt, France	2
2	Baker Lake, NWT 64°19'N; 96°02'W	Joseph Svoboda University of Toronto	2
7	Arviat, NWT 61°07'N; 94°04'W	Nicolas Tremblay McGill University	2
10	Churchill, Manitoba 58°47'N; 94°11'W	Nicolas Tremblay McGill University	2
16	Stanton, NWT 69°48'N; 128°42'W	Nicolas Tremblay McGill University	2
19	Kittagazuit, NWT 69°21'N; 138°41'W	Nicolas Tremblay McGill University	2
20	Eskimo Lake, NWT 68°40'N; 132°53'W	Nicolas Tremblay McGill University	2

23	Whitehorse, Yukon 61°N; 135°W	Nicolas Tremblay McGill University	2
28	Muncho Lake, British Columbia 58°40'N; 126°10'W	Nicolas Tremblay McGill University	2
30	Sunwapta Flats, Alberta 52°30'N; 117°W	Nicolas Tremblay McGill University	2
32a	Big Snowy Montains, Montana 47°N; 109°W	Nicolas Tremblay McGill University	2
35a	Mingan Islands, Québec 50°23'N; 64°12'W	Patrick Nantel, Danielle Cantin McGill University	2
36	Banks Is., NWT 72°15'N; 124°50'W	Evan G. Cooch Simon Fraser University	1
37	Coppermine River, NWT 67°13'N;113°26'W	Stephen Wolfe Geological Survey of Canada	2
40	Cambridge Bay, NWT 69°07'N; 105°03'W	Rick Gillis, Mark Calvez Atmospheric Environment Service	2
41	Albert County, New Brunswick 45°N; 64°W	Stephen R. Clayden New Brunswick Museum	2

42	Fosheim Peninsula, NWT 79°55'N; 84°11W	Wayne Pollard McGill University	2
43	Somerset Is., NWT 72°55'N; 93°27'W	Anne Gunn, Judy Dragon Government of the Northwest Territories	1
44	Iqaluit, NWT 63°45'N; 68°31'W	Yvonne Earle Iqaluit, NWT	2
46	Kent Peninsula, NWT 68°21N; 108°5'W	Martin Predavec University of British Columbia	2
48	Bylot Is., NWT 73° N; 80°W	Monique Poulin Université Laval	2
49	Thelon River, NWT 64 °16'N; 101°54'W	Christofer Shank Government of the Northwest Territories	2
50	Garry Is., NWT 69°28'N; 135°39'W	Alan Fehr Science Institute of the Northwest Territories	2

51	Igloolik, NWT 69° 22'N; 81°48' W	John MacDonald, Carolyn MacDonald Igloolik Research Centre	2
53	Caw Ridge, Alberta 53°50'N; 119°W	Steeve Côté Université de Sherbrooke	2
54	Truelove Lowland, NWT 75°33'N; 84°40'W	Lawrence Bliss, Warren Gold, Julia Gold University of Washington	2
55	Prudhoe Bay, Alaska 69°48'N; 148°44'W	Michael Hunt Jones Institute of Arctic and Alpine Research	2
57	Mould Bay, NWT 76°N; 119°W	Sylvain Juneau Atmospheric Environment Service	2
58a	Weasel River, NWT 66°53'N; 64°53'W	Yves Bossé, Mark Heathcott, Michael Keenaina Auyuittuq National Park Reserve	2
59	Perry River, NWT 67°43'N; 102°12'W	Ray T. Alisauskas, Stuart Slattery, Mike Schwitters, Glen Mack Environment Canada	2

65	Coats Is., NWT 63°N; 83°W	Anthony J. Gaston Canadian Wildlife Service	2
68f	Tanquary Fjord, NWT 81°24'N;76°52'W	Barry Troke Ellesmere Island National Park Reserve	2
71	Nanisivik, NWT 73°02'N; 84°33'W	Ron Sutherland, Lois Sutherland Nanisivik, NWT	2
88	Bathurst Is., NWT 75°26'N; 97°56 W	Jan Bednarski, Anne Williams Canadian Wildlife Service	1
95	Cape Dorset, NWT 64°14'N; 76°32'W	Norman Hallendy Tukilik Project	2
98a	Disko Is., Greenland 69°N; 53°W	Per Mølgaard Royal Danish School of Pharmacy	2
101	Schefferville, Québec 54°36'N; 66°41'W	Oksana Choulik, Martine Giangioppi McGill Subarctic Research Station	2
102	Kuujjuarapik, Québec 55°20'N; 77°45'W	Nicolas Tremblay McGill University	1

103	Povungnituk, Québec 60°05'N; 77°20' W	Marcel Blondeau Trois-Rivières, Québec	2
106a	Kuujjuaq, Québec 58°16'N; 68°13'W	Nicolas Tremblay McGill University	2
108c	Søndre Strømfjord, Greenland 67°09'N; 50°18'W	Ole Bennike Geological Survey of Denmark and Greenland	2
112b	Gros Morne National Park, Newfoundland 49°30'N; 57°50'W	Anne Marceau Gros Morne National Park	1

[a] The number of individuals collected was 30 on average, but only ca. two per population were analysed for molecular variation in the chloroplast genome.

Table 2. Primers pairs used for this study, and the restriction enzyme used to digest the corresponding amplified fragment. The digested fragments exhibited one or two polymorphic sections, whose various lengths are also described below. Polymorphic fragments are given a number. The most common fragment is given the number 0. For a given cpDNA molecule, the various combinations of polymorphic fragments make up the different haplotypes (see Table 3).

Primers pair[a]	Restriction enzyme used	Polymorphic section (size in base pairs)[b]
*trn*T-*trn*F[1]	*Mse* I	I- 524 (0); 498 (1)
*trn*C-*trn*D[2]	*Nla* III	II- 729 (0); 768 (1); 666 (2)
*trn*F-*trn*Vr[3]	*Rsa* I	III- 508 (0); 503 (1)
		IV[c]- 304 (0); 294 (1); 283 (2); 312 (3)
*trn*H-*trn*K[2]	*Alu* I	V- 275 (0); 266 (1)
*trn*Q-*trn*Rr[3]	*Rsa* I	VI- 500 (0); 498 (1)
*trn*D-*trn*T[2]	*Alu* I	VII- 638 (0), 617 (1)

[a] Primer sequences published in: 1- Taberlet et al. 1991, 2- Demesure et al. 1995, 3- Dumoulin-Lapègue et al. 1997b.

[b] The roman numeral is an arbitrary number used to distinguish each variable subsegment. The number in parentheses corresponds to the possible lengths of each subsegment.

[c] The amplified fragment *trn*F-*trn*Vr, once digested, had two different sets of polymorphic fragments. They are considered as two different characters.

Table 3. Haplotype characteristics as defined by the presence of particular restriction length variants for each polymorphic section of digested PCR amplified fragments (polymorphic fragments and sections defined in Table 2).

	Polymorphic section						
Haplotype	I	II	III	IV	V	VI	VII
A	0	1	0	0	0	1	0
B	0	2	1	1	0	0	0
C	0	2	0	0	0	0	0
D	0	1	0	0	1	0	0
E	0	1	0	2	0	0	0
G	0	0	1	0	0	0	0
H	0	1	0	3	0	0	0
I	1	1	0	0	0	1	0
K	0	1	0	0	0	0	0
L	0	1	0	0	1	0	1
M	0	3	1	0	0	0	0
N	0	1	0	2	0	1	0
O	0	1	1	0	0	1	0
P	1	1	0	0	0	0	0
Q	0	1	0	0	0	0	1
R	0	1	0	3	0	2	0
S	0	1	2	0	0	0	0
T	0	2	2	0	0	0	0
U	0	2	0	1	0	1	0
V	0	2	1	0	0	0	0

Table 4. Analysis of population substructure in *Dryas integrifolia*.

Diversity parameter	Estimate	Standard error[a]
h_S	0.50	0.07
h_t	0.90	0.02
G_{st}	0.44	0.08
v_S	0.39	0.08
v_t	0.74	0.06
N_{st}	0.47	0.10
N_{st}-G_{st}	0.03	0.06

[a] Standard errors calculated by bootstrap resampling (1000 trials). All estimates were significantly different from zero at the P<0.001 level except that of *Nst-Gst*.

Table 5. Analysis of heterogeneity of haplotype frequencies across different parts of the range of *Dryas integrifolia*. Significant results are emphasized with an asterisk.

Regions defined[a]	$\chi 2$	Significance level (standard error)[b]
Ecological regions		
High Arctic vs. Low Arctic vs. boreal forest	62.9774	P=0.0004*
(15 vs. 17 vs. 10)		(0.0002)
Glaciated vs. unglaciated areas		
18,000 years BP	20.3104	P= 0.3996
(34 vs. 8)		(0.0049)
12,000 years BP	28.7839	P= 0.0141*
(21 vs. 21)		(0.0012)
8,400 years BP	21.3851	P=0.2782
(15 vs. 27)		(0.0045)

[a] Figures in parentheses represent the number of populations in each category.

[b] Standard errors calculated by bootstrap resampling (10,000 trials).

APPENDICES A-D

APPENDIX A

Leaflet explaining the collecting protocol. Note that initially, the research proposal also included analysis of *Eriophorum angustifolium*, a boreal species whose migrational history would have been constrasted with the history of *Dryas integrifolia*, an arctic species. Thus, samples of *E. angustifolium* were collected in the same time as samples of *D. integrifolia*, but the molecular analysis was eventually abandoned because of time constraints.

McGill

PLANT MIGRATION

AFTER THE

ICE AGE

About the project...

18 000 years ago, a huge ice cap extended over Canada and the northern United States. It is believed that many arctic species survived the glaciation north of the ice, in Beringia, an area including Alaska and eastern part of Siberia. Other species are believed to have found a refugium south of the ice. When that huge ice cap started to melt, it is thought that species started to spread over the arctic to their present distribution.

This project aims to test this hypothesis by studying the past migrational history of two common and widespread arctic species, *Dryas integrifolia* and *Eriophorum angustifolium*, using the tools of molecular genetics. We will reconstruct the phylogenetic tree of different sampled populations and then identify the region where the ancestral population(s) occurred.

We will need samples from all over the Arctic in order to carry out this investigation. Because we can't afford to fly everywhere, we wish to ask for the collaboration of other people to get samples from places that we would not otherwise be able to reach. By helping me, you contribute to the success of this research and I thank you in advance.

About collecting...

Sampling is easy :

-First, locate a patch of *D. integrifolia* and/or *E. angustifolium* (see Fig. 1 and Fig. 2 for descriptions).

-Then collect seed samples from **30 different plants.**

Pick the whole head (Fig. 1C and 2B).

-Try to pick the seed samples from plants located not too close to one another (**2 feet apart** is enough).

-Before collecting, make sure the seeds are **ripe** by pulling on the long hairs coming out of the flower stem. If the hairs (to which the seed is attached) come out easily, then it is ready to be collected. If they resist, then it is not ripe.

-Put seeds of different plants in different envelopes.

-Apart from the seeds, collect **one whole plant** specimen for each species, so we will be able to confirm identification later on. For *D. integrifolia*, a simple stem is enough. Put that specimen in the plant-press, inside the paper towel sheet (one specimen per layer of cardboard).

-Note the **date** and the **location** of collection for each species (as precise as possible).

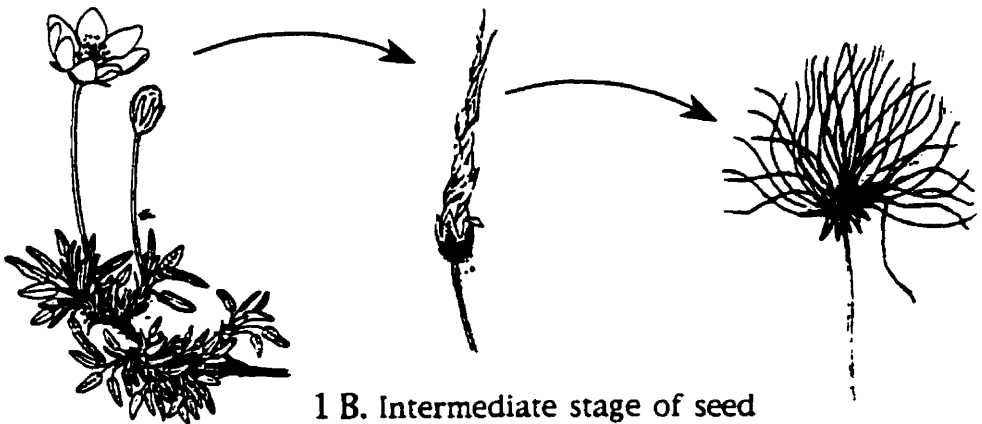
-Put everything in the pre-addressed *XPRESSPOST* envelope and mail it directly (**postage is already paid**).

For more information, please contact me :

Nicolas Tremblay
McGill University, Department of Biology
1205 Docteur Penfield Avenue
Montreal, Quebec
H3A 1B1

Tel: (514) 398-4084 **Fax**: (514) 398-5069
E-Mail: Tremblay@bio1.lan.mcgill.ca

Thank you very much!



1A. Whole plant (half of actual size)

1 B. Intermediate stage of seed development (actual size)

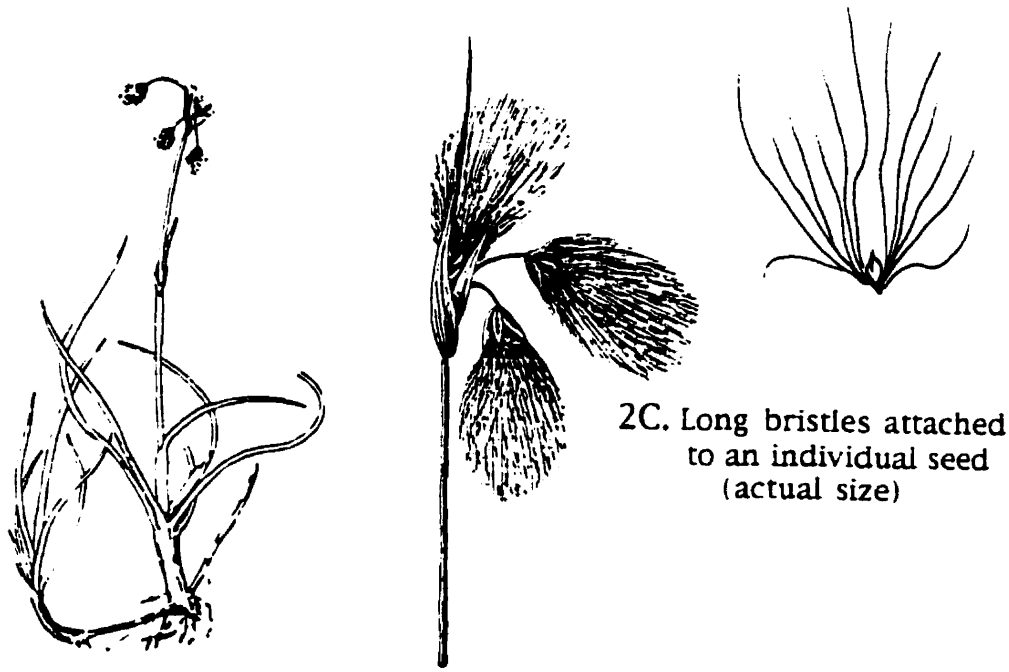
1C. At maturity (actual size)

1D. Individual seed with long plumose style (actual size)

1 E. Leaves of *Dryas integrifolia* (actual size).

Figure 1 : *Dryas integrifolia*, also known as White Mountain Avens.

Description : Dwarf plant (less than 10 cm high) forming large mats **on dry rocky or gravelly places**. It has **white flowers** with 8 petals, numerous stamens and many carpels (Fig 1A). At maturity, petals fall. The styles then start to elongate, all twisted together at first (Fig. 1B) and all spread out at the end (Fig. 1C). The seeds are at the base of the **long plumose style.** (Fig 1D).
D. integrifolia has dark green, simple leaves, shaped like a **lance-head**, three times longer than broad; the **margin is entire** or with a few teeth in the lower half; there are white matted hairs beneath the leaf. You can tell *D. integridolia* from other related species by the shape of its leaves (Fig. 1E). In flower generally around end of June, early July; ripe around end of July, early August.



2A. Whole plant
(1/4 of actual size)

2B. Detail of flower spikes
(actual size)

2C. Long bristles attached to an individual seed
(actual size)

Figure 2 : *Eriophorum angustifolium*, also known as Tall Cottongrass.

Description : Grass-like plant with solid, somewhat 3-angled **smooth stem** (peduncle glabrous), 20 to 40 cm high, with **flat leaves** (Fig. 2A). Produces **several flower spikes** (2 to 10) (Fig. 2B - that is what needs to be collected). Flower spikes 1-2 cm long, with 2-5 cm long silky **white bristles** when mature (Fig. B & C). In the High Arctic, a smaller form occurs : it is often called *Eriophorum triste*. It can be considered as an arctic race of *Eriophorum angustifolium*, and it can be collected as well. Very common and abundant in **wet bogs** or by the **edge of ponds**, where it often forms pure stands. In general, young spikes appear in late June, white cotton visible around mid-July, seed ripe and dispersing in early August.

APPENDIX B

Details of the DNA Extraction and PCR Amplifications

Detailed protocol of genomic DNA extraction modified from Jobes (1995)

Tissue grinding:

- weigh 10-20 mg of fresh tissue
- place in a microcentrifuge tube (1.5 mL)
- dip tube in liquid nitrogen for 5 minutes
- grind with pestle grinder, preferably using a drill
- add 0.75 mL of warm 2% CTAB (60°C) with Proteinase K (0.1 mg/mL) (add Proteinase K at the last moment)
- incubate 60 min. at 60°C
- spin 10 min.
- keep supernatant in new tube, discard pellet

Potassium acetate cleaning:

- add 0.25 mL of 5M potassium acetate pH 4.8
- keep 20 min. at -20°C
- spin 10 min.
- keep supernatant in new tube, discard pellet

First DNA precipatation:

- add cold isopropanol to fill the tube
- keep 20 min. (or overnight) at -20°C
- spin 10 min.

•keep pellet, discard supernatant

Polysaccharide removal:

•dissolve pellet in 0.4 mL of ddH_2O (double distilled water)

•add 0.2 mL of 5 M NaCl, mix well

•add 0.8 mL of cold 95% ethanol

•keep 20 min. at -20°C

•spin 10 min.

•keep pellet, discard supernatant

RNA removal:

•dissolve pellet in 0.5 mL ddH_2O

•add 0.17 mL of 8 M LiCl

•keep overnight at -20°C

•spin 15 min.

•keep supernatant in new tube, discard pellet

Protein removal in three steps:

•first phenol/chloroform extraction (to remove contaminating proteins)

.add equal volume of phenol/chloroform (1:1, v/v) to supernatant

.mix gently

.spin 10-15 min.

.keep supernatant in a new tube

•second phenol/chloroform extraction

.add equal volume of phenol/chloroform (1:1, v/v) to supernatant

.mix gently

.spin 10-15 min.

.keep supernatant in new tube

•chloroform/isoamyl extraction (to remove trace amounts of phenol and proteins)

.add 1/2 volume of chloroform/isoamyl (24:1, v/v)

.mix gently

.spin 6 min.

.keep supernatant in new tube

Last DNA precipitation:

•add cold isopropanol

•keep 20 min. at -20°C

•spin 10 min.

•discard supernatant

Pellet cleaning:

•wash pellet with 70% ethanol (about 1 mL)

•spin 5 min.

•discard supernatant

•air dry pellet (approximately 2 hours)

Final DNA dissolution in buffer:

•resuspend pellet in TE buffer (0.05 to 0.10 mL)

•keep at 4°C or -20°C

APPENDIX B (CONTINUED)

Details of the PCR procedure and gels parameters

Primers pair	Approximate length[a] (kb)	PCR variables: Annealing temperature (°C); Extension time (minutes)	Amount of PCR product used for digestion (µL)	Polyacrylamide gels : Voltage (V); Concentration of gel (%); Migration time (h)
*trn*C-*trn*D	3.2	57; 2	10	160; 4; 5:30
*trn*D-*trn*T	1.2	62; 2	5	170; 4; 5:00
*trn*F-*trn*Vr	3	57; 2	8	170; 8; 8:00
*trn*H-*trn*K	1.6	40; 2	12	170; 8; 6:30
*trn*Q-*trn*Rr	3	62; 3	5	160; 4; 6:00
*trn*T-*trn*F	2.1	55; 2	10	170; 8; 6:30

[a] Total length of uncut amplified fragment, estimated by visual comparaison with molecular marker.

APPENDIX C

Description of the Fossil Localities of *Dryas Integrifolia*, *Dryas Drummondii* and *Dryas* sp.

Site	Coordinates (Latitude; Longitude)	Age[a] (radiocarbon years before the present)	Reference
Dryas integrifolia			
Greenland, Qagssiarssuk	61°09'N; 45°31'W	985-500	Fredskild 1978
Greenland, Thule	76°27'N; 69°35'W	130,000-74,000	Bennike and Böcher 1992
Illinois, Wedron	41°25'30"N; 88°47'W	21,460±470	Garry et al. 1990
Iowa, Conklin site	42°N; 91°20'W[b]	18,090±190-16,710±270	Baker et al. 1986
Maine, Carrying Place Cove (A)	44°44'N; 66°58'W	11,000-10,000	Tolonen and Tolonen 1984
Maine, Chalk Pound	44°50'N; 68°05'W	>13,760±100 (estimation probably too old)	Tolonen and Tolonen 1984
Maine, Gould Pond	44°59'N; 69°19'W	13,280±410-11,330±260	Anderson et al. 1992

Maine, Upper South Branch Pond	46°5'N; 68°54'W	<12,500-10,965±230	Anderson et al. 1986
Massachusetts, Cambridge	41°N, 71°30'W[b]	13,000-11,000	Argus and Davis 1962
Minnesota, Cary-Port	45°N; 85°W[b]	13,300-12,500	Miller and Benninghoff 1969
Minnesota, Northeastern	46°30'N; 92°34'W	10,630±500	Wasylikowa and Wright 1970
Minnesota, Norwood	44°45'N; 93°55'W	12,400±60-11,200±250	Ashworth et al. 1981
Minnesota, Spider Creek	47°N; 93°W[b]	12,000	Baker 1965
Minnesota, Wolf Creek	46°07'N; 94°07'W	20,500±400- 14,700	Birks 1976
New Brunswick, Mayflower Lake	45°18'10"N; 66°04'15"W	>12,030±110-<10,880±110	Mayle and Cwynar 1995
New Brunswick, Pine Ridge Pond	45°33'N; 67°05'W	>11,490±80-9,500±100	Levesque et al. 1994
New Brunswick, Spland Pond	45°15'20"N; 67°19'50"W	11,640±90-10,090±70	Mayle and Cwynar 1995
New Hampshire	43°56'N; 71°42.5'W	12,800	Spear et al. 1994
New Jersey, Allamuchy Pond	40°55'N; 74°50'W	>12,260±220	Peteet et al. 1993
New York, Central Western	42°30'N; 78°33'W	24,900±1000-24,180±900	Miller and Calkin 1992
Northwest Territories (NWT), Banks Is.	71°57'N; 125°40'W	Preglacial[c]; 730,000	Matthews et al. 1986
NWT, Bathurst Is.	99°N; 76°W	>50,000	Blake 1974
NWT, Devon Is.	75°33'N; 84°40'W	<2,540±90	Jankovska and Bliss 1977

NWT, Ellesmere Is., Hot Weather Creek	79°58'N; 84°28'W	7,910±120-3,850±80	Garneau 1992
NWT, Ellesmere Is., Piper Pass	82°12'N; 68°31'W	6,315±110-4,720±100	Lafarge-England et al. 1991
NWT, Ellesmere Is., Twin Glacier	78°53'N; 75°55'W	430±90; 410±45; 400±140	Bergsma et al. 1984
NWT, Meighen Is.	79°50'N; 99°W	Pliocene	Matthews 1987
Nova Scotia, Chase Pond	45°39'05"N; 60°40'30"W	>14,010±100-9,600±80	Mayle and Cwynar 1995
Nova Scotia, Lac à Magie	44°15'50"N; 66°04'45"W	12,140±90-10,480±80	Mayle and Cwynar 1995
Nova Scotia, Little Lake	44°40'05"N; 63°56'20"W	>11,820±90	Mayle and Cwynar 1995
Ontario, Brampton	43°42'30"N;79°45'W	12,320±360	Terasmae and Matthews 1980
Ontario, Clarksburg	44°32'02"N; 80°30'W	>36,000; >35,000; 31,500±1,000	Warner et al. 1988
Ontario, Essex	42°05'17"N; 82°38'15"W	13,225±200; 13,150±100	Morris et al. 1993
Ontario, Gage Street Bog	43°30'N; 81°W	13,000-12,500	Schwert et al. 1985
Ontario, Hudson Bay	54°19'20"N; 84°33'30"W	8,000-6,500; 6,500-2,870±90	McAndrews et al. 1982
Ontario, Lake Erie	42°30'N; 80°30'W[b]	13,360±100	Warner and Barnett 1986
Ontario, Manitoulin Is.	46°56'N; 81°55'W	9,930±90	Warner et al. 1984
Ontario, Scarborough Bluffs	43°40'00"N; 79°20'00"W	>80,000[d]	P.J.H. Richard, personal communication

Ontario, Toronto	43°39'N; 79°20'W[b]	28,000-24,000	Berti 1975
Ontario, Toronto	43°39'N; 79°20'W[b]	28,300±600	Berti 1975
Ontario, Toronto	43°39'N; 79°20'W[b]	32,000±690	Berti 1975
Ontario, Toronto	43°39'N; 79°20'W[b]	48,000-36,000	Berti 1975
Ontario, Toronto	43°39'N; 79°20'W[b]	>53,000	Berti 1975
Pennsylvania, Longswamp	40°29'N; 75°40'W	12,400-12,200	Watts 1979
Pennsylvania, Tannersville Bog	41°02'N; 75°16'W	>13,300	Watts 1979
Québec, Chaudière River	45°55'N; 70°45'W	Mid-Wisconsinan	Matthews et al. 1987
Québec, Couchepaganiche	48°21'43"N; 71°50'49"W	>8,386	P.J.H. Richard, pers. comm.
Québec, Desautels	49°27'28"N; 73°15'00"W	>8,983	P.J.H. Richard, pers. comm.
Québec, Iles Charles	62°40'00"N; 74°15'00"W	not available	P.J.H. Richard, pers. comm.
Québec, Lac à Euloge	49°14'45"N;65°22'20"W	>9,730-7,400	Marcoux and Richard 1995
Québec, Lac à la Fourche	47°58'30"N; 69°12'28"W	10,200-9,200±160	Richard et al. 1992
Québec, Lac Leonard	49°12'22"N;65°48'45"W	9,040±140	Labelle and Richard 1984
Québec, Lac Bromont	45°15'53"N; 72°40'12"W	>10,946	P.J.H. Richard, pers. comm.
Québec, Lac Caribou	48°11'52"N; 64°56'24"W	>9,591	Jetté and Richard 1992 & P.J.H. Richard, pers. comm.
Québec, Lac de la Montagne ronde	48°19'15"N;68°33'55"W	>9,630±780-9,220±150	Richard and Larouche 1994

Québec, Lac du Diable	48°54'39"N; 66°07'30" W	>8,971-6,543	P.J.H. Richard, pers. comm.
Québec, Lac Gallant	48°25'05"N; 68°28'14"W	>9,730 ±110; 8,070±70	Richard and Larouche 1994
Québec, Lac j'Arrive	49°14'56"N;65°22'35"W	10,400-9,200	Marcoux and Richard 1995
Québec, Lac Memphremagog	45°00'00"N; 73°20'00"W	not available	P.J.H. Richard, pers. comm.
Québec, Lac Mimi	47°29'49"N;70°22'33"W	not available	P.J.H. Richard, pers. comm.
Québec, Lac Turcotte	49°09'30"N;65°45'45"	10,360±170	Labelle and Richard 1984
Québec, Petite Vallée	49°12'47"N; 65°02'12"W	not available	P.J.H. Richard, pers. comm.
Québec, Rivière Foucault	62°07'00"N; 75°45'00"W	not available	P.J.H. Richard, pers. comm.
Québec, Site Hinchinbrook	45°00'51"N; 74°04'08"W	10,480±140	Delage et al. 1985
Québec, St. Eugene	47°04'20"N; 70°19'40"W	11,500±130	Mott et al. 1981
Québec, St-Joseph de la rive	47°28'10"N; 70°20'09"W	not available	P.J.H. Richard, pers. comm.
Québec, Témiscamingue	47°04'35"N; 78°47'00"W	9,530	Richard and Larouche 1989
Vermont, Columbia Bridge site	44°50'50"N; 70°38'30"W	11,540±110-11,390±115	Miller and Thompson 1979
Yukon, Coastal Plain	69°04'30"N; 137°51'W	14,400± 180	Rampton 1982
Yukon, Northern Coast	69°36'14"N; 140°36'12"W	10,900 ± 80	Matthews 1975
Yukon, Northern Interior	65°34'30"N; 135°30'W	36,900±300	Hughes et al. 1981

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Ontario, Eighteen Mile River	44°N; 81°W[b]	10,600±160	Karrow et al. 1975
Ontario, Gage Street Bog	43°30'N; 80°30'W[b]	13,000-12,500	Schwert et al. 1985
Ontario, Marathon	48°N; 86°W	8,310 ±100	Bajc et al. 1986
Quebéc, Mont St-Hilaire	45°31'48"N; 73°08'30"W	10,100±150	Mott et al. 1981
Vermont, Columbia Bridge site	44°50'50"N; 70°38'30"W	11,540±110-11,390±115	Miller and Thompson 1979

***Dryas* sp.**

Greenland, Kap København	82°27'N; 20°45'W	Last interglacial[e]	Fredskild and Røen 1982
Maine, Gould Pond	44°59'N; 69°19'W	>13,280±410-10,670±140	Anderson et al. 1992
Minnesota, Kylen Lake	47°21'N; 91°48'W	15,850±240-12,390±120	Birks 1981
Minnesota, Wolf Creek	46°07'N; 94°07'W	20,500-14,700	Birks 1976
New Brunswick, Pine Ridge Pond	45°33'N; 67°05'W	>11,490±80-9,500±100	Levesque et al. 1994
NWT, Banks Is.	71°57'N; 125°40'W	730,000	Matthews et al. 1986
NWT, Beaver Pond	78°30'N; 82°15'W[b]	Pliocene (2-5 MyBP)	Matthews and Ovenden 1990

NWT, Bluefish Section B	67°23.1'N; 140°21.7'W	Pliocene (2.5-3.0 MyBP)	Matthews and Ovenden 1990
NWT, Ch'ijee's Bluff 2	67°28'N; 139°54'W	Pliocene (2 MyBP)	Matthews and Ovenden 1990
NWT, Fish Creek	70°16'N; 152°01'W	Quaternary (1-2 MyBP)	Matthews and Ovenden 1990
NWT, Meighen Is.	80°N;100°W[b]	Pliocene (2-5 MyBP)	Matthews and Ovenden 1990
NWT, Niguanak	69°49.3'N; 143°05.2'W	Pliocene (2.5-3.0 MyBP)	Matthews and Ovenden 1990
NWT, Prince Patrick	76°N;120°W[b]	Pliocene (2-5 MyBP)	Matthews and Ovenden 1990
NWT, Wolf Valley	79°33'N; 82°40'W[b]	Quaternary (1-2 MyBP)	Matthews and Ovenden 1990
Ontario, Gage Street Bog	43°30'N; 80°30'W[b]	13,000-12,500	Schwert et al. 1985
Ontario, Lake Six	48°24'N; 81°19'W	7,500-7,100	Liu 1990
Québec, Lac Caribou	48°11'52"N;64°56'24"W	>10,000 ±400	Jetté and Richard 1992

[a]Age based on radiocarbon dating method (with estimate of error when available), or on estimations from stratigraphic evidence. The symbols ">" and "<" mean "older than" and "younger than" respectively, depending on whether the fossils were found below or above the dated horizon.

[b]Approximate coordinates.

[c]"Preglacial" refers to before the last glacial age which started around 1.8 to 2.4 million years before the present.

[d]Date disputed.

[e]The last interglacial lasted from 130,000 to 65,000 years before the present.

APPENDIX D

List of All Sampled Individuals Studied and their Corresponding Haplotype

Individual[a]	Haplotype	Individual	Haplotype	Individual	Haplotype
1-10	A	40-1	H	58a-4	K
1-18	A	40-4	O	58a-7	K
2-3	B	41-7	D	59-3	D
2-7	B	41-8	K	59-4	B
7-19	C	42-3	H	65-1	K
7-21	C	42-5	A	65-4	A
10-1	B^b	43-7	D^b	68f-4	R^b
10-3	D	44-4	P^b	68f-5	E
16-3	E	44-8	I	71-1	A
16-4	K	46-2	K	71-4	A
19-11	K	46-5	D	88a-11	H
19-8	E	48-1	A	95-1	A
20-2	A	48-2	A	95-3	A
20-4	A	49-4	B	98a1	L
23-4	G	49-6	K	98a-2	Q
23-7	M	50-6	E	101-1	K
28-1	E^b	50-7	E	101-2	K
28-5	N	51-1	D	102-3	A
30-4	B^b	51-2	H	103-1	S
30-5	B	53-4	B^b	103-2	S
32a-3	E	53-5	E	106a-1	T
32a-5	E	54-5	H	106a-2	P

35a-5	K	54-9	K	108c-1	L
35a-6	K	55-1	C	108c-2	U
36-1	A[b]	55-3	C	112b-1	V
37-5	A	57-5	H[b]		
37-7	A	57-9	A		

[a]The first number corresponds to the population (see Table 1) and the second number corresponds to an individual within that population.

[b]Individuals with incomplete haplotype characterization (because of missing data for one or two polymorphic sections). In these cases an individual was assigned the most closely related haplotype.