FROM FOREST TO TUNDRA: HISTORICAL BIOGEOGRAPHY, FLORISTIC DIVERSITY AND NUCLEOTIDE VARIATON IN BALSAM POPLAR

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A

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Abstract

The North America boreal forest extends across more than 10° of latitude from central Labrador to interior Alaska. Periods of major climate fluctuations, including glacial and interglacial cycles, drove major migrations in the Quaternary history of the boreal forest. Beringia, the unglaciated region between the Lena and Mackenzie rivers, is recognized as an important refugium for arctic plants during the last ice age, but its role for boreal trees remains controversial. The paleobotanical record indicates *Populus balsamifera* (balsam poplar) survived within Beringia during the last glacial. My research employed an interdisciplinary approach, combining techniques in the fields of ecology, evolution and population genetics, to reconstruct the late Quaternary migration history of balsam poplar and to describe and classify balsam poplar plant communities in the Alaskan Arctic.

Chapter 1 describes the motivation for the research. Chapter 2 addresses whether a demographically-detectable population of balsam poplar was present within Beringia during the most recent ice age. I found that patterns of variation in chloroplast DNA are most consistent with the presence of a single population of balsam poplar south of the continental ice sheets through the Late Quaternary. Chapter 3 is an analysis of floristic diversity in balsam poplar communities across the Arctic Slope, Interior Alaska and the Yukon Territory and asks whether one balsam poplar-associated plant community spans the arctic and boreal regions, or if these communities differ. I found that arctic communities are dominated by arctic-alpine taxa, whereas boreal communities are dominated by boreal taxa. A strong linkage between climate and the occurrence of balsam poplar also was observed on the Arctic Slope. Chapter 4 is a study of nucleotide diversity in three nuclear loci across the range of balsam poplar. This was the first study to document geographic structure in genetic variation within the species. It also showed that diversity in three North American poplars (*P. balsamifera*, *P. deltoides* and *P. trichocarpa*) was substantially less than that of three Eurasian poplars (*P. alba*, *P. nigra* and *P. tremula*). Chapter 5 summarizes the research and points toward future research directions.

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CHAPTER ONE: HISTORICAL PERSPECTIVE AND OVERVIEW OF RESEARCH

The present-day distributions of high latitude plants were significantly influenced by the advance and retreat of ice sheets during the Quaternary (2 Ma or millions of years before present). Early biogeographers, notably Darwin (1859), believed the entire circumpolar region was ice-covered during Pleistocene glaciations and survival of arctic and boreal plants depended upon migration southward, ahead of advancing ice sheets, to southern refugia. Dawson (1894), however, noting the shallow depths of the Bering and Chukchi Seas later proposed Alaska and Northeast Siberia remained ice-free and were joined by a "wide terrestrial plain' during the late Quaternary. The similarity between the flora and fauna of the Eurasian and American Arctic prompted other biogeographers such as Wallace (1876) to support Dawson's hypothesis and argue for a land bridge between the two regions. Hultén in his classic analysis (1937) proposed the term, "Beringia" for the land bridge and suggested it served as a migration route and northern refugium for arctic and boreal plants and animals. Although the role of Beringia as an ice-age refugium for arctic herbs and shrubs has been accepted since then, it is unclear whether this region also served as a refugium for boreal trees and shrubs through the last glacial maximum (*c.* 28-15 thousand calibrated years before present, hereafter referred to as Ka) (Hopkins *et al.* 1981, Brubaker *et al.* 2005).

Balsam poplar, *Populus balsamifera* L. (Salicaceae), is a common forest tree throughout boreal North America. In addition, it occurs in isolated stands in the otherwise treeless Alaskan Arctic and is reported from the Chukotka Peninsula in northeast Siberia (Katenin 1980). These stands are thought to support range disjunctions of boreal plant species and are described as biodiversity hotspots (Walker *et al.* 2000). The plant biomass at these sites may be orders of magnitude greater than the surrounding vegetation and is vital to wildlife such as moose and the locally rare Siberian Tit. The occurrence of these communities has intrigued ecologists since they were first described in the literature over twenty years ago (Walker *et al.* 2000). Contrasting hypotheses suggest these populations either: 1) persist from an earlier late-glacial landscape, 2) migrated from western Canada during the early Holocene, 11.5-8.5 Ka (thousands of years before present), or 3) originate from recent and continual colonization events from the southern taiga (Ritchie 1984, Mann *et al.* 2002, Oswald *et al.* 2003, Williams *et al.* 2004). Fossil evidence suggests balsam poplar survived within a northern refugium through the late Pleistocene (50 Ka) (Murray 1980, Hopkins *et al.* 1981, Brubaker *et al.* 2005) causing several authors to suggest that northern populations persist from an earlier late-glacial landscape. Murray (1980) writes:

> *It is very tempting to assign relict status to the some of the well developed balsam poplar stands, since they would so satisfactorily supply the source from which migrants could have reached the Mackenzie River Delta in late glacial and early Holocene (10 Ka) time, prior to the arrival of spruce from the southern ice-free corridor.*

Furthermore, Hopkins *et al.* (1981) writes:

> *Small groves of balsam poplar are found today far beyond the limits of spruce, growing on deeply thawed ground along riverbanks and near springs and seepages. Riparian sites must always have been present, and if threshold amounts of summer warmth were available, as they seemingly must have been in highly continental Beringia, then it is hard to imagine that balsam poplar would have ever become extinct.*

In my dissertation research, I set out to survey levels of genetic variation and population differentiation in chloroplast DNA to reconstruct the late Quaternary history of balsam poplar across its range in North America and to describe and classify balsam poplar plant communities in the Alaskan Arctic. I also compare estimates of baseline population genetic parameters of nuclear loci with studies of other poplar species in North American and Europe. I therefore employed an interdisciplinary approach combining techniques in the fields of ecology, evolution and population genetics in my research. The material presented herein is organized in three main data chapters that examine historical biogeography, floristic diversity and nucleotide variation in balsam poplar.

In Chapter 2, I assess patterns of variation in chloroplast DNA across the range of balsam poplar to determine whether there is a demographic signal of survival of this tree species survived in Beringia through the Last Glacial Maximum (LGM; *c.* 29-19 Ka). I present results from analyses designed to detect whether distinct allele frequency or genealogical and demographic patterns indicate prolonged independent population histories, as would be expected if balsam poplar survived the LGM both within Beringia and south of the continental ice sheets.

In Chapter 3, I describe balsam poplar plant communities on the Arctic Slope, interior Alaska and Yukon Territory. I use the Braun-Blanquét sorted-table method to classify balsam poplar communities and nonmetric multidimensional scaling (NMDS) to identify the ecological gradients underlying community differentiation. In addition, I present a map of balsam poplar occurrences in arctic Alaska compiled from the literature, herbarium records and arctic scientists' personal observations.

In Chapter 4, I present results from a survey of nucleotide sequence variation in balsam poplar of three nuclear loci and compare estimates of baseline population genetic parameters of these loci with studies of other poplar species, particularly European aspen (*P. tremula*).

In the final Chapter, I summarize the results of the studies presented herein and discuss the broader implications of my findings.

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CHAPTER TWO: USING CHLOROPLAST DNA TO RECONSTRUCT THE LATE PLEISTOCENCE AND HOLOCENE HISTORY OF BALSAM POPLAR (*Populus balsamifera L.*) IN NORTH AMERICA[1]

2.1 Abstract

Aim: Beringia, the unglaciated region encompassing the former Bering Land Bridge and the land between the Lena and Mackenzie rivers, is recognized as an important refugium for arctic plants during the last ice age. Beringia's role as a refugium specifically for boreal trees, however, remains controversial. Here, we assess patterns of variation in chloroplast DNA across the range of balsam poplar (*Populus balsamifera* L.) to determine whether this tree species survived in Beringia through the Last Glacial Maximum (LGM; *c.* 29-19,000 calibrated yr BP).

Location: North America.

Methods: We sequenced three variable non-coding regions of the chloroplast genome (cpDNA) from 40 widely distributed populations of balsam poplar. Our analyses were designed to detect whether distinct allele frequency or genealogical and demographic patterns indicate prolonged independent population histories, as would be expected if balsam poplar survived the LGM both within Beringia and south of the continental ice sheets.

Results: Levels of regional allelic diversity were consistently greater for populations in the South ($\theta_W = 0.00122$) than in the Central ($\theta_W = 0.00086$) or Northern ($\theta_W = 0.00034$)

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[1] Breen, A. L., Murray, D. A. & Olson, M. S. Using chloroplast DNA to reconstruct the Late Pleistocene and Holocene history of balsam poplar (*Populus balsamifera* L.) in North America. Submitted to *Journal of Biogeography*

regions of balsam poplar's distribution, and diversity decreased significantly with increasing latitude ($R^2 = 0.49$, $P < 0.01$). The cpDNA genealogy was shallow, showing an absence of highly differentiated chloroplast haplotypes. Patterns of the frequencies of rare alleles, as estimated by Tajima's *D*, were complex. Northern populations harbored few rare alleles, whereas Southern and Central populations harbored significantly higher frequencies of rare alleles than expected by chance.

Main conclusions: Patterns of variation in chloroplast DNA are most consistent with the presence of a single population of balsam poplar south of the continental ice sheets through the Late Quaternary that migrated north during the Holocene to colonize Alaska and northwestern Canada. We cannot, however, statistically dismiss the possibility northern populations also persisted *in situ* within Beringia through the LGM. Coalescent analyses suggest historical divergence occurred between Southern and Northern populations of this tree species in response to ice age conditions and events. Nonetheless, if balsam poplar did persist within Beringia, the refugium was not a significant reservoir of cpDNA diversity.

2.2 Introduction

The North American boreal forest spans the continent, encompassing > 10° latitude extending from interior Alaska eastward through Canada to central Labrador. Major climate fluctuations, including the glacial and interglacial intervals of the ice ages, altered the latitudinal and longitudinal extent and floristic composition of the boreal forest. During the last interglacial (Sangamon or Marine Isotype Stage 5, *c.* 130,000 years before present, hereafter yr BP; Muhs *et al.*, 2001), the northern boreal forests were more extensive than today, perhaps even approaching the arctic coast of Alaska but certainly into areas north of the Brooks Range that are today arctic tundra (Hopkins *et al.*, 1981). During the Last Glacial Maximum (LGM; 29-19,000 calibrated yr BP; Elias & Crocker, 2008), however, ice sheets covered the present-day boreal region with the exception of Beringia, the area between the Lena and Mackenzie rivers where ice sheets did not form thus providing a refugium for biota throughout the Pleistocene (Fig. 2.1). While a component of the Sangamon flora persisted within the Beringian refugium throughout glacial intervals, elsewhere in glaciated North America the flora was extirpated or displaced south of the continental ice margins and thus what we see today is entirely postglacial (Chapin *et al.*, 2006). Elements of the forest then migrated northward when the climate warmed and glaciers receded. The role of Beringia as a northern glacial refugium for arctic flora has long been accepted since Hultén's classic analysis (Hultén, 1937). It is unclear, however, whether boreal trees persisted through the LGM within Beringia and served as a source for the reconstitution for the present-day Alaskan and Canadian boreal forests.

Pollen and macrofossil remains in sediments of LGM and post-glacial age yield ambiguous results as to whether boreal trees from the Last Interglacial survived *in situ* within Beringia. Brubaker *et al.* (2005) hypothesized that forest refugia were present on the Bering Land Bridge during the LGM. Trace amounts of tree pollen in the LGM records have been interpreted as representing distant sources while others have thought LGM trees could have occurred in gallery forests along streams, similar to boreal tree populations observed north of treeline in North America today. Pollen and macrofossil records are difficult to obtain from either the Land Bridge, now under water, or riparian sites where sediments have been eroded and re-deposited. In addition, small populations of trees are unlikely to produce sufficient pollen to be observed in palynological samples. For example, in northern Alaska today *Populus balsamifera* (balsam poplar) occurs in isolated stands well beyond latitudinal treeline, adjacent to spring-fed rivers (Bockheim *et al.*, 2003). A study of surface pollen samples collected at increasing distance from one of these balsam polar stands showed the frequency of poplar pollen was greatest within the stand (70%) and then sharply declined to < 5 % within 100 m of the stand (Edwards & Dunwiddie, 1985). Balsam poplar surface pollen samples in the boreal forest show a similar trend (Lee *et al.*, 1996). Pollen remains from lake sediments, therefore, are inadequate for assessing temporal-spatial patterns for under-represented taxa such as balsam poplar, whose pollen preserves poorly. Palynology is a 'blunt instrument' for reconstructing past vegetation (Colinvaux, 1967; Birks & Birks, 2000). Interpretations of the pollen record for the LGM in eastern Beringia (Alaska and western Yukon and northwesternmost Northwest Territories) therefore suggest the landscape was some combination of polar desert, mesic tundra, and tundra-steppe, but in any case treeless (Elias & Crocker, 2008).

Analysis of DNA sequence variation from living plants provides a method independent of pollen and macrofossils to examine long-standing paleoecological questions such as whether Beringia harbored populations of boreal tree taxa through the LGM. Glacial refugia have been inferred in a phylogenetic context by detecting regions of high allelic diversity for neutral alleles in a variety of plants (Hewitt, 1996). Theoretical studies predict that populations having survived glaciations within a refugium will have retained higher genetic variation and harbor more unique alleles relative to recently colonized localities. This is because populations established through colonization by short and long distance dispersal tend to be genetically depauperate due to founder effects and subsequent genetic drift (Jansson and Dynesius, 2002). That is, an expanding range front is expected to harbor only a subset of a species overall genetic diversity (Excoffier *et al.*, 2009). Genetic studies show that populations of various boreal tree species, including *Picea glauca* (white spruce; Anderson *et al.*, 2006), *Pinus banksiana* (jack pine; Godbout *et al.*, 2005) and *Picea mariana* (black spruce; Jaramillo-Correa *et al.*, 2004), survived the LGM adjacent to the southern boundaries of the Laurentide and Cordilleran ice sheets in North America. Of these taxa, only the extant ranges of white and black spruce include Beringia. To date, a chloroplast DNA (cpDNA) survey of white spruce is the only molecular study to provide support for tree populations surviving the LGM within Beringia (Anderson *et al.*, 2006). This study found unique cpDNA haplotypes and high neutral allelic diversity in Alaskan populations that are consistent with persistence in a glacial refugium. Testing refugial hypotheses, however, is complex because variation in allele frequencies across the geographic range of any species can result from a variety of historical scenarios (Nielsen & Beaumont, 2009). For example, refugia comprised of few individuals can harbor low levels of diversity because of low effective population size. If subsequent demographic changes include large-scale immigration and admixtures from other persisting populations, the genetic consequence of refugial existence can be lost. Moreover, the period of isolation may be too short for the generation of distinct polymorphisms or lineage sorting; therefore, in such cases diversity statistics cannot identify refugia.

Here, we assess patterns of variation in maternally inherited cpDNA across the geographic range of *Populus balsamifera* L. to investigate its history during the Late Pleistocene and Holocene. *Populus* first becomes a consistent component of Late Quaternary Beringian pollen assemblages 18,000 yr BP, prior to large-scale glacial retreat in North America (Fig. 2.1; Williams *et al.*, 2004; Brubaker *et al.*, 2005). *Populus* fossil pollen cannot be separated at the species level, so it is unknown whether the pollen is derived from balsam poplar or its congener *Populus tremuloides*, which is also widespread today in boreal Alaska. The earliest definitive evidence for the presence of balsam poplar in Alaska is based on dated macrofossils, is 12-9,000 yr BP (Hopkins *et al.*, 1981; Mann *et al.*, 2002). Persistence of small populations of balsam poplar through the LGM, analogous to the isolated stands found in the Arctic today, would provide the source for poplar pollen 18,000 yr BP in the paleobotanical record (Williams *et al.*, 2004; Brubaker *et al.*, 2005). The authors of these works stress that the belief *Populus* was present within Beringia 18,000 yr BP should be considered a working hypothesis that invites testing by future research as these data rely largely on low accuracy conventional bulk dating of sediments (Gove, 1992).

The presence of balsam poplar within Beringia at the early date of 18,000 yr BP is difficult to explain by rapid post-glacial dispersal from a southern refugium since the massive Laurentide and Cordilleran ice sheets were intact and formed a significant barrier to terrestrial migration by a standard stepping-stone model. There has been a long-standing debate in the scientific community as to whether plant propagules have the capacity to disperse over long distances such as across large oceans or ice sheets. A recent molecular study in the Arctic, however, showed long-distance plant colonization to the remote archipelago of Svalbard has occurred repeatedly and from several distant source regions (Alsos *et al.*, 2007). The authors of this work suggest propagules were likely dispersed by wind and drifting sea ice. Wind may be an especially efficient long-distance vector in the Arctic due to extensive snow and ice cover, open landscapes and strong air currents; an idea championed by D. B. O. Savile (1972). This scenario in the Arctic is analogous to the late-glacial landscape of North America 18,000 yr BP. The identification of wind as a probable long-distance vector, together with recent theoretical developments in modeling plant migration, support the occurrence of rare long-distance dispersal events. The likelihood of balsam poplar colonization of Beringia from a source at the southern margin of the continental ice sheets is far greater than previously realized (Cain *et al.,* 2003; Nathan, 2005; Nathan, 2006; Bullock & Nathan, 2008).

It should be mentioned that current taxonomy (cf. Furlow, 1997) links Asian and American alder (*Alnus fruticosa* Ruprecht) and tree birch (*Betula neoalaskana* Sargent), which makes probable that the earliest occurrences for both taxa in Alaska were derived from late glacial migrants along the southern edge of the land bridge. For *Populus*, however, the closest Asiatic candidate is *P. suaveolens* Fischer, which is not a close relative to *P. balsamifera*, and thus an Asian origin is doubtful.

For this study, we sequenced three variable non-coding regions of chloroplast DNA (cpDNA) in balsam poplar from 40 populations distributed across its entire range. Because the chloroplast genome is maternally inherited and is non-recombining, geographic patterns of neutral genetic variation are only influenced by seed dispersal (Mejnartowicz, 1991). Thus, these patterns are likely to harbor migration-related signals unaffected by past and present gene flow through pollen dispersal (McCauley, 1994). We chose to survey cpDNA because it has potential for greater population structure than the biparentally inherited nuclear genome that is influenced by both seed and pollen dispersal (Hu *et al.*, 2009). Our goal was to test the hypothesis that balsam poplar survived the LGM in two regions, one within Beringia and the other south of the continental ice sheets. Alternatively, balsam poplar may have been restricted to a single southern population and then migrated northward to Alaska following deglaciation. Our analysis addresses whether distinct allele frequency or genealogical and demographic differences exist between widely disjunct populations of balsam polar growing today at its southern and northern limits of distribution and whether these differences indicate prolonged independent histories, as would be expected if balsam poplar survived the LGM both within Beringia and south of the continental ice sheets.

2.3 Methods

2.3.1 Sampling and cpDNA sequencing

Balsam poplar is a fast-growing deciduous tree that is common throughout the boreal region of North America (Fig. 2.2; Little, 1971; U. S. Geological Survey, 2006).

Balsam poplar has also been reported from two localities on the Chukotka Peninsula in far northeastern Russia where it grows as a prostrate shrub (Katenin, 1980). Balsam poplar is a clonal, early-successional species that grows today primarily on floodplains. It is dioecious, wind pollinated, and produces tiny seeds that are wind dispersed. Poplar seeds are short-lived, typically viable less than a single growing season (Stanton & Villar, 1996). The ideal plant for most successful dispersal would be one with self-compatible, bisexual flowers. *Populus,* by virtue of the very large number of plumed seeds produced and clonal habit, compensates for obstacles to colonization by dioecious plants. Poplar has emerged as a model tree system with the recent release of the complete genome of *Populus trichocarpa* (Tuskan *et al.*, 2006).

Leaf or bud tissues were collected from two to five individuals from each of 40 populations of balsam poplar from Prince Edward Island westward, through Canada to Alaska (Table 2.1, Fig. 2.2). Twelve populations were derived from the AgCanBaP collection of the Agriculture and Agri-Food Canada, Agroforestry Division, Indian Head, Canada (Soolanayakanahally *et al.*, 2009). We recognize three regions across the contemporary range of balsam poplar: 1) "Northern," the ice-free area of Beringia within Alaska and northwestern Canada, 2) "Central," the region covered by continental ice sheets in central North America during the LGM, and 3) "Southern," the region below 50° N at the southernmost distribution of balsam poplar. Although parts of the southern region were also glaciated, if there were populations of balsam poplar at the southern margin of the ice sheets as indicated by pollen records, then we would expect the descendents of these populations to be located today near the southern extent of its present-day range. We also sampled western black cottonwood (*Populus trichocarpa*), eastern cottonwood (*Populus deltoides*), and quaking aspen (*Populus tremuloides*) to determine whether populations at the margins of balsam poplar's distribution were influenced by introgression from these closely related taxa (Hamzeh & Dayanandan, 2004). Sampled trees were separated by at least 15 m within each population. Our data from autosomal loci showed trees sampled in this manner differ in their multi-locus genotypes, indicating this distance was sufficient to sample distinct genets (Breen *et al.*, 2009). Specimens were stored at -80° C until DNA extraction at the University of Alaska Fairbanks.

We surveyed three variable non-coding regions of the balsam poplar chloroplast genome: 1) *trn*L intron: a group I intron within the *trnL* gene, 2) *trn*L-*trn*F spacer: intergenic spacer regions between the *trnL* and *trnF* genes, and 3) *rpl20-rps12* spacer: intergenic spacer between the ribosomal protein genes *S12* and *L20* (Taberlet *et al.*, 1991; Hamilton, 1999). Genomic DNA was extracted from frozen leaf or bud tissue with DNeasy Plant Extraction kits (Qiagen, Valencia, CA, USA). To generate PCR products, we used the universal primers of Taberlet *et al.* (1991) to amplify the *trn*L-*trn*F spacer (primers ucp-e and ucp-f) and the trn-L intron (ucp-c and ucp-d). The *rpl20-12* intergenic spacer was amplified using *P. trichocarpa* specific variations of the primers rpl20 (5'-TTT GTT CTA CGC CTC CGA GC-3') and rps12 (5'-CCT AGT ACA TGT CCC TCG GC-3') of Hamilton (1999) based on the chloroplast genome of *Populus trichocarpa* available at http://genome.jgi-psf.org/.

We generated bidirectional sequence data from PCR fragments of the three chloroplast regions. These regions were amplified using TaKaRa Ex Taq polymerase (Takara Bio Inc., Madison, WI, USA) and column purified (Qiagen, Valencia, CA, USA). PCR products were sequenced using the above primers and BigDye Terminator Cycle Sequencing chemistry (v. 3.1; Applied Biosystems, Foster City, CA, USA) on an ABI3100 Genetic Analyzer.

2.3.2 Analyses

We used Aligner (v. 2.0.5, CodonCode Corporation, Dedham, MA, USA) to curate and trim sequences based on Phred quality scores. Sequence ends were trimmed until the average quality value was Phred > 25 in a 10 base pair (bp) window. We visually inspected sequence chromatograms and aligned bidirectional sequences separately for each chloroplast region for all trees sampled using Sequencher (v. 4.7, Gene Codes, Ann Arbor, MI, USA). Mononucleotide repeats and associated insertion/deletion polymorphisms (indels) in our data set resulted in a high quality (Phred > 30) chromatogram in one direction up to the repeat and in a lower quality chromatogram thereafter for some chloroplast regions for select trees. For these sequences, base calls in the flanking regions were then determined by Phred > 30 scores from a single directional read. We changed to 'missing data.' the base calls of difficult to resolve mononucleotide repeats and indel regions. The sequence data from the three regions of the chloroplast genome were concatenated to derive alleles. For each individual tree, we aligned 1,828 bp including 691, 314 and 823 bp for the *trn*L intron and *trn*L-*trn*F and *rpl20-rps12* spacers, respectively. All sequences were deposited in the EMBL/GenBank nucleotide sequence database.

Levels of regional genetic variation were estimated by the number of segregating sites (*S*), haplotypes (h), haplotype diversity (H_d) and Watterson's and Tajima's estimators θ_W and π per site, respectively (Watterson, 1975; Tajima, 1989) in DnaSp (v. 5.0, Rozas *et al.*, 2003). To infer the colonization history of balsam poplar, we plotted latitude versus θ_W calculated for each population from the observed data using linear regression implemented in JMP (v. 7, SAS Institute, Cary, NC, USA). Under a stepping stone model of northern migration from populations south of the continental ice sheets, we expect a significantly negative relationship between diversity and increasing latitude. We present these data including only populations harboring greater than one haplotype ($\theta_W > 0$). We exclude populations fixed for a single haplotype because including zeros violates the assumption of normality for linear regression. To examine the signature of alternative hypotheses on tree topology and to determine the extent of introgression among *Populus* species, we constructed a statistical parsimony network using a median-joining algorithm within the program Network (v. 4.510, http://www.fluxus-engineering.com; Bandelt *et al.*, 1999).

To assess changes in population size as expected with colonization from refugia, we estimated Tajima's *D* and Fu & Li's *F* to test for departure from a model of constant population size using coalescent simulations in DnaSP (Tajima, 1989; Fu & Li, 1993). To calculate Fu & Li's *F*, we used our sequence data from *P. deltoides* as an outgroup.

The associated one-tailed *P*-values were obtained by computing 10,000 simulations based on the number of segregating sites assuming no recombination. We also tested for population expansion by generating mismatch distributions (Rogers & Harpending, 1992) in the program Arlequin (v. 3.11, Excoffier *et al.*, 1992; Schneider *et al.*, 2000). The raggedness statistic was used to evaluate the fit of the observed mismatch distribution to an expected distribution modeled via 10,000 parametric bootstrap replicates using a hypothesis of sudden population expansion (Harpending, 1994). If the model of sudden expansion was supported, we then approximated the timing (t) of the inferred expansion using the estimated parameter τ ($\tau = 2\mu t$; where μ is the mutation rate for the locus and t is the number of generations since the expansion). Because the mutation rate for the *Populus* chloroplast genome is unknown, we used the substitution rate for noncoding cpDNA from *Arabidopsis* (2.9×10^{-9} substitutions per site per year; Säll *et al.,* 2003;) to approximate the mutation rate for our data. This is an imprecise estimate, yet it falls within the range of the synonymous substitution rates most commonly cited for angiosperm cpDNA (Wolfe *et al.*, 1987; $1.1 - 2.9 \times 10^{-9}$ substitutions per site per year) and allows us to approximate the historic context of demographic events. We assumed a 15 year generation time (Ingvarsson, 2008). Therefore, for 1828 bp, $\mu = 7.95 \times 10^{-5}$ substitutions per locus per generation ($\mu = 2.9 \times 10^{-9}$ substitutions per site per year * 1828 sites per locus * 15 years per generation).

We examined patterns of genetic subdivision between regions of balsam poplar's current distribution by an analysis of molecular variance (AMOVA) implemented in the Arlequin software package (v. 3.11, Excoffier *et al.*, 1992; Schneider *et al.*, 2000). We used this method to partition the genetic variance among the Southern, Central and Northern regions of balsam poplar's range (F_{CT}), among populations within regions (F_{SC}) and among populations (F_{ST}). Significance levels were determined using uncorrected pairwise differences between haplotypes through 10,000 random permutation replicates.

2.4 Results

In total, 15 distinct cpDNA haplotypes were detected among the four *Populus* species surveyed (Figs. 2.2 & 2.3). We observed 13 haplotypes in *P. balsamifera* (A-M), and 1-3 haplotypes in *P. deltoides* (M), *P. tremuloides* (N) and *P. trichocarpa* (A, B, O). The network diagram for the relationships among poplar chloroplast haplotypes shows *P. balsamifera* and *P. trichocarpa* haplotypes are clearly diverged from *P. deltoides* and *P. tremuloides* chloroplast haplotypes. Nine haplotypes were unique to *P. balsamifera* (C-K), and two were shared with the Alaskan population of *P. trichocarpa* (A, B). In addition, two individuals identified as *P. balsamifera*, one from Prince Edward Island (M) and the other from central Colorado (L), were more closely related to a clade that does not include balsam poplar, suggesting recent introgression from these closely related poplar species or their congeners. These two individuals, because they showed evidence of introgression based on shared sequence similarity, were removed from all subsequent analyses.

Chloroplast haplotype A was the most frequent haplotype detected throughout *P. balsamifera*'s distribution, occurring in almost every population sampled (36/40 populations; Table 2.1, Fig. 2.2). Haplotype B was the second most frequent; occurring in over half of the populations sampled (23/40 populations). Chloroplast haplotypes unique to balsam poplar (C - K) were less common and largely confined to a single region of balsam poplar's range including: Southern (E, G-I), Central (C, D) and Northern (K). The Central region shared haplotypes F and J with the Southern and Northern regions, respectively.

Levels of regional genetic variation within *P. balsamifera*, as estimated by S, h, H_d, θ_W, and π, were consistently greater for populations in the south than for those in the Central or Northern regions of balsam poplar's range (Table 2.2). For example, the Southern region exhibited levels of allelic diversity, as estimated by θ_W, over 3-fold greater than found in the Northern region ($0_W = 0.00122$, Southern; 0.00086, Central; 0.00034, Northern). Levels of variation, as estimated by π however, were less than 2-fold greater in the South than in the other regions. The Central region was the most depauperate ($\pi = 0.00096$, Southern; 0.00038, Central; 0.00055, Northern). We detected a significantly negative relationship between latitude and diversity (0_W) suggesting postglacial expansion to the north from populations south of the continental ice sheets (Fig. 2.4; $R^2 = 0.49$, $P < 0.01$).

Tajima's D and Fu & Li's F values were negative for the overall sample (Tajima's $D = -1.30$, $p = 0.07$; Fu & Li's $F = -2.43$, $p = 0.02$), and in the Central (Tajima's $D = -1.58$, $p = 0.04$; Fu & Li's $F = -2.30$, $p = 0.03$) and Southern (Tajima's $D = -0.62$, $p = 0.37$; Fu & Li's $F = -0.73$, $p = 0.27$) regions of *P. balsamifera*'s range indicating recent population expansion (Table 2.2). In the Northern region, however, both parameters were positive (Tajima's $D = 1.12$, $p = 0.14$; Fu & Li's $F = 1.08$, $p = 0.12$) showing a deficit of rare alleles. The observed mismatch distribution for the entire range and for haplotypes from the Southern and Central regions are a close fit with the expected distribution under a model of population expansion (sum of squared deviations = 0.02-0.07, $P > 0.12$; raggedness index = 0.09-0.30, $P > 0.16$ for all tests). Based on our estimate of *tau* for all sampled alleles ($\tau = 2.5$; 95% CI = 0.3-6.0) and the substitution rate for noncoding cpDNA for *Arabidopsis* (Säll *et al.,* 2003), balsam poplar began expanding approximately 235,000 yr BP at the start of the Yarmouth or Marine Isotope Stage 7 Interglacial (*c.* 186,000-245,000 yrs BP; Imbrie *et al.*, 1984). Inferences drawn from mismatch distributions are imprecise however, and at this time it is unclear whether this date represents the most influential or the most recent population expansion. The mismatch distribution for the Northern region, in contrast, significantly differs from a model of population expansion (sum of squared deviations = 0.13; $P = 0.02$, raggedness index = 0.51, $P = 0.10$), which is consistent with a population at demographic equilibrium or a recent bottleneck during immigration.

We detected low, but significant, genetic subdivision between the Southern, Central, and Northern regions of balsam poplar's range ($F_{CT} = 0.05$, $P = 0.05$). Most of the variation, however, was partitioned among populations within regions and within populations, accounting for 73% and 22% of the genetic variation in cpDNA, respectively ($F_{SC} = 0.23$, $P < 0.01$; $F_{ST} = 0.27$, $P < 0.01$).

2.5 Discussion

Beringia is recognized as an important refugium for arctic plants during the last ice age, although its role as a refugium specifically for trees remains controversial. We assessed patterns of variation in cpDNA across the range of *Populus balsamifera* to determine whether this boreal tree species survived in Beringia through the Last Glacial Maximum (LGM). Our aim was to test the hypothesis that balsam poplar survived the LGM in two disjunct localities, one within Beringia and the other south of the continental ice sheets. Alternatively, balsam poplar may have been restricted to a single southern population that migrated northward to Alaska and Canada following deglaciation.

Patterns of chloroplast nucleotide diversity in our survey are consistent with the presence of a balsam poplar population south of the continental ice sheets through the Late Quaternary. We found no definitive evidence for the occurrence of a balsam poplar glacial refugium within Beringia. If balsam poplar survived the LGM both within Beringia and south of the continental ice sheets, we expected distinct phylogroups indicative of long-term isolation during the Late Quaternary. However, we found an absence of highly differentiated chloroplast haplotypes in the north and observed predominantly two common haplotypes in Alaska. From these results, coupled with the trend in decreasing molecular diversity with increasing latitude, we infer that rare haplotypes observed in the south were lost through recurrent founder events during range expansion to the north following deglaciation. This winnowing of genetic variation may have generated the low, but significant structure, observed among regions of *P. balsamifera*'s distribution ($F_{CT} = 0.05$, $P = 0.05$).

2.5.1 cpDNA variation in P. balsamifera

The network diagram depicting the relationship among poplar chloroplast haplotypes shows *P. balsamifera* and *P. trichocarpa* are clearly diverged from *P. deltoides* and *P. tremuloides* haplotypes (Fig. 2.3). We detected shared haplotypes between *P. balsamifera* and our Alaskan population of *P. trichocarpa*, although the haplotype observed in our Washington population of *P. trichocarpa* was unique to that taxon. Given that *P. balsamifera* and *P. trichocarpa* are sister species estimated to have diverged approximately 780,000 yr BP in western North America at the beginning of the Middle Pleistocene, it is possible that these are shared ancestral haplotypes that have not yet reciprocally sorted into the *P. balsamifera* and *P. trichocarpa* lineages (N. Levsen, pers. comm.). Because *P. balsamifera* and *P. trichocarpa* are known to hybridize where their ranges overlap (Viereck and Foote, 1970), introgression also could account for the observed patterns of shared cpDNA haplotypes.

Levels of variation observed in balsam poplar were similar to those reported for other boreal tree species in North America. Haplotype number (h) and total diversity (H_d) in the chloroplast genome of *P. balsamifera* ($h = 11$, $H_d = 0.54$) is similar to that detected in maternally inherited mtDNA of *Picea mariana* ($h = 10$, $H_d = 0.56$; Jaramillo-Correa *et al.*, 2004), *Pinus banksiana* ($h = 14$, $H_d = 0.33$; Godbout *et al.*, 2005), *Pinus contorta* ssp. *contorta* ($h = 19$, $H_d = 0.79$; Godbout *et al.*, 2008) and paternally inherited cpDNA of *Picea glauca* ($h = 17$, $H_d = 0.64$; Anderson *et al.*, 2006). Average pairwise sequence diversity in *P. balsamifera* in non-coding regions of the single locus chloroplast genome ($\pi = 0.0006$) is at the low end of the range observed in the bi-parentally inherited nuclear genome (mean $\pi_{syn} = 0.0045$ for 515 nuclear loci in *P. balsamifera*; for loci with ≥ 50 synonymous sites, 27.1% (96/354) had $\pi < 0.0006$; Olson *et al.*, 2010). For neutral loci, the expected polymorphism at mutation-drift equilibrium is proportional to the effective population size (Hartl & Clark, 1997). The difference in sequence diversity between cpDNA and nuclear DNA (nDNA) for dioecious balsam poplar, in which only females transmit the chloroplast genome, can most likely be attributed to the effective population size of the chloroplast genome being approximately one quarter that of the nuclear genome. Alternatively, cpDNA and nDNA sequence diversity can differ because the mutation rate varies between the two genomes (Wolfe *et al.*, 1987), or because the chloroplast genome is physically linked, and the effects of hitchhiking may reduce diversity (Charlesworth *et al.*, 1993).

The site frequency spectrum differed among regions of balsam poplar's range indicating that trees in these areas may have different demographic histories. Estimates of Tajima's *D* and Fu & Li's *F* were elevated, but not significantly different from zero, in the North (> 1) showing a loss of rare alleles suggestive of a recent bottleneck, whereas Tajima's D was negative in the Center (< -1.5) and South (< -0.5) showing an excess of rare alleles consistent with a recent population expansion. The trend in regional estimates of Tajima's *D* is consistent with two other studies of nucleotide diversity in balsam poplar in which *D* was more positive in northern populations than southern populations (Breen *et al.*, 2009; Keller *et al.*, 2010). The site frequency spectrum results are analogous to the pattern we observed in mismatch distributions wherein the model of population expansion was rejected in the North, but supported in the Central and Southern regions.

2.5.2 Population structure in P. balsamifera

A significant level of overall population structure was detected across balsam poplar's range ($F_{ST} = 0.27$; $P < 0.01$); this was approximately five-fold greater than reported for random single nucleotide polymorphisms (SNPs) in the nuclear genome (412 SNPs, mean $F_{ST} = 0.05$; Keller *et al.*, 2010). The disparity in levels of population differentiation between the chloroplast and nuclear genomes is likely the result of differing modes of transmission and dispersal between the two genomic compartments (McCauley, 1994). Overall higher levels of dispersal in the nuclear genome are expected to decrease differentiation among populations, relative to that found in the chloroplast. Similar trends in population structure between maternally inherited mtDNA and bi-parentally inherited nDNA occurs in other North American boreal trees, including *Picea mariana* (mtDNA $G_{ST} = 0.68$, Jaramillo-Correa *et al.*, 2004; nDNA $F_{ST} = 0.03$, Isabel *et al.,* 1995) and *Pinus contorta* (mtDNA $F_{ST} = 0.66$, Dong & Wagner, 1993; nDNA $F_{ST} = 0.03$,Wheeler & Guries, 1982).

2.5.3 Coalescent Model Testing

Although the pattern of variation in cpDNA across the range of *P. balsamifera* is consistent with persistence within a single region south of the continental ice sheets through the LGM, we have not statistically excluded the possibility that balsam poplar also persisted within a Beringian refugium. Historical biogeography studies are increasingly turning to multiple types of information acquired from DNA sequence variation to address questions of where refugial populations occurred (Knowles & Maddison, 2002; Nielson & Beaumont, 2009). A common approach is to use coalescent-based models to specifically test genealogical hypotheses and estimate demographic parameters within a statistical framework (Kuhner, 2008). We tested for divergence between regions of balsam poplar's range utilizing a demographic model implemented in the program Isolation with Migration (IM), in an attempt to exclude the possibility balsam poplar persisted within a Beringian refugium.

The IM model assumes samples represent two populations that diverged in the past, but are still potentially connected via dispersal (Hey & Nielsen, 2004). We assumed balsam poplar had a continent-wide distribution, similar to its range today, for the duration of the Last Interglacial. During subsequent glaciations including the LGM, continent-wide glaciers would have formed a significant barrier dividing the larger population into two isolated populations if balsam poplar persisted both south of the ice sheets and within Beringia. We allow for secondary contact between these disjunct regions of balsam poplar's range today by estimating gene flow. The model allows estimates of effective population sizes of current and ancestral populations (N_e), the timing of divergence between populations (*t*), and migration rate between populations (*m*). If balsam poplar persisted south of the continental ice sheets and within a Beringian refugium through the LGM, we expected the model estimate for the timing of divergence between Southern and Northern populations to occur prior to the onset of the LGM (> 29,000 calibrated yr BP).

Our IM analyses were implemented on the Life Science Informatics portal at the University of Alaska Fairbanks. Multiple runs were performed using a burn-in of 1,000,000 steps. We recorded results every hour allowing the program to run until the lowest effective sample sizes (ESS) for each parameter were greater than 500. We began by assigning uninformative prior distributions to estimate appropriate priors and then carried out three independent runs with identical conditions and different random number seeds to assure convergence. We report here the results of the model with the highest ESS, which was run for over 100,000,000 steps after the initial burn-in (minimum ESS > 1,000). To approximate the timing of divergence, we assumed a Hasegawa, Kishino and Yano (HKY; Hasegawa *et al.*, 1985) mutation model and inheritance scalar of 0.25 for cpDNA, scaled parameters to the substitution rate, and converted t to time in years. We used the substitution rate indicated in the methods above ($\mu = 5.30 \times 10^{-6}$ substitutions per locus per year) and assumed a 15-year generation time (*g*; Ingvarsson, 2008).

The IM model did not converge when attempting to estimate demographic parameters for the South-Central nor the North-Central pairs of populations, but it did converge for the Southern-Northern comparison suggesting we had insufficient data to produce reliable estimates of divergence times for two of the comparisons. For the South-North comparison, the estimate of *t* did not overlap with zero, assuming a constant mutation rate comparable to that of *Arabidopsis* (Säll *et al.*, 2003). The model estimated present-day balsam poplar populations south and north of continental ice sheets began diverging in the Middle Pleistocene (peak of *t* = 316,226 yr BP; 95% CI = 135,094 - 1,244,528 yr BP) during the Marine Isotope Stage 9 Interglacial (*c.* 339,000-303,000 yr

BP; Imbrie *et al.*, 1984). The estimated population size of the southern population was six-fold greater than that for the northern population (N_{North} = 6,336; 95% CI = 3,077-18,647; N_{South} = 45,865, 95% CI = 31,381-4,613,077), but only ¼ the size of the ancestral population ($N_{Ancestral}$ = 176,219, 95% CI = 98,972-4,535,831). We estimated *M*, the effective number of female migrants per generation between Southern and Northern regions from divergence to the present, using $M = (\theta_{South} + \theta_{North})\ m / 2$, where θ for each region = $N_e * \mu * g$ (Hey & Nielson, 2004). Because we estimated a single migration parameter in the model, *M* is summed over both populations and therefore reflects the number of females that migrate between the Southern and Northern populations per generation. The posterior distribution of *m* (effective migration rate; per gene per generation) reached a maximum at 8.18 (95% CI = 2.02-32.46). Using the equations above, we converted values of *m* to estimate the effective number of migrants per generation to be approximately 17 females per generation (M = 16.97; 95% CI = 4.19-67.35 migrants per generation).

Taken at face value, the IM modeling results showing that Southern and Northern populations diverged *c.* 300,000 yr BP are suggestive of balsam poplar persistence south of the continental ice sheets and in a small, but demographically detectable, refugium within Beringia throughout the Late Quaternary. Because we were skeptical of the long divergence time, we confirmed that species-wide patterns in nucleotide diversity (π) and Tajima's *D* found in our data could be generated using neutral genealogies generated by the software program ms (Hudson, 2002). Although variance was high, it was possible to generate patterns very similar to those we observed under the model parameters estimated by IM (results not shown, but available by request). Therefore, it remains unclear whether a small population could have persisted within Beringia during the last LGM. Based on general patterns of cpDNA diversity we would conclude that Beringia was recently colonized, but we also were able to generate demographic scenarios whereby a small population could have persisted in Beringia through the last LGM. Thus, we conclude that single locus data from the cpDNA alone is insufficient for discriminating between these two hypotheses. A proper test of this hypothesis, regrettably, will require a robust statistical phylogeography approach including many loci, which is beyond the scope of the current single locus cpDNA data set.

2.5.4 Conclusions

Our findings demonstrate molecular analyses have potential to contribute toward understanding long-standing paleoecological questions such as the role of Beringia as a refugium, specifically for boreal trees. The cpDNA genealogy is shallow and lacks distinct phylogroups that would be indicative of long-term isolation during the Late Quaternary. Balsam poplar exhibits a pattern of chloroplast nucleotide diversity consistent with the presence of a single, large, widespread population south of the continental ice sheets through the Late Quaternary. We observed a loss of allelic richness along a northern route of colonization indicative of balsam poplar being a recent migrant to Alaska and northern Canada. If we accept dates provided by the pollen record, the presence of balsam poplar within Beringia 18,000 yr BP might be best explained by long-distance dispersal from the south across the massive Laurentide and Cordilleran ice sheets since an ice-free migration corridor did not open until *c.* 14,000-12,000 calibrated yr BP (Fig. 2.1; Brubaker *et al.*, 2005). This conclusion is consistent with a recent study of 11 nuclear genes that showed the nuclear genome of individuals from a single population of balsam polar in interior Alaska was strongly influenced by migration from populations in the central region of the contemporary range of balsam poplar (Keller *et al.*, 2010). The authors suggest this pattern, and differences in regional levels of diversity, are consistent with a single southern population that colonized the northern tier of balsam poplar's range following deglaciation.

We cannot discount, however, that northern populations also were re-colonized from populations that persisted *in situ* within Beringia through the LGM. Contrary to our expectations, coalescent analyses did not statistically exclude the possibility of balsam poplar having persisted within a Beringian refugium. Despite the findings of Keller *et al.* (2010), to further lend credence to, or refute alternative hypotheses for the presence or absence of a balsam poplar northern refugium, future studies designed to increase the numbers of loci and populations analyzed are required. Our study and that of Keller *et al.*'s sampled different population sets; whereas Keller *et al.* sampled seven populations for coalescent analyses, we sampled 32 populations for our IM analysis. Although there was some overlap between the two studies for sampled populations, we sampled more extensively in Alaska and along the southern edge of balsam poplar's range. In contrast, Keller *et al.* sampled only a single Alaskan population and did not include any populations from the southernmost limit of balsam poplar's distribution. Nonetheless, if balsam poplar did persist within Beringia, we can conclusively state the refugium was not a significant reservoir of cpDNA diversity.

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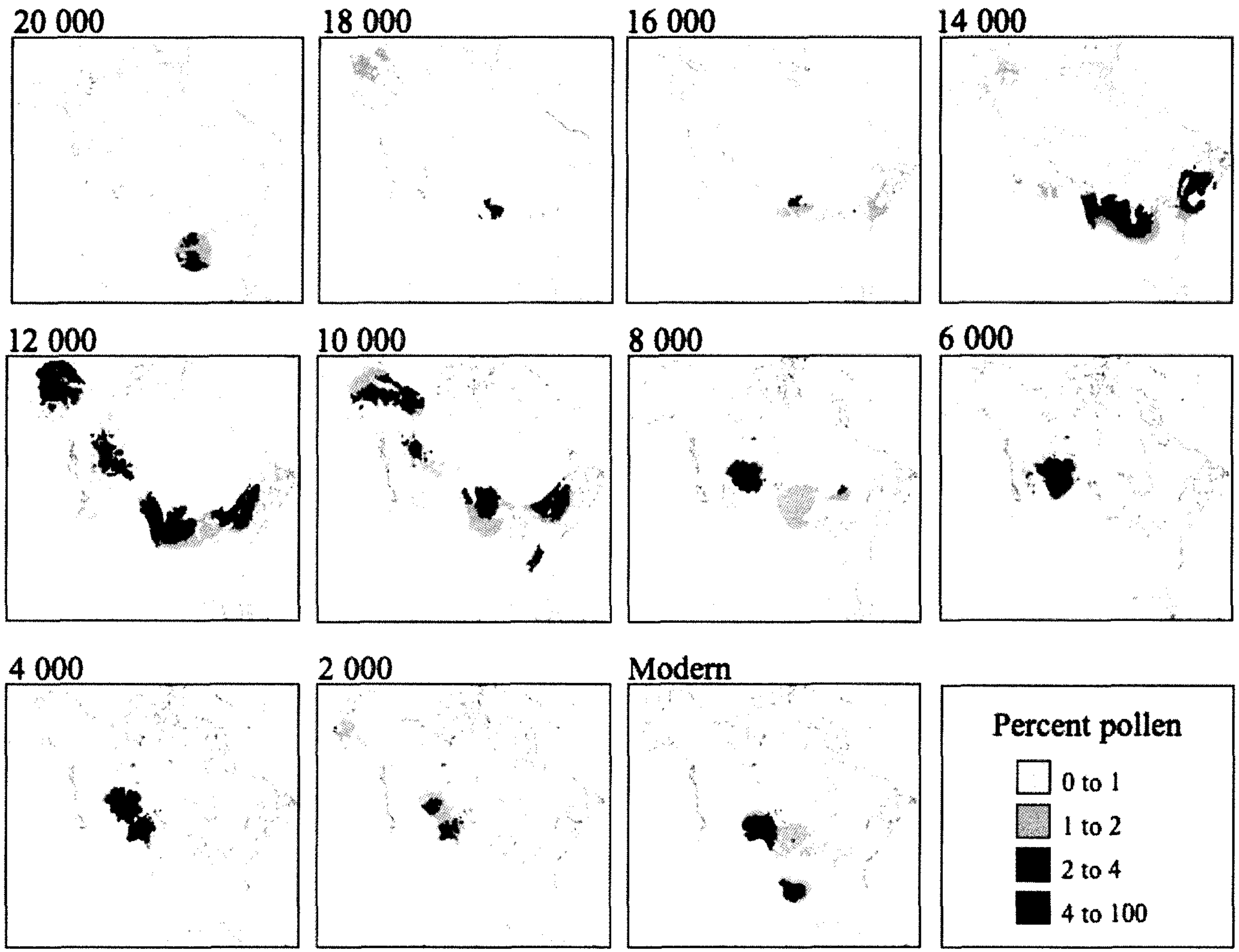

Figure 2.1 Pollen maps showing inferred poplar range shifts in North America from 20,000 calibrated years ago to the present (maps courtesy of J. Williams, University of Wisconsin). The extent of continental glaciers is shown in light blue and the abundances (> 1%) of poplar pollen are shown in green.

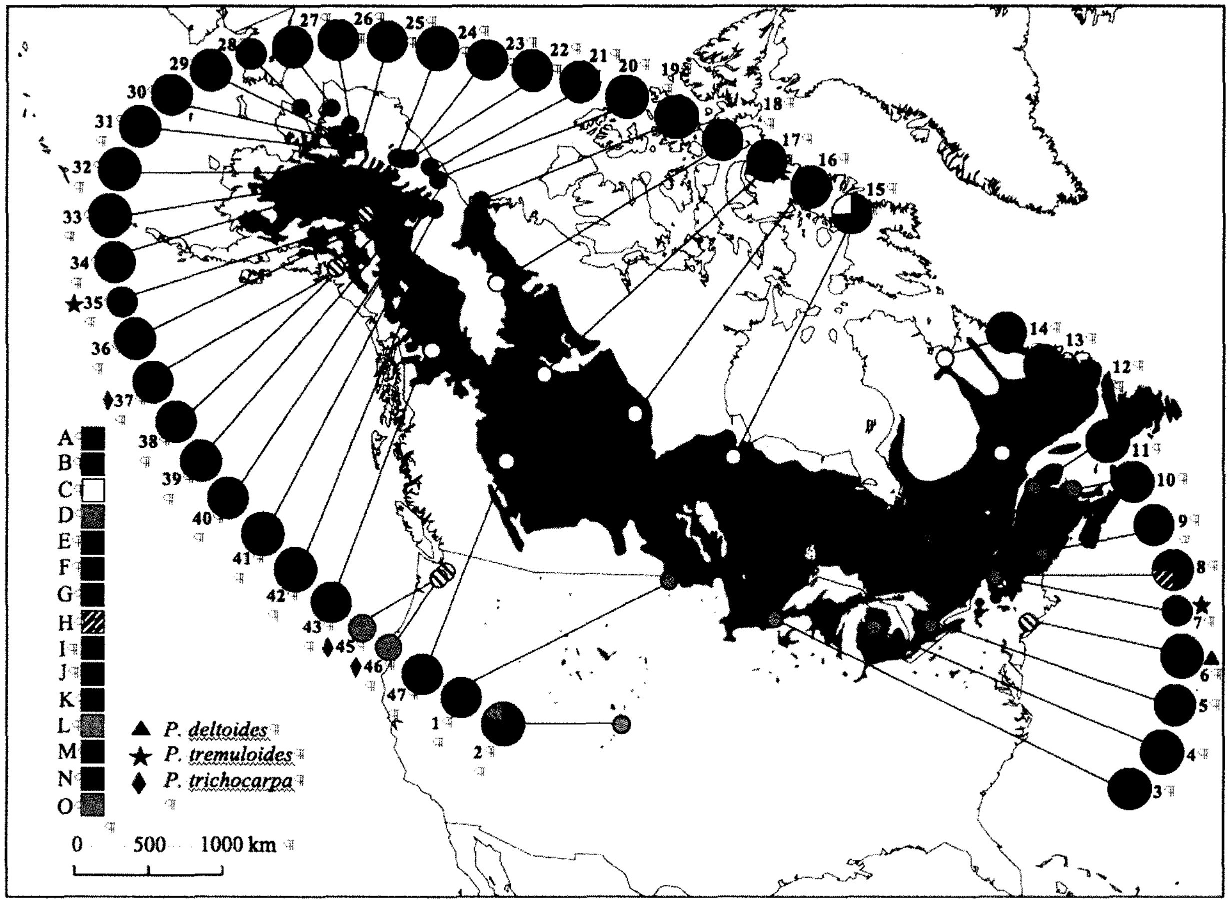

Figure 2.2 Distribution of chloroplast haplotypes observed in *Populus*. Pie charts indicate frequency of haplotypes within each population, the size of the chart indicates the number of alleles sampled (1-5) and alleles are indicated by different colors. Numbers adjacent to pie charts correspond to the populations listed in Table 1. The region in which a population occurs is indicated by symbols: Southern (gray), Central (white), Northern (black). The range of *P. balsamifera* in North America is show in green and the locality of other *Populus* species is indicated by black and white population symbols (U. S. Geological Survey, 2006). Map depicts an Albers equal-area conic projection.

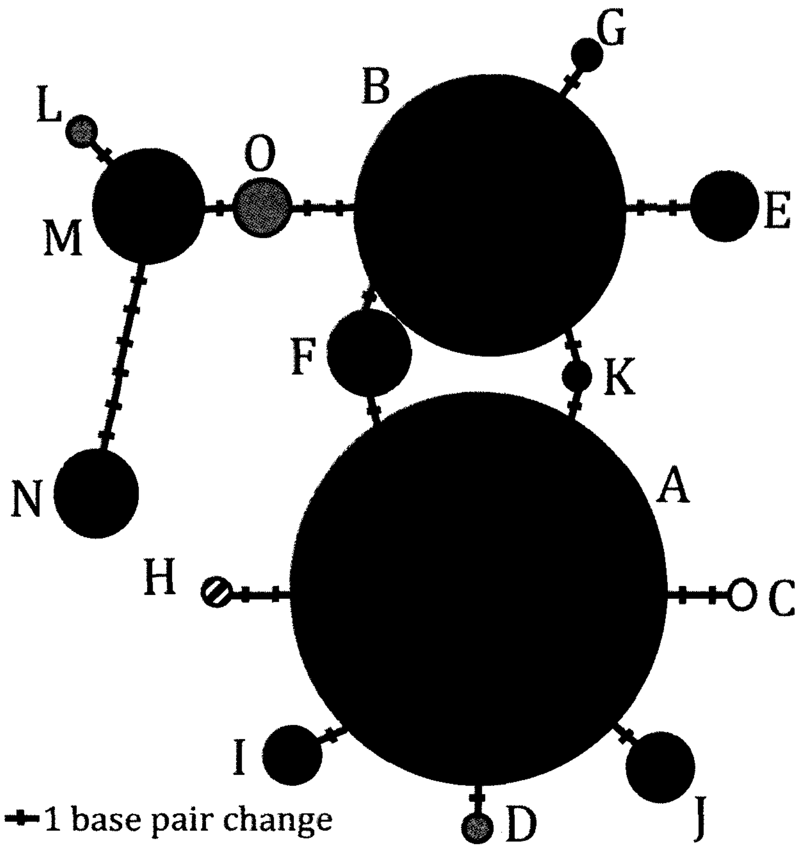

Figure 2.3 Network of 15 cpDNA haplotypes observed in *Populus*. The relative frequency of haplotypes observed is indicated by the size of the circles. The colors correspond to the alleles depicted in Figure 2.2.

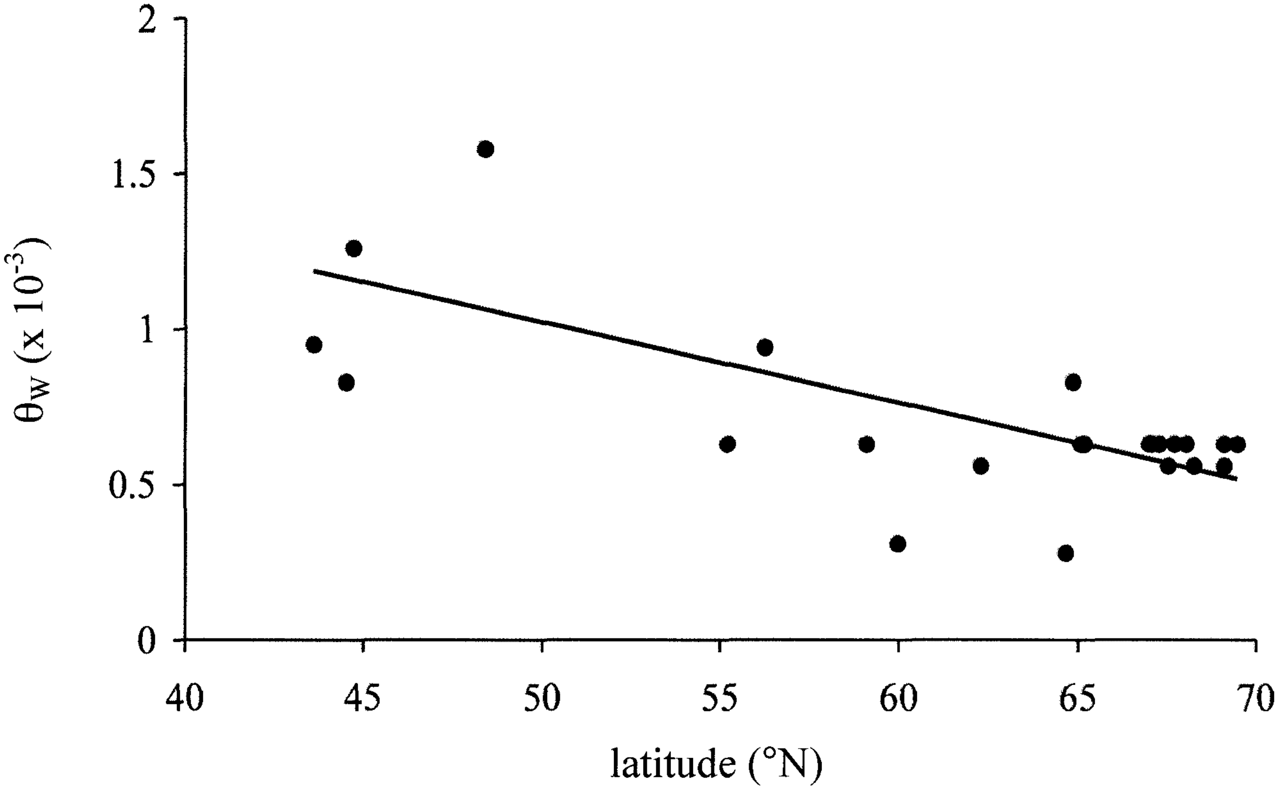

Figure 2.4 Linear regression analysis between latitude and nucleotide diversity (θ_W) in *Populus balsamifera* ($R^2 = 0.49$, $P < 0.01$).

Table 2.1 Localities, sample size and observed cpDNA haplotypes in the *Populus* study populations.

Species	Population	Locality	Latitude, longitude (°N, °W)	N	Haplotypes
P. balsamifera	***Southern***				
	1	Canada, SASK, Carnduff*	49.14, 101.57	4	A
	2	USA, CO, Meeker Mountain	40.23, 105.53	5	A, L
	3	USA, MN, General C. C. Andrews State Forest	46.34, 92.80	5	B
	4	USA, MI, N. Higgins Lake State Park	44.51, 84.76	5	A, F, G
	5	Canada, ONT, Guelph Lake	43.60, 80.26	4	B, F, I
	8	USA, NY, Dannemora	44.72, 73.71	4	A, B, H
	9	USA, ME, Pittsfield	44.79, 69.49	4	B
	10	Canada, PEI, Roseville*	46.69, 64.21	4	A, M
	11	Canada, QUE, Matane*	48.38, 67.19	4	A, E, F
	12	Canada, QUE, Rouyn-Noranda*	48.41, 77.94	4	A, B, E
	total			43	
	Central				
	13	Canada, QUE, Mount Grouix*	51.33, 68.09	4	A
	14	Canada, QUE, Kuujjuaq*	58.01, 68.35	4	A
	15	Canada, MAN, Gillam*	56.25, 94.36	4	A, C, D
	16	Canada, SASK, Stony Rapids*	59.11, 105.42	4	A, B, F
	17	Canada, NWT, Hay River*	60.49, 115.47	4	A
	18	Canada, NWT, Norman Wells*	65.16, 126.44	4	A, B
	43	Canada, BC, Partridge Creek	59.97, 131.22	4	B, J
	47	Canada, BC, Saskatoon Island	55.21, 119.10	4	A, B
	total			32	
	Northern				
	19	Canada, NWT, Inuvik*	68.26, 133.49	5	A, B
	20	USA, AK, Kongakut River	69.11, 141.99	5	A, B
	21	USA, AK, Hulahula River	69.47, 144.37	4	A, B
	22	USA, AK, Cottonwood Creek	69.10, 147.89	4	A, B
	23	USA, AK, Sagavanirktok River	68.78, 148.85	4	A
	24	USA, AK, Toolik Lake	68.63, 149.65	5	A
	25	USA, AK, Noatak River	67.71, 155.89	4	A, B
	26	USA, AK, Noatak River	68.04, 158.76	4	A, B

Table 2.1 (cont.)

Species	Population	Locality	Latitude, longitude (°N, °W)	N	Haplotypes
	27	USA, AK, Noatak River	67.81, 162.74	4	B
	28	USA, AK, Seward Peninsula	66.35, 165.67	2	A
	29	USA, AK, Kobuk River	67.05, 158.95	4	A
	30	USA, AK, Kobuk River	67.00, 157.65	4	A, B
	31	USA, AK, Kobuk River	66.86, 156.32	4	A
	32	USA, AK, Yukon River	64.65, 156.47	5	A, J
	33	USA, AK, Yukon River	64.85, 155.15	5	A, B, J
	34	USA, AK, Yukon River	65.19, 152.75	4	A, B
	36	USA, AK, N. Fork Chena River	65.07, 146.08	4	A, B
	38	USA, AK, Porcupine River	66.79, 144.11	4	A
	39	USA, AK, Porcupine River	67.07, 142.50	4	A, B
	40	USA, AK, Porcupine River	67.28, 141.63	4	A, B
	41	Canada, YT, Porcupine River	67.53, 139.92	5	A, B, K
	42	Canada, YT, Yukon River	62.28, 136.31	5	A, B
	total			93	
	species total			168	
P. deltoides	6	USA, CT, Hubbard Park	41.55, 72.84	5	M
P. tremuloides	7	USA, NY, Dannemora	44.72, 73.71	2	N
	35	USA, AK, Fairbanks	64.86, 147.85	2	N
P. trichocarpa	37	USA, AK, Valdez	61.13, 146.35	4	A
	45	USA, WA, Seattle	47.65, 122.31	1	O
	46	USA, WA, Kent	47.40, 122.24	1	O

*AgCanBaP collection

Table 2.2 Levels of nucleotide polymorphism and neutrality in the chloroplast genome of *Populus balsamifera*. One-tailed *P*-values for Tajima's *D* and Fu and Li's *F* calculated by coalescent simulation are in parentheses. Significant neutrality estimates are in bold type ($P \leq 0.05$).

	Region			
	Northern	Central	Southern	Total
N	93	32	41	166
S^a	3	6	9	13
H^b	4	6	7	11
H_d^c	0.49	0.39	0.70	0.54
$\theta_W (\mathrm{x}\ 10^{-3})^d$	0.34	0.86	1.22	1.33
$\pi (\mathrm{x}\ 10^{-3})^e$	0.55	0.38	0.96	0.64
Tajima's *D*	1.12 (0.14)	**-1.58** (0.04)	-0.62 (0.37)	-1.30 (0.07)
Fu and Li's *F*	1.08 (0.12)	**-2.30** (0.03)	-0.73 (0.27)	**-2.43** (0.02)

[a] segregating sites

[b] haplotypes

[c] haplotype diversity

[d] Watterson estimator

[e] average pairwise nucleotide diversity

CHAPTER THREE: BALSAM POPLAR COMMUNITIES ON THE ARCTIC SLOPE OF ALASKA[1]

3.1 Abstract

Trees are absent from the Arctic Slope of Alaska except for isolated stands of balsam poplar (*Populus balsamifera* L., Salicaceae) disjunct by over 100 km from the boreal forest south of the Brooks Range. The occurrence of balsam poplar is anomalous because the arctic region is characterized by continuous permafrost and tundra vegetation. Here, I describe balsam poplar plant communities on the Arctic Slope and interior Alaska and Yukon Territory. I used the Braun-Blanquét sorted-table method to classify balsam poplar communities and nonmetric multidimensional scaling (NMDS) to identify the ecological gradients underlying community differentiation. The NMDS ordination revealed a clear differentiation between arctic and boreal communities (stress = 14.73, $R^2 = 0.73$). Ecological gradients, reflected by ordination axes, correspond to a complex productivity gradient and a complex gradient in slope angle and aspect. I identify one new association, Eurybiae sibirica-Populetum balsamiferae ass. nov., and two subassociations: (1) subass. salicetosum alaxensis var. alaxensis (arctic communities) with two variants (var. *Androsace chamaejasme* on south-facing slopes and var. *Cystopteris montanum* near perennial springs), and (2) subass. rositosum acicularis (boreal communities). In all communities, species richness derives from herbaceous and woody species, which make up 85% of the total species. Arctic

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[1] Breen, A. L. Balsam poplar communities on the Arctic Slope of Alaska. Prepared for submission to *Phytocoenologia*

communities are dominated by arctic-alpine taxa (47- 55%), while boreal communities are dominated by boreal taxa (37%). Arctic communities have lower tree canopy and shrub height, stand basal area, litter depth and summer air temperature than communities in the boreal forest. A strong linkage between summer warmth index (SWI), the sum of mean monthly temperatures > 0° C, and the presence of balsam poplar is observed for the Arctic Slope (SWI > 25 for ~80% of the stands). Alteration of temperature regimes caused by global climate change will likely lead to an increase in the abundance and distribution of balsam poplar on the Arctic Slope of Alaska.

3.2 Introduction

Climate influences the distribution of plant communities, and climate change causes shifts in plant distributions, as evidenced by the fossil record (DAVIS & SHAW 2001) and recently observed trends (WALTHER *et al.* 2002; PARMESAN 2006; POST *et al.* 2009). Over the past 30 years, the Arctic has warmed ~2° C per decade and this trend is predicted to continue over the coming years (OVERPECK *et al.* 1997, SERREZE *et al.* 2000, ACIA 2004). Moreover, the rapid retreat of summer ice cover in the Arctic Ocean threatens the region with climatic conditions without recent analogues (BHATT *et al.* 2010). Vegetation changes predicted for the Arctic Slope of Alaska include increased abundance of shrubs and northward migration of treeline (SERREZE *et al.* 2000).

Trees are absent from the Arctic Slope of Alaska except for isolated stands of balsam poplar (*Populus balsamifera* L., Salicaceae) (BLISS & CANTLON 1957, SPETZMAN 1959, VIERECK & FOOTE 1970, MURRAY 1980, EDWARDS & DUNWIDDIE 1985, Bockheim *et al.* 2003) disjunct by over 100 km from boreal forest populations south of the Brooks Range. The northern foothills of the Arctic Slope, with mean July temperatures of 10-12°C, lie near latitudinal treeline today (Zhang *et al.* 1996). The occurrence of balsam poplar, however, is anomalous because the Arctic Slope is characterized by continuous permafrost and tundra vegetation.

Balsam poplar occurs preferentially on floodplains of braided rivers in areas with a sharp change in relief from the Brooks Range to the Arctic Foothills (Bockheim *et al.* 2003). The importance of climate for persistence of balsam poplar on the Arctic Slope is suggested by several arctic ecologists. Viereck (1979) noted well-developed balsam poplar stands occur at warm spring localities, while Murray (1980, 1992) observed that stands often persisting at certain sheltered sites or near perennial springs where groundwater is abundant throughout the year. Bockheim *et al.* (2003) developed a model to explain the origin and distribution of balsam poplar on the Arctic Slope. Their model hypothesizes that groves occur preferentially in areas of braided streams, geothermal springs, aufeis deposits and faulting. While Bockheim *et al.* (2003) don't explicitly include climate in their model, they do discuss the importance of favorable thermal and moisture conditions. Specifically, they note the deeper thaw bulb and warmer soil temperatures relative to the surrounding tundra that occurs adjacent to spring-fed rivers. A better understanding of the relationship between the distribution of balsam poplar and climate on the Arctic Slope is of interest, especially in light of predicted warming that will accompany climate change in the region.

The origin of balsam poplar on the Arctic Slope is unknown. The paleoecological record and balsam poplar's capacity to persist long-term through clonal reproduction suggests stands on the Arctic Slope may be relicts from a warmer period during the last interglacial (Sangamon, *c.* 130,000 yr BP; MUHS *et al.*, 2001) when balsam poplar was more widespread in northern Alaska (MURRAY 1980, HOPKINS *et al.* 1981, EDWARDS & DUNWIDDIE 1985). Others (RITCHIE 1984, MANN *et al.* 2002) suggest the paleoecological record is indicative of migration by balsam poplar following the Last Glacial Maximum into the Alaskan Arctic during the early Holocene (*c.* 11,000 yr BP). Regardless of timing, the occurrence of balsam poplar on the Arctic Slope need not be explained by recent, chance dispersal over the Brooks Range. Balsam poplar likely has a long history on the Arctic Slope.

In this study, I classify and describe balsam poplar plant communities on the Arctic Slope and interior Alaska and Yukon Territory. MURRAY (1980, 1992), from a limited number of observations, suggested the understory of arctic balsam poplar communities is derived primarily from taxa occurring in the surrounding tundra. Given the potential for a long history of balsam poplar in northern Alaska, I was particularly interested in the extent to which Arctic Slope stands differ floristically from stands in the boreal forest. The aim of this study is to analyze floristic variation in balsam poplar communities, classify vegetation types, and identify the ecological gradients underlying community differentiation. I discuss the implications of these relationships with respect to a changing arctic climate.

3.3 Study Area

This study was conducted in the Arctic Foothills of Alaska and the interior boreal forests of Alaska and Yukon Territory. The arctic study area is bounded by the Noatak River (162°W) to the west and the Kongakut River (142°W) to the east. Broad sloping valleys with elevations up to 350 m characterize the foothills of the Arctic Slope. Mean annual air temperatures range from -7 to -10° C, and mean annual precipitation ranges from 140 to 270 mm, of which 40% falls as snow (HAUGEN 1982). The boreal forest study area was bounded to the east by the Kobuk River (159°W) and to the west by the headwaters of the Yukon River (137°W). The landscape of the boreal forest consists of rolling hills, lowlands and nearly flat bottomlands along major rivers. In interior Alaska, temperature ranges from extremes of -50 to > 33° C and mean annual precipitation is 269 mm in Fairbanks, of which 30% falls as snow (HINZMAN *et al.* 2005).

3.4 Methods

3.4.1 Sampling methods

A total of 32 plots, or relevés, were established north and south of the Brooks Range in northern Alaska and Yukon Territory during the summers of 2003-2006 (Table 3.1, Fig. 3.1). Sampling localities were selected subjectively in areas of homogeneous vegetation dominated by balsam poplar. The minimum sampling area was approximately 100 m^2. I scored the occurrence of vascular, bryophyte and lichen species using the Braun-Blanquét cover-abundance scale (r, +, 1-5; BRAUN-BLANQUÉT 1965, MUELLER-DOMBOIS & ELLENBERG 1974) and recorded the height and actual cover of separate plant functional types (trees, shrubs, herbs). I also estimated the percent cover of standing dead and dead woody debris, and litter. Voucher specimens were collected for identification and a representative specimen for each taxon was deposited in the Herbarium of the University of Alaska Museum of the North (ALA). Vascular plant nomenclature follows the Panarctic Flora checklist (ELVEN *et al.* 2005) except for few boreal taxa that follow Missouri Botanical Garden's TROPICOS nomenclature database (2010). Non-vascular plant nomenclature follows ESSLINGER (2008) for lichens and generally follows TROPICOS (2010) for mosses.

At each relevé, I quantified several aspects of the site, stand and soils. The physical characteristics of each site were described by the following variables: elevation, slope, aspect, site stability, exposure, parent material and geomorphology. Site and soil moisture and snow duration were categorized on scales of 1 to 10 (KOMÁRKOVÁ 1983). Summer warmth index (SWI), the sum of monthly mean air temperatures above freezing from May to September 1982-2003 calculated from AVHRR temperature data, was used to characterize the amount of summer warmth available for plant growth at each site (COMISO 2006, RAYNOLDS *et al.* 2008). SWI is calculated from monthly climate summaries from arctic stations and is strongly correlated with the distribution of many arctic plant species (YOUNG 1971, EDLUND 1990, WALKER *et al.* 2003a, 2003b, 2005) and vegetation productivity (RAYNOLDS *et al.* 2008). I followed the point centre quarter method to estimate stand density (trees/ha), basal area and canopy height (MUELLER-DOMBOIS & ELLENBERG 1974). Five of the largest trees at each site were cored at a height of 1 m to estimate stand age. I dug soil pits at each relevé, recorded the depth of litter and the organic horizon and collected soil samples from the upper 10 cm of the mineral horizon. Tree core and soil samples stored in the laboratory freezer were inadvertently discarded prior to analysis, and therefore these results are not presented herein.

3.4.2 Classification and ordination methods

Vegetation was classified according to the Braun-Blanquét sorted table method (BRAUN-BLANQUÉT 1965) and the specific protocol of DANIËLS (1982) for distinguishing vegetation types. I arranged the relevés in phytosociological tables to characterize community types. The differentiation of vegetation types was based on diagnostic species. I followed the criteria proposed in DIERSCHKE (1994) to assess the degree of fidelity of character species and determine differential species. The nomenclature of the described syntaxa is in accordance with WEBER *et al.* (2000).

To examine the relationship between species composition and the local environment, I used Nonmetric Multidimensional Scaling (NMDS; MATHER 1976, KRUSKAL 1964). NMDS is considered the most effective ordination technique for ecological community data as it is well suited for non-normal data, or data that occur on arbitrary or discontinuous scales (MCCUNE & GRACE 2002). Because inclusion of a single abundant species can bias the results (MCCUNE & GRACE 2002), I removed all balsam poplar occurrences from the data prior to analysis. A preliminary analysis using a Monte Carlo test of significance for a 6-dimensional solution stepping down to a 1-dimensional solution was used to determine the appropriate dimensionality, or axes, for the ordination. The preliminary run included 500 iterations, with 50 runs of real data and

50 runs of randomized data, and an instability criterion of 0.00005. Based on preliminary results, I chose a 3-dimensional solution for the final analysis. The final run included 500 iterations, with 1 run of real data and an instability criterion of 0.00005. All analyses used the Sorenson distance measure and random starting configurations. Pearson correlation coefficients between metric environmental variables and ordination axes were explored in ordination space by examining the angle and length of vectors indicating the direction and strength (*r* value) of the relationships, respectively. NMDS was implemented using the software program PC-ORD (v. 5.10; McCune & Mefford 2006).

3.4.3 Plant functional types and floristic analyses

To examine growth form distributions (trees, shrubs, forbs, graminoids, ferns & fern allies, mosses and lichens) and the floristic affinities of the vegetation types, I used a multi-character approach modified from criteria by Walker (1985). I assigned vascular species to floristic units using Hultén's (1968) distribution maps and the Panarctic Flora checklist (Elven *et al.* 2005). Vascular species were classified according to: 1) major regional unit, or the principal environmental region in which the plant occurs (arctic, alpine, boreal, arctic-alpine, arctic-boreal and boreal-alpine), and 2) geographic range, or the global range of distribution of the plant (Beringia, North America, North America-Asia, North America-Asia-Europe, Circumpolar/boreal).

3.4.4 Linkage between climate and balsam poplar on the Arctic Slope

To examine the influence of climate on the presence of balsam poplar on the Arctic Slope, I constructed a comprehensive map of balsam poplar stands in northern Alaska. The area of interest is restricted to the region north of treeline, or the northern limits of *Picea glauca* (white spruce), that is characterized by an arctic climate, arctic flora and tundra vegetation. Occurrence data were compiled from the literature, the Herbarium of the University of Alaska Museum of the North and observations of the author and her colleagues. Summer warmth index (SWI), the sum of mean monthly temperatures > 0° C, was used as a proxy for climate. The balsam poplar occurrence data are presented overlain on a map of northern Alaska showing SWI at a resolution of 12.5 km pixels (RAYNOLDS *et al.* 2008).

3.5 Results and discussion

3.5.1 Ordination and classification

Balsam poplar plant communities in arctic Alaska differ in their characteristic species composition from communities in the boreal forest. The floristic differentiation between arctic and boreal vegetation types is clearly reflected in the NMDS ordination diagram; boreal relevés are clustered on the left and arctic relevés are clustered on the right (Figs. 3.2 & 3.3; stress = 14.73, $R^2 = 0.73$). Both arctic and boreal relevés occupy a broad range within ordination space showing relatively heterogeneous species composition. In the Arctic, community types can be further differentiated by habitat. Distinct arctic vegetation types occur among riparian, south-facing slopes and spring relevés. A similar pattern was not observed in the boreal forest where balsam poplar occurs predominantly on floodplains (VIERECK *et al.* 1992)

The NMDS ordination reflects not only the floristic similarity structure of balsam poplar stands, but also shows the relationship between plant communities and principal environmental factors. Three axes captured 73% of the variance in species composition in balsam poplar communities. The strongest environmental gradients, axes 1 and 3, captured most of the variance in balsam poplar communities (cumulative $R^2 = 0.63$). I therefore present only axis 1 versus axis 3 (Figs. 3.2 & 3.3).

Axis 1, describing 46% of the variation in community composition, is interpreted as a complex productivity gradient which corresponds to the south-to-north transition from boreal forest to arctic tundra in northern Alaska. Latitude and elevation increase along axis 1, while air temperature as inferred from SWI, canopy and shrub height, stand basal area, litter depth, and various cover estimates all decrease. Boreal communities occupy the left end of the gradient and arctic communities occupy the right end. Axis 3 was much weaker than the first, describing 17% of the variation in community composition. Axis 3 primarily represents increasing slope angle among arctic relevés. Relevés with the greatest slope angle, which are all south-facing, are clustered in the bottom right corner of the diagram.

In accordance with the ordination analyses, the classification of balsam poplar communities resulted in one association, two subassociations and two variants. I place the association in the class Salicetea purpureae (MOORE 1958) supporting SCHICKHOFF et al.'s (2002) proposal to extend the range of the class of riparian willow shrub and poplar forest vegetation of northern Europe to North America. LOOMAN (1987) proposed a separate class, Betulo-Populetea, for the riparian poplar forests of North America. He noted the woods of the floodplains, or gallery forests, do not have a true counterpart in Eurasia. The exception may be the basal community Populus suaveolens-Chosenia arbutifolia in the Russian far north, which is assigned to Salicetea purpureae, as indicated by SUMINA & MIRONOVA (2004). The structural, floristic and ecological affinities of the community Populus suaveolens-Chosenia arbutifolia to balsam poplar communities on the Arctic Slope support the assignment of the described association to Salicetea purpureae. The class Betulo-Populetea, however, was listed as invalid shortly after its proposal (THEURILLAT & MORAVEC 1990).

I assign the balsam poplar communities to the order Populetalia balsamiferae Krajina (KRAJINA 1969; LOOMAN 1987) and the alliance Junipero-Shepherdio-Populion tremuloidis Hoefs *et* Krajina (KRAJINA 1969, HOEFS *et al.* 1975). The plant communities of the order Populetalia balsamiferae occur on alluvial floodplains or more recent terraces and therefore include the riparian *Populus balsamifera* communities that occur across boreal North America. RIVAS-MARTÍNEZ *et al.* (1999) also proposed the order Populetalia angustifolio-deltoidis for cottonwood and riparian forests of North America. The authors place this order within class Linnaeo americanae-Piceetea marianae, their North American boreal forest counterpart to the Eurasian class Vaccinio-Piceetea (BRAUN-BLANQUÉT *et al.* 1939). Linnaeo americanae-Piceetea marianae primarily includes boreal coniferous forests and their deciduous secondary forests and woodlands, although for the present also includes riparian cottonwood communities and tall tundra vegetation (RIVAS-MARTÍNEZ *et al.* 1999). The proposed class Populetalia angustifolio-deltoidis subsumes Populetalia balsamiferae, however it does not include the forests of the arctic or boreal regions. As none of the characteristic species of the class Populetalia angustifolio-deltoidis occur within the balsam poplar communities described herein, I chose to assign these communities to the class Populetalia balsamiferae.

3.5.2 Description of the plant communities

Eurybiae sibirica-Populetum balsamiferae

Breen ass. nova

Nomenclatural type relevé: Table 3.2, rel. 1

The Eurybiae sibirica-Populetum balsamiferae association is primarily distributed on floodplains in northern Alaska and Yukon Territory (Fig. 3.4, Tables 3.2). In the Arctic, this community occurs in isolated stands associated with north-flowing rivers, primarily braided streams and aufeis deposits (BOCKHEIM *et al.* 2003), although the community also occurs infrequently on south-facing slopes in the foothills of the Brooks Range. Trees are the most prominent growth form of the association, with a mean balsam poplar canopy cover of approximately 50% (mean = 51.25% and range = 30-70%). Canopy height ranges from 1.69-18.70 m (mean = 9.76 m) and tree diameters range from 3.18-32.05 cm (mean = 14.26 cm). Soils are well-drained unmodified cryofluvents lacking permafrost in the boreal forest, and with deep active layers in the Arctic (VIERECK *et al.* 1992; PING et al. 1998). The association has thin leaf litter (range = 0.25-3.70 cm) and organic soil horizons because of frequent flooding at spring snowmelt. Similarly, ground lichen and moss cover is limited. Disturbance by moose is common within this association, especially those stands occurring on floodplains. The degree of moose disturbance is greatest in the arctic communities, including scat, discarded antlers, browsing and noticeable trampling.

Several taxa are faithful/differential within this community, despite the wide range of the association from the boreal forest northward to latitudinal treeline on the Arctic Slope. *Populus balsamifera*, the name giving taxon of the association, occurs consistently across all relevés. The well-developed shrub and herbaceous layers comprise the remaining taxa differential for the community: *Eurybia sibirica*, *Hedysarum americanum*, *Chamerion angustifolium* ssp. *angustifolium*, *Equisetum arvense*, *Anticlea elegans* and *Shepherdia canadensis*.

Previous descriptions of balsam poplar communities in Alaska show similar findings supporting the use of the Braun-Blanquét approach to define community types based on floristic and ecological characteristics. Notably, VIERECK *et al.* (1992) describe three broad classes of balsam poplar communities (closed broadleaf forest, open broadleaf forest and closed mixed forest) in interior and northern Alaska. Their classification is based on stand structural characteristics, such as canopy cover and stand density. The physiognomic approach is thought to be a better predictor of processes dominated by the tree canopy such as productivity and energy exchange (CHAPIN *et al.* 1996), than composition of understory floristic communities. In the ordination analyses, however, I found variation in overall species composition was best described as a complex productivity gradient corresponding to the south-to-north transition from boreal forest to arctic tundra in northern Alaska. The classification, accordingly, resulted in two distinct regional subassociations. In the arctic, I describe the subassociation Salicetosum alaxensis var. alaxensis that is similar to VIERECK et al.'s balsam poplar open broadleaf forest occurring at treeline in interior and northern Alaska. In the boreal forest, I describe the subassociation Rositosum acicularis that is similar to VIERECK *et al.*'s balsam poplar closed broadleaf forest occurring on floodplains in interior Alaska.

1) **Eurybiae sibirica-Populetum balsamiferae salicetosum alaxensis var. alaxensis**

Breen et al. subass. nova

Nomenclatural type relevé: Table 3.2, rel. 12

This community is distributed on floodplains and south-facing slopes in the arctic foothills of northern Alaska (Fig. 3.5). Tundra stands of balsam poplar are stunted (mean height ± SE = 7.52 ± 0.75 m) and far less dense (mean ± SE = 1841.82 ± 293.24 trees/ha) than in the boreal forest (mean height ± SE = 13.05 ± 0.93 m; mean density ± SE = 2780.87 ± 623.09 trees/ha). Basal area per stand of the arctic subassociation (mean ± SE = 141.13 ± 17.48 m^2/ha) is less than half observed in the boreal subassociation (Rositosum acicularis; mean ± SE = 283.39 ± 84.30 m^2/ha). Stands grow slowly, are even-aged, and individual trees range from 90-230 years in age (EDWARDS & DUNWIDDIE 1985, BOCKHEIM et al. 2003). Moreover, the subassociations are further differentiated by physical characteristics such as higher latitude, elevation and reduced summer warmth available for plant growth, and site characteristics including comparatively lower litter depth and various cover estimates.

Salix alaxensis var. *alaxensis*, the name giving taxon of the subassociation, occurs nearly exclusively in the arctic subassociation (Table 3.2). Previous ecological studies delimiting arctic riparian vegetation identify remarkably similar *Salix alaxensis* communities, however, balsam poplar is absent (BLISS & CANTLON 1957; MOORE 1982;

GOULD 1998; SCHICKHOFF *et al.* 2002). BLISS & CANTLON (1957), however, do anecdotally note that balsam poplar occurs in the older parts of stands of *Salix alaxensis* var. *alaxensis* on alluvial fans in river valleys in the eastern part of the Arctic Slope.

Balsam poplar apparently originates from rare long distance dispersal by seed over the Brooks Range (MURRAY 1980), although local reproduction occurs both asexually and presumably from seed (EDWARDS & DUNWIDDIE 1985). I observed female catkins in fruit in ~40% of the arctic relevés, although seed production was not evaluated. The occasional occurrence of balsam poplar within a common arctic pioneer community of active floodplains and gravel bars is likely associated with colonization history, rather than with floodplain successional trajectories.

Several other species further differentiate this subassociation including *Senecio lugens*, *Arctous rubra*, *Dasiophora fruticosa*, *Festuca altaica*, *Anemone parviflora*, *Pyrola grandiflora* and *Delphinium chamissonis*. Two moss species, *Dicranum dispersum* and *Sciuro-hypnum ornellanum*, occur within this subassociation that have not been reported previously from North America (AFONINA & BREEN 2009). Interestingly, the nearest record of *Sciuro-hypnum ornellanum* to northern Alaska is from the Chukotka Peninsula in Penkignei Bay. It is in this same locality that balsam poplar was discovered for the first time in Chukotka (KATENIN 1980).

We identify two variants of the subassociation that differ in species composition and habitat. The variant of *Androsace chamaejasme* (Fig. 3.6) occurs on south-facing slopes and is identified by the name giving taxon and the differential/faithful taxa *Carex obtusata*, *Buplureum arcticum*, and *Anemone drummondii*. These faithful taxa are shared with the Poa glauca-Arnica angustifolia community that occurs locally on ridge tops or other high points in the landscape and is often associated with dens of the arctic ground squirrel (WALKER *et al.* 1994). The spring variant of *Cystopteris montanum* (Fig. 3.7) includes the differential/faithful taxa *Carex podocarpa* and *Carex atrosquama.*

2) Eurybiae sibirica-Populetum balsamiferae rositosum acicularis

Breen et al. subass. nova

Nomenclatural type relevé: Table 3.2, rel. 26

This subassociation is widely distributed in the boreal forests of interior Alaska and Yukon Territory (Fig. 3.8). It represents the typical form of the association, occupying moist well-drained sites on floodplains that are often part of a successional sequence. Balsam poplar stands on floodplains in interior Alaska are highly productive (551 $g/m^2/yr$; VIERECK *et al.* 1992), as is reflected in the measurements of stand characteristics which all reach their greatest extent within this subassociation (Table 3.3). Differential/faithful taxa of this community are *Rosa acicularis*, *Picea glauca*, *Artemisia tilesii*, *Galium boreale* and *Viburnum edule.*

The Rosa acicularis subassociation is floristically similar to previous descriptions of floodplain balsam poplar communities in the boreal region. A *Populus/Salix/Sherpherdia* community was identified by LA ROI (1967) in his description of floristic attributes of the North American boreal forest. GILL (1971) identifies a *Populus balsamifera* community of the Mackenzie River Delta in Northwest Territories in which he cites the dominance of *Salix alaxensis*, *Arctous rubra*, *Artemisia tilesii*, *Hedysarum alpinum*, *Equisetum arvense*. HOEFS *et al.* (1975) describe the Roso-Shepherdio-Populetum balsamiferae association in southwest Yukon Territory. Finally, YARIE (1983) described three communities from Porcupine River floodplains in eastern-interior Alaska: *Populus balsamifera-Alnus-Salix/Rosa acicularis/Equisetum*, *Populus balsamfiera/Rosa acicularis/Equisetum* sp.-*Pyrola* spp. and *Populus balsamifera-Arctostaphylos uva-ursi/Peltigera* spp.

3.5.3 Plant functional types and floristic analysis

The analyses of plant functional types show moderate differences between balsam poplar communities. The floristic richness, including vascular plants, lichens and mosses, of the Eurybiae sibirica-Populetum balsamiferae association is 203 species, and is higher within the Salicetosum alaxensis var. alaxensis subassociation (155 species) than the Rosa acicularis subassociation (111 species) (Fig. 3.9). The greater floristic richness in the arctic subassociation is likely due to the inclusion of variants that differ by habitat and species composition. For example, when comparing floristic richness between arctic (96 species) and boreal (111 species) riparian relevés, levels of diversity are similar. In all communities, species numbers are driven by herbaceous and woody species, which make up about 85% of the total species. The species richness of lichens and bryophytes is greatest in the upland south-facing communities of the *Androsace chamaejasme* variant with 15 species, and lowest in the spring communities of the *Cystopteris montanum* variant with 2 species. The low diversity of cryptogams in the latter community is likely due to shading in the understory from high percent cover (180%), especially of forbs (42%), which is the highest observed in any of the balsam poplar communities. Forb rich vegetation in the Arctic is typically confined to local situations, often on south-facing slopes classified as meadow or steppe (WALKER *et al.* 1994). Here, it is of interest that we observe forb rich vegetation within spring communities that is greater than upland south-facing communities. In general, however, total plant cover is similar across all communities (~150%).

The analysis of the phytogeographic spectra of vascular plants revealed arctic and boreal balsam poplar communities differ in their floristic affinities (Fig. 3.10). The Eurybiae sibiricae-Populetum balsamiferae association is dominated by arctic-alpine species (38%), and less so by boreal species (25%) and arctic-boreal species (22%). Arctic communities are dominated by arctic-alpine species, which proportion ranges from 47-55%, while the boreal Rosa acicularis subassociation is dominated by boreal species (37%). All communities are dominated by species with circumpolar or circumboreal ranges (Fig. 3.11). Taxa with a Beringian distribution were least frequent within all communities, although occurred most frequently in the arctic south-facing slope *Androsace chamaejasme* variant (11% of the observed vascular flora).

3.5.4 Linkage between climate and balsam poplar on the Arctic Slope

The distribution of balsam poplar on the Arctic Slope is predominantly in warm areas of the Arctic Foothills and Brooks Range (Fig. 3.12). In a previous study, BOCKHEIM *et al.* (2003) identified 28 balsam poplar stands on the Arctic Slope. I significantly expand upon their work to identify an additional 43 balsam poplar groves for a total of 71 occurrences in arctic Alaska (Table 3.4). Approximately 75% of the balsam poplar stands occur in the eastern foothills, from 154° W to 141° W longitude, or from the Killik (stands 19-21) to the Kongakut (stands 69-71) rivers. North of the Brooks Range, west of the Killik River, we identified only 4 occurrences, including trees on the Utukok, Etivluk, Kigalik and Oolamnagavik Rivers. At this time, it is uncertain whether the infrequent occurrence of balsam poplar in the western foothills is accurate or an artifact of fewer collectors having visited this area. The northern-most occurrence of balsam poplar is at Sadlerochit Springs (69.6583° N, stand 61) where the Brooks Range reaches its northern limit.

The presence of balsam poplar in northern Alaska is strongly linked to climate; balsam poplar occurs predominantly in sites with high summer warmth indices (SWI > 25 for ~80% of the stands; Fig. 3.13). Most stands (~40%) occur in the 30-35 SWI class. Given the importance of microclimate and the coarse resolution of 12.5 km pixels for the SWI data in arctic Alaska, it is of interest that an overall positive relationship between balsam poplar occurrence and high SWI was observed. This finding confirms the hypothesis of early workers of the importance of climate for persistence of balsam poplar on the Arctic Slope (VIERECK 1979, MURRAY 1992). Over the past 30 years, the Arctic has warmed ~2° C per decade and this trend is predicted to continue over the coming years (OVERPECK *et al.* 1997, SERREZE *et al.* 2000, ACIA 2005). Climatic change is expected to have major effects on vegetation patterns, including shifts in plant distributions, community composition and northward migration of treeline (SERREZE *et al.* 2000). An alteration of temperature regime caused by climate change will likely result in an increase in the abundance and distribution of balsam poplar on the Arctic Slope of Alaska.

3.6 Conclusions

Based on over 30 relevés, I identify and describe five balsam poplar communities on the Arctic Slope and interior Alaska and Yukon Territory. The classification resulted in one association, two subassociations and two variants. The Eurybiae sibirica -Populetum balsamiferae association described herein is placed in Salicetea purpureae (MOORE 1958), the class of riparian willow shrub and cottonwood forest vegetation known primarily from northern Eurasia. Despite the wide range of the association from the boreal forest north to the Arctic Slope, the well-developed woody and herbaceous layers share many faithful taxa within the community in addition to *Populus balsamifera* including: *Eurybia sibirica*, *Hedysarum americanum*, *Chamerion angustifolium* ssp. *angustifolium*, *Equisetum arvense*, *Anticlea elegans* and *Shepherdia canadensis*. Arctic and boreal balsam poplar communities are further differentiated into the subassociations salicetosum alaxensis var. alaxensis and rositosum acicularis, respectively. Faithful taxa for the arctic subassociation include: *Senecio lugens*, *Arctous rubra*, *Dasiophora fruticosa*, *Festuca altaica*, *Anemone parviflora*, *Pyrola grandiflora* and *Delphinium chamissonis* and faithful taxa for the boreal subassociation include: *Rosa acicularis*, *Picea glauca*, *Artemisia tilesii*, *Galium boreale* and *Viburnum edule*. Distinct arctic vegetation types occur among south-facing slopes and spring relevés and are recognized by the variants *Androsace chamaejasme* and *Cystopteris montanum*, respectively. A similar pattern was not observed in the boreal forest where balsam poplar occurs predominantly on floodplains.

The principle environmental factors resulting in the characteristic vegetation types of the communities described above are latitude, elevation, SWI, tree canopy and shrub height, stand basal area, litter depth, slope angle and aspect, and various cover estimates. The NMDS ordination displayed the relationship between plant communities and these environmental gradients. Three axes captured 73% of the variance of in species composition in balsam poplar communities. The strongest environmental gradients, axes 1 and 3, captured most of the variance in balsam poplar communities (cumulative R^2 = 0.63). The first axis corresponds to a complex productivity gradient which reflects the south-to-north transition from boreal forest to arctic tundra in northern Alaska. Latitude and elevation increase along axis 1, while air temperature as inferred from SWI, canopy and shrub height, stand basal area, litter depth, and various cover estimates all decrease. The third axis corresponds to a complex gradient in slope and aspect.

In all communities, species richness is driven by herbaceous and woody species, which make up 85% of the total species. Species richness of lichens and mosses is low throughout the vegetation types, most likely because of annual flooding and shading by the balsam poplar overstory. Two moss species, *Dicranum dispersum* and *Sciuro-hypnum ornellanum*, that occur within the arctic subassociation are of interest, however, because they are the first occurrences reported from North America (AFONINA & BREEN 2009).

Arctic communities are dominated by arctic-alpine taxa (47-55%), while boreal communities are dominated by boreal taxa (37%). All communities are dominated by species with circumpolar or circumboreal ranges. Trees in arctic communities are generally dwarfed and elfin, with lower tree canopy heights and stand basal area than trees in the boreal forest. Litter depth and summer air temperature are far greater in the boreal forest communities than in the arctic communities.

A map of balsam poplar occurrences in arctic Alaska is presented showing its distribution primarily follows the Arctic Foothills of the Brooks Range. A total of 71 occurrences were compiled from the literature, herbarium records and observations of the author and her colleagues. Most of the balsam poplar stands occur in the eastern foothills, from 154° W to 141° W longitude. Only four occurrences were identified from the western foothills. A strong linkage between summer warmth index (SWI), the sum of mean monthly temperatures > 0° C, and the presence of balsam poplar was observed (SWI > 25 for ~80% of the stands). Alteration of temperature regimes caused by global climate change will likely lead to an increase in the abundance and distribution of balsam poplar on the Arctic Slope of Alaska.

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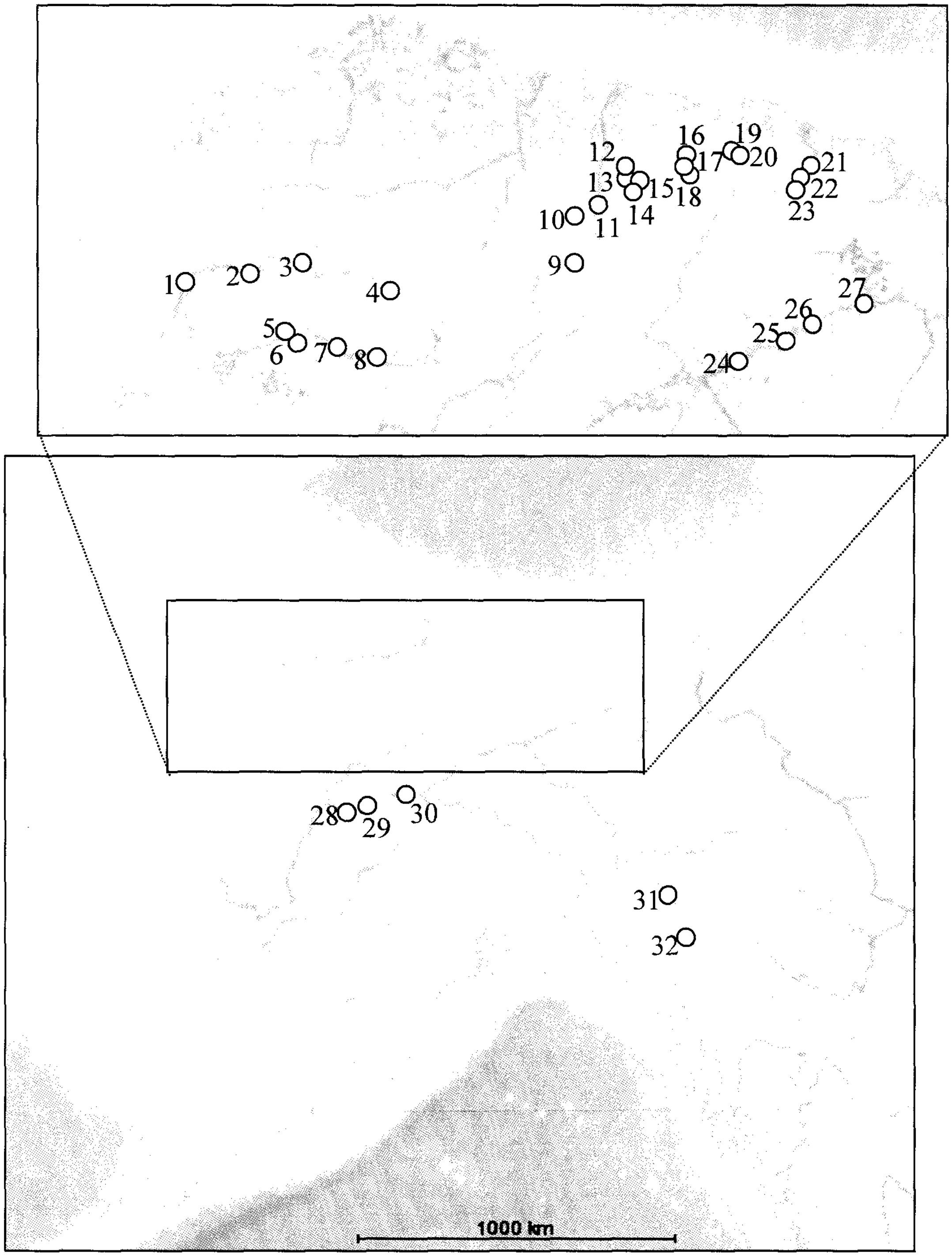

Figure 3.1 Location of study sites in Alaska and Yukon Territory. The relevés are numbered as in Table 3.1.

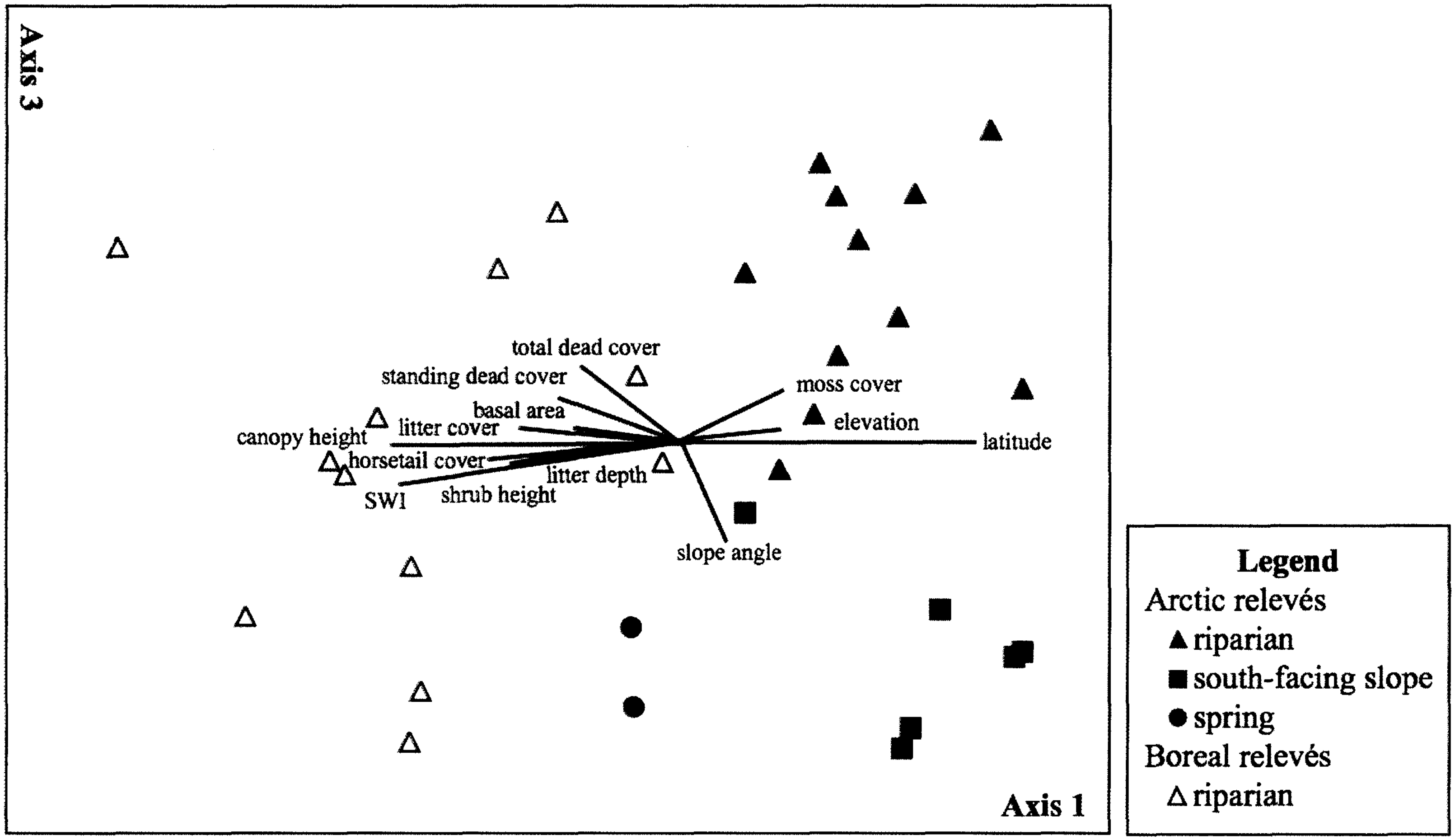

Figure 3.2 Nonmetric multidimensional scaling ordination of all relevés. The biplot diagram shows metric environmental variables correlated with the relevé distribution. Lines indicate the direction and strength correlations with $r^2 > 0.20$.

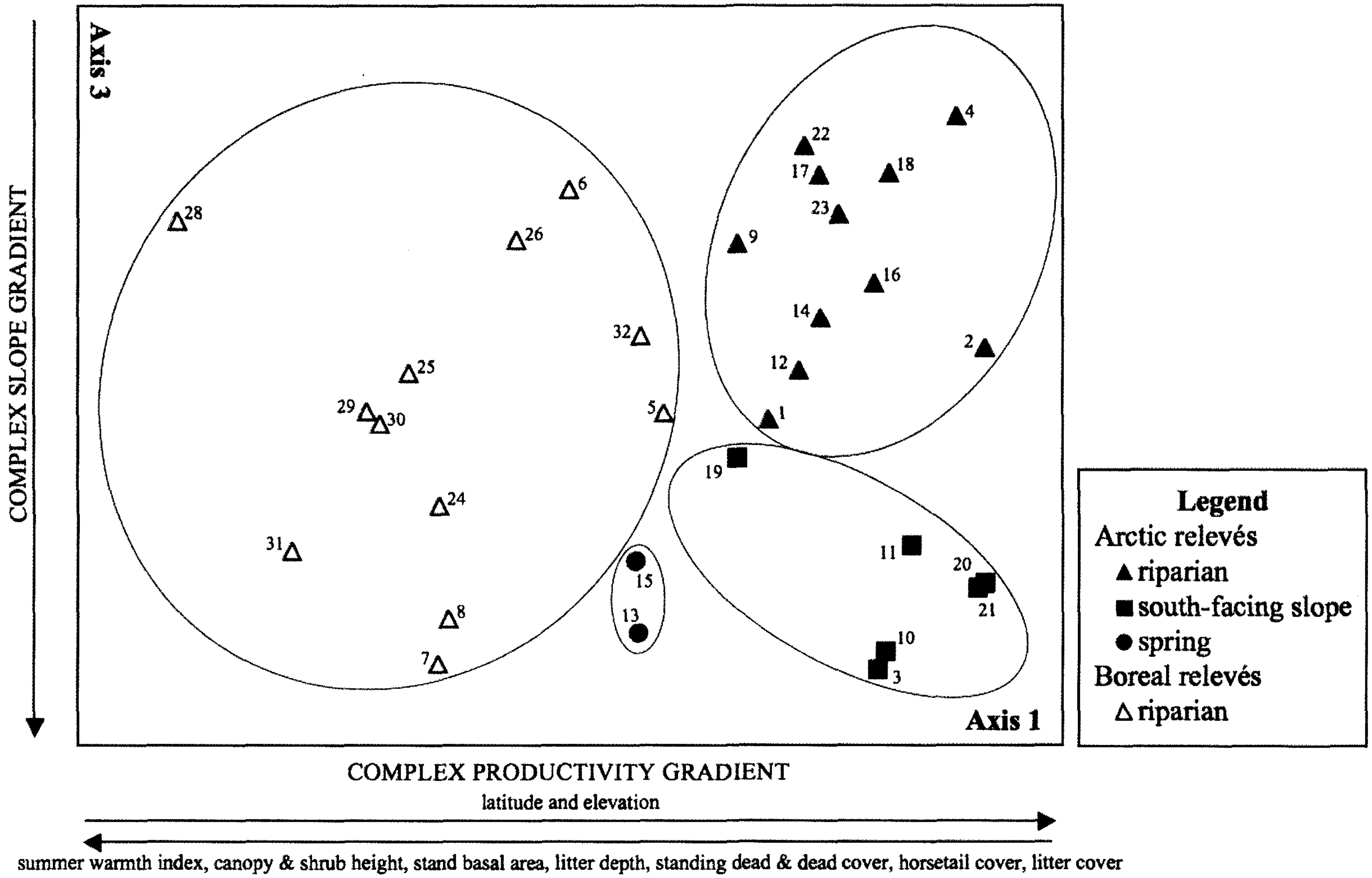

Figure 3.3 Nonmetric multidimensional scaling ordination of all relevés. The sample plots are grouped according to plant community. Arrows along each axis indicate the direction of principal environmental gradients. The relevés are numbered as in Table 3.1.

Figure 3.4 Eurybiae sibirica-Populetum balsamiferae association on the lower Yukon River, Alaska (Relevé 29).

Figure 3.5 Salicetosum alaxensis var. alaxensis subassociation on a tributary of the Kongakut River in Arctic Alaska (Relevé 22).

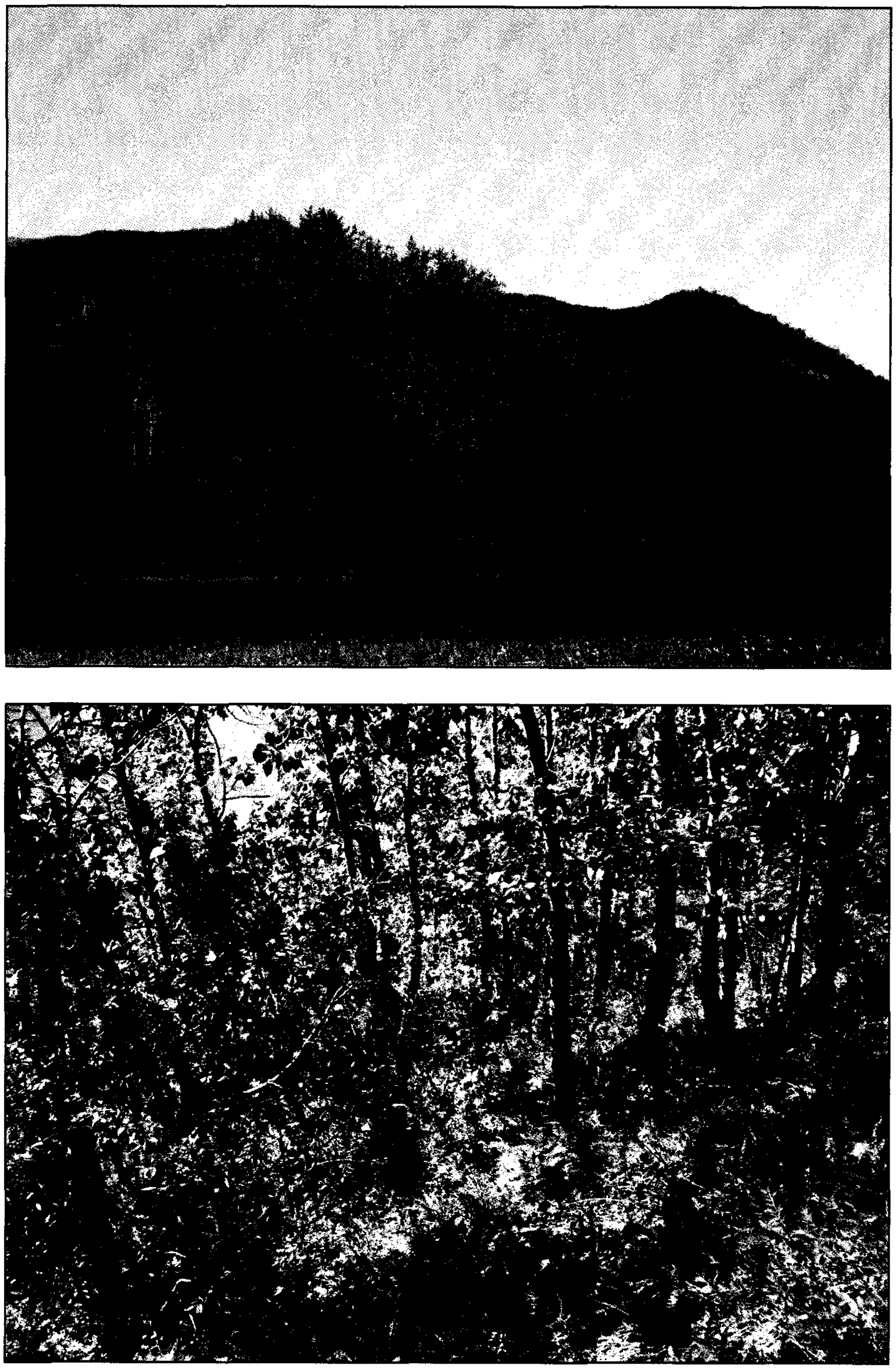

Figure 3.6 *Androsace chamaejasme* variant of the Salicetosum alaxensis var. alaxensis subassociation on a south-facing slope adjacent to the Noatak River in Arctic Alaska (Relevé 3).

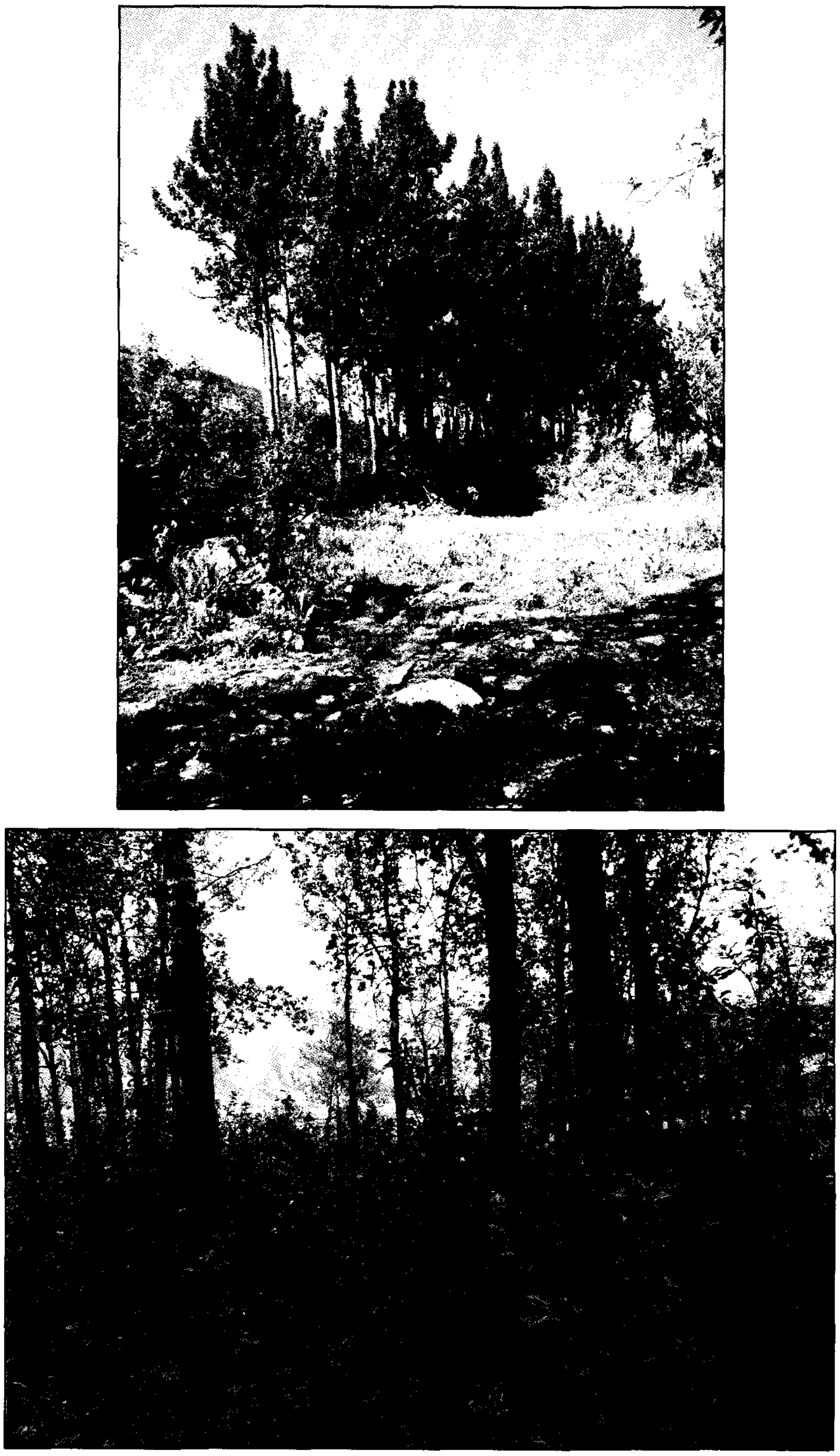

Figure 3.7 *Cystopteris montanum* variant of the Salicetosum alaxensis var. alaxensis subassociation at Ivishak Hot Springs in Arctic Alaska (Relevé 15).

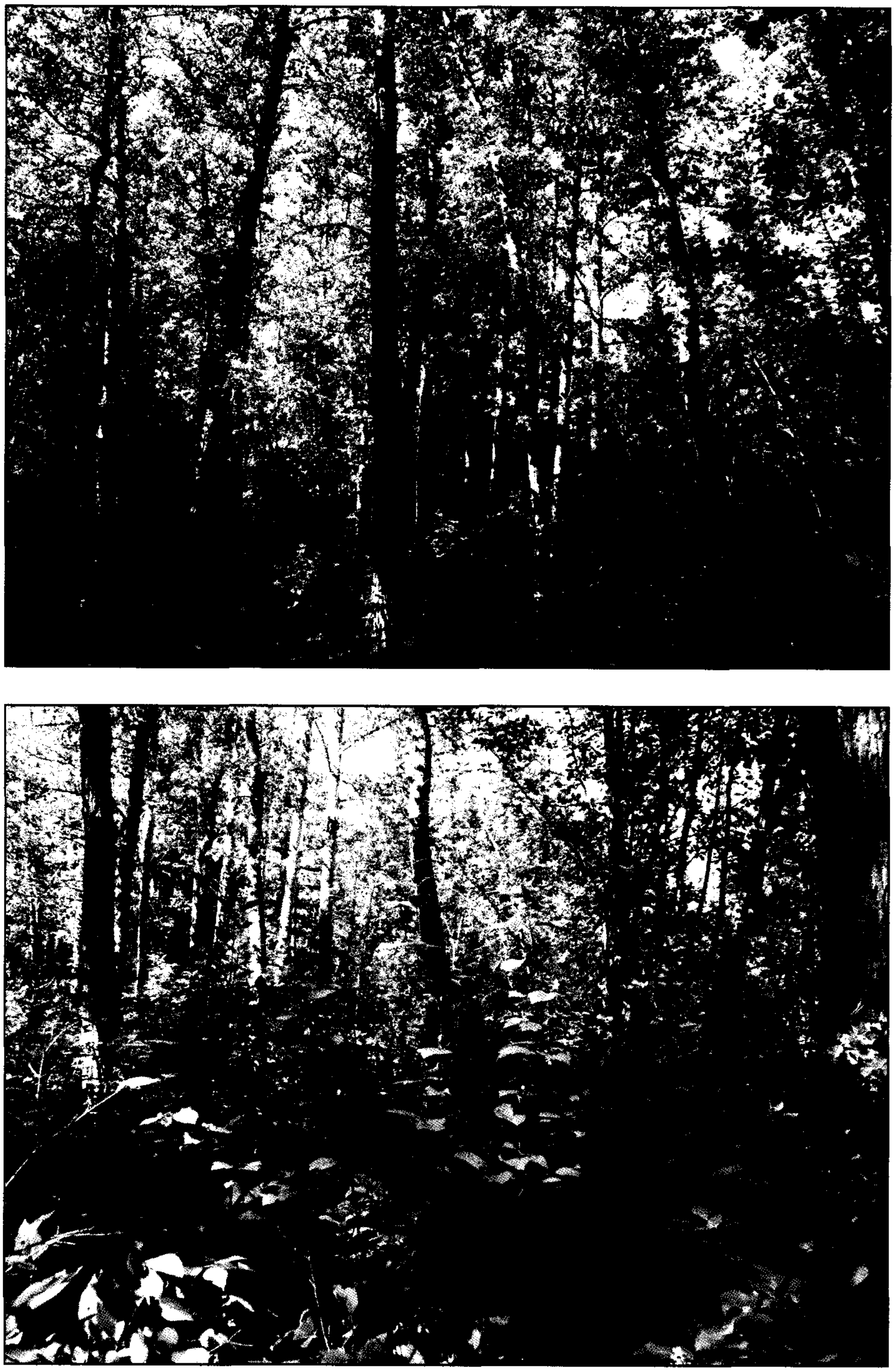

Figure 3.8 Rositosum acicularis subassociation within the boreal forest on the upper Yukon River, Yukon Territory (Relevé 31).

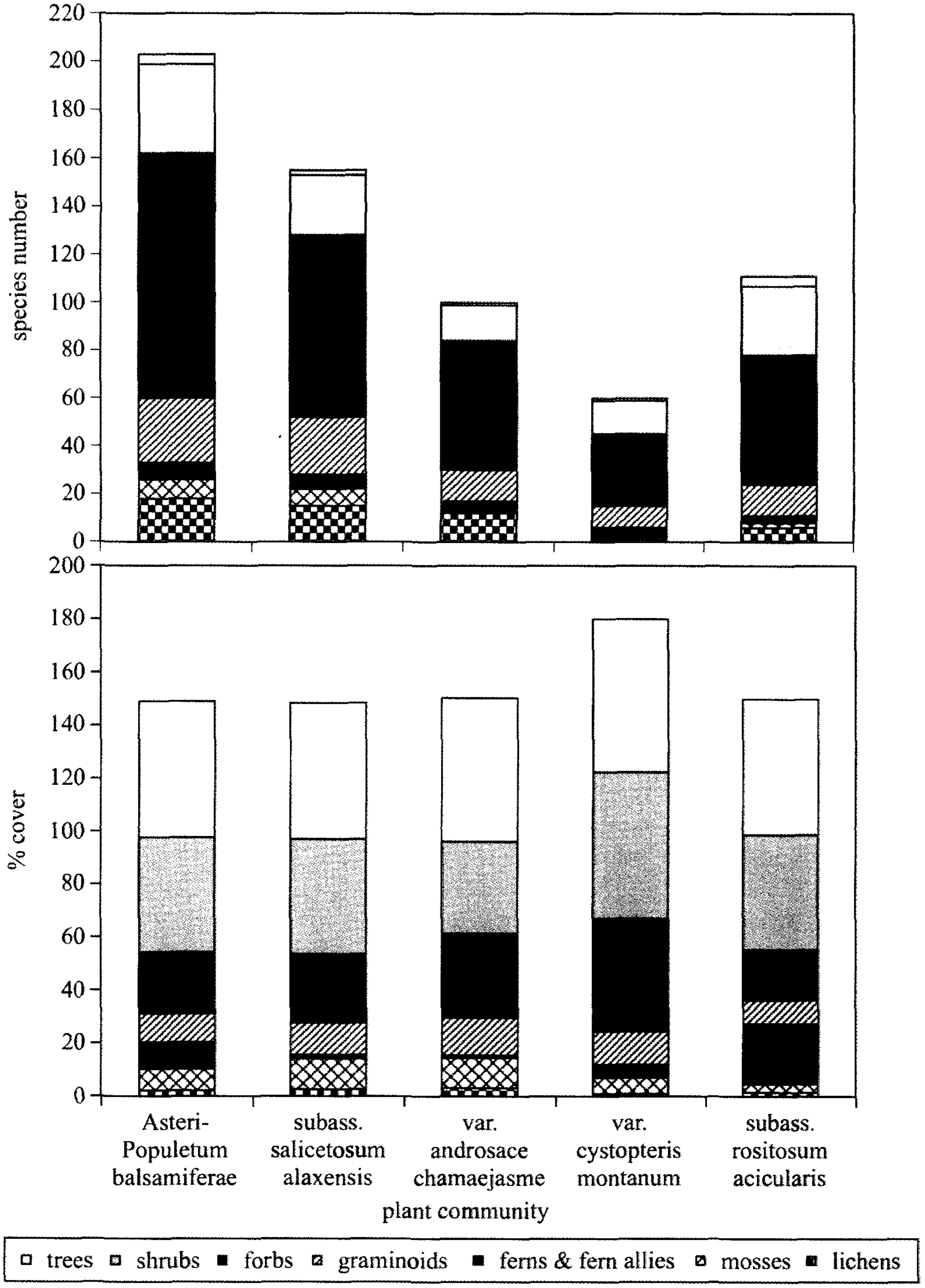

Figure 3.9 Analysis of species richness and functional types in the *P. balsamifera* communities. Plant functional types are show as total species numbers and percent cover values.

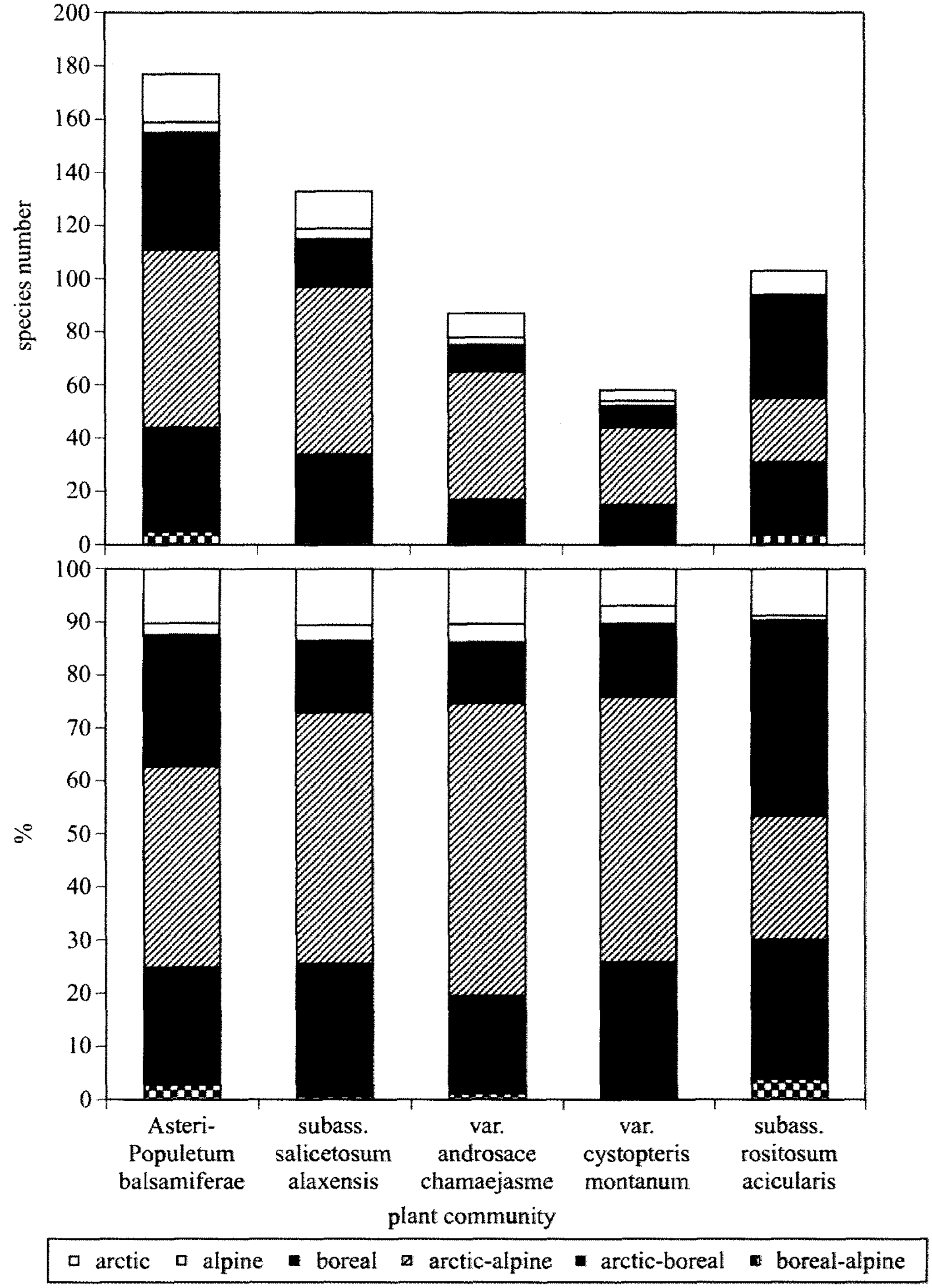

Figure 3.10 Floristic analysis for vascular species of the *P. balsamifera* communities. Major regional untis are show as total species numbers and percent values.

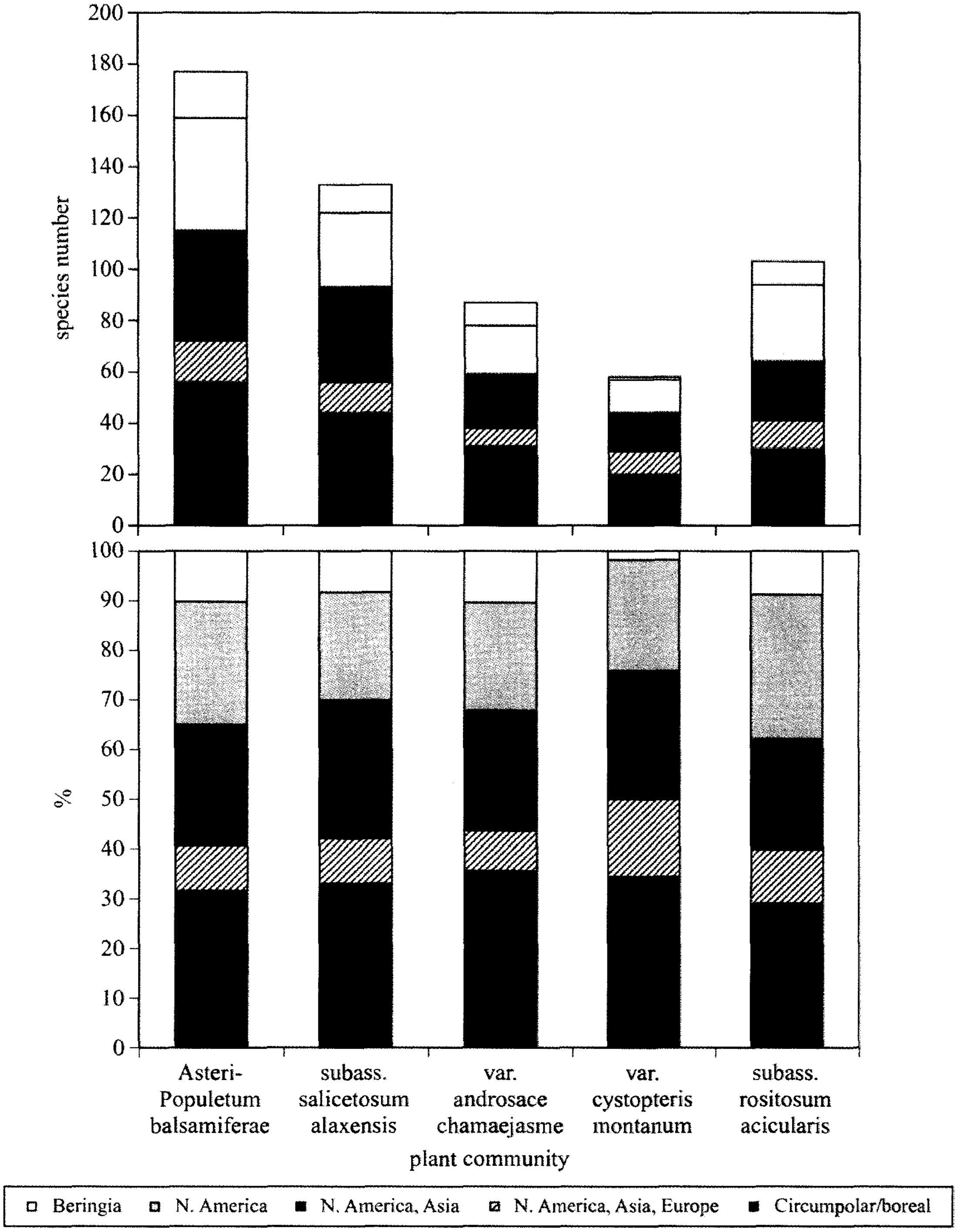

Figure 3.11 Floristic analysis for vascular species of the *P. balsamifera* communities. Geographic ranges are shown as total species numbers and percent values.

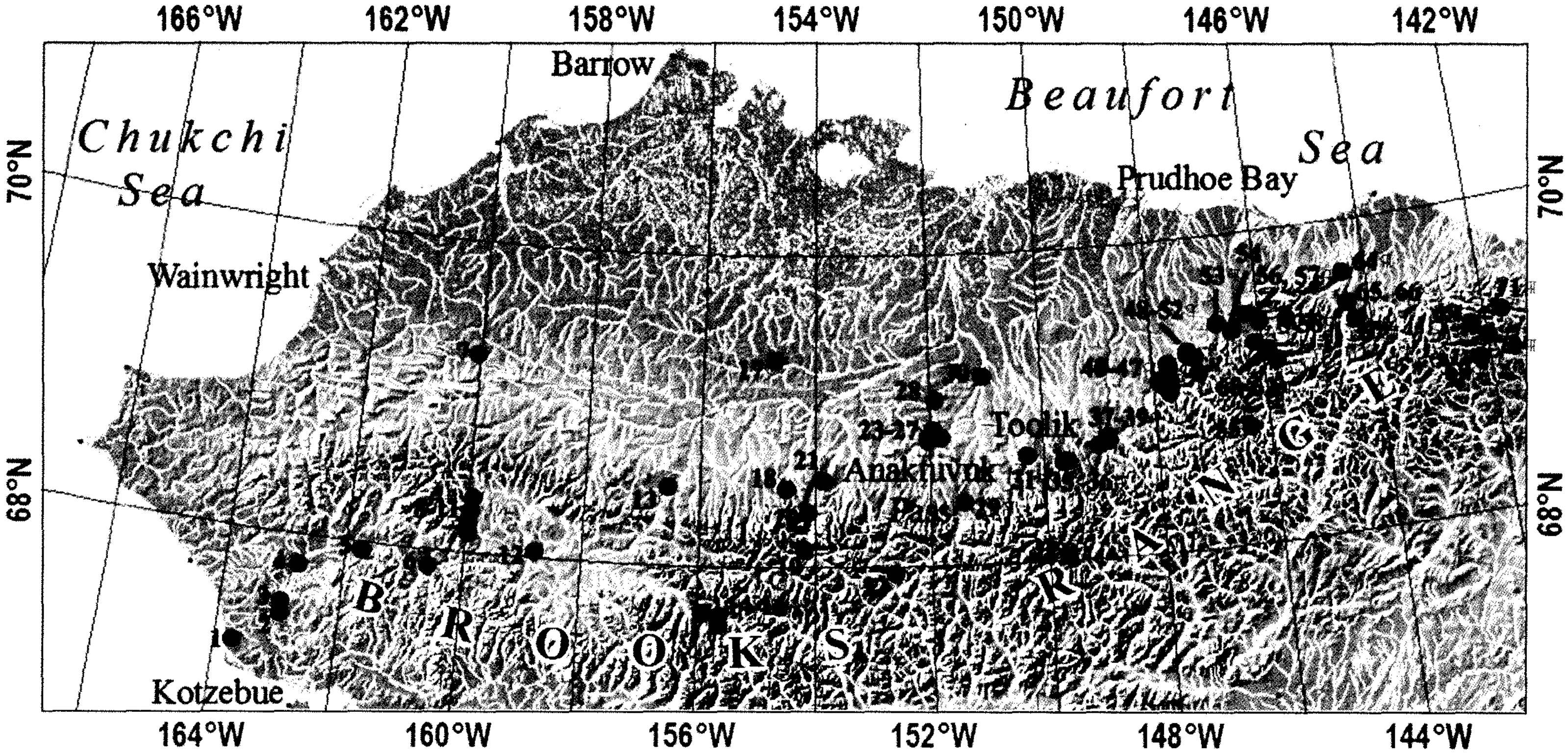

Figure 3.12 Map showing topography and balsam poplar occurrences in arctic Alaska. The balsam poplar groves are numbered as in Table 3.4.

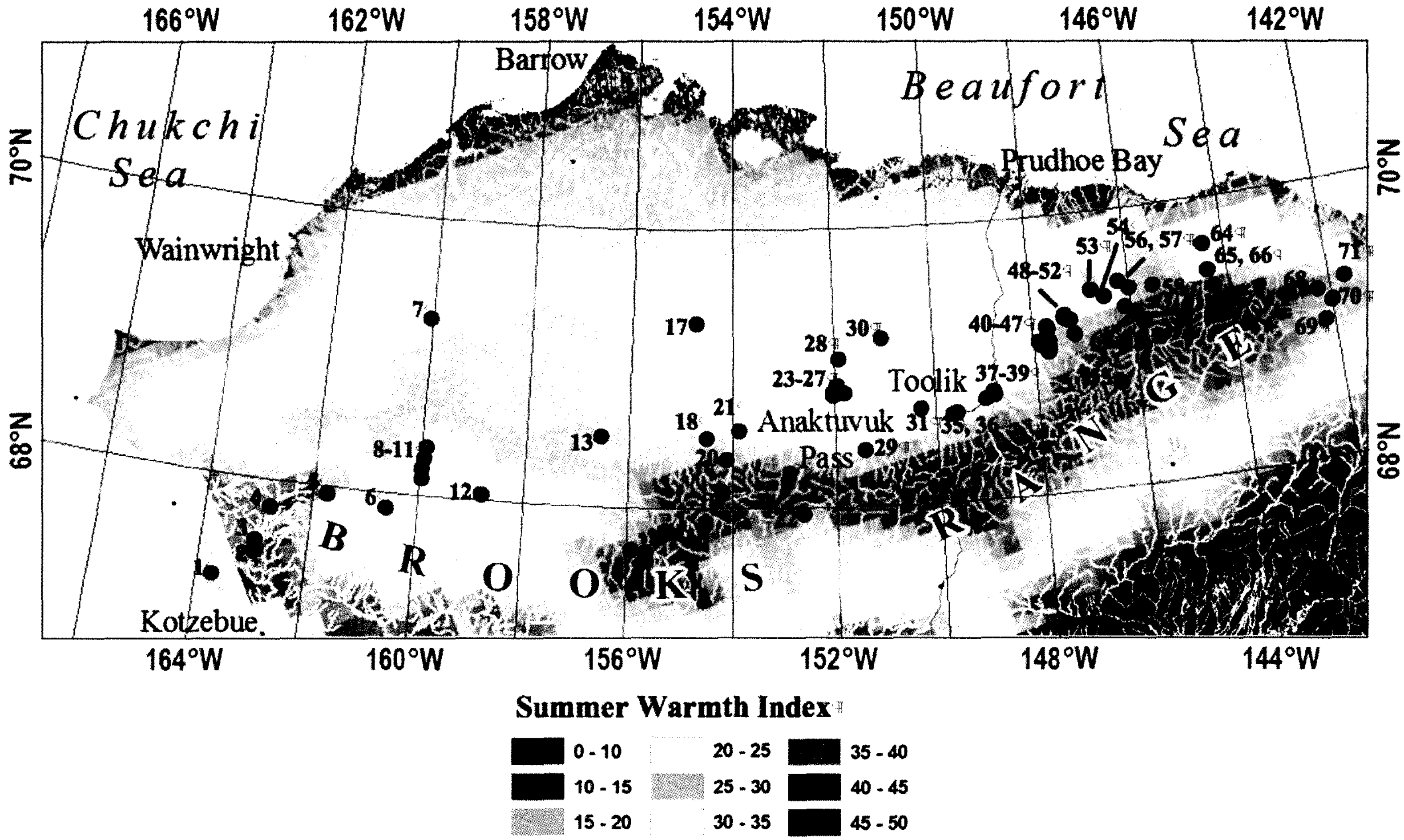

Figure. 3.13 Map showing summer warmth index and balsam poplar occurrences in arctic Alaska. Summer warmth index is the sum of mean monthly temperatures > 0° C from May to September and was used to characterize the amount of summer warmth available for plant growth at each site. The balsam poplar groves are numbered as in Table 3.4.

Table 3.1 Location of *Populus balsamifera* relevés.

Reléve No.	Locality	Field relevé reference	Latitude, longitude (°N, °W)	Elevation (m)
1	Noatak River	NOA4	67.8084,-162.7422	41
2	Noatak River	NOA3	67.8973,-160.5698	157
3	Noatak River	NOA2	68.0438,-158.7587	293
4	Noatak River	NOA1	67.7051,-155.8914	524
5	Kobuk River	KOB4	67.1862,-159.3250	12
6	Kobuk River	KOB3	67.0460,-158.9506	50
7	Kobuk River	KOB2	66.9971,-157.6482	20
8	Kobuk River	KOB1	66.8569,-156.3168	65
9	Dietrich River	DIE1	68.0351,-149.6573	792
10	Toolik Lake	TOO1	68.6289,-149.6529	761
11	Sagavanirktok River	SAG1	68.7769,-148.8480	480
12	Ivishak River	IVI4	69.1024,-147.8944	318
13	Ivishak River	IVI3	69.0476,-147.7095	386
14	Ivishak River	IVI2	69.0540,-147.7110	417
15	Ivishak River	IVI1	69.0238,-147.7187	369
16	Canning River	CAN3	69.4147,-145.8812	351
17	Canning River	CAN2	69.2419,-145.8886	458
18	Canning River	CAN1	69.1607,-145.7605	450
19	Hulahula River	HUL1	69.4674,-144.3665	412
20	Hulahula River	HUL2	69.4694,-144.3705	428
21	Kongakut River	KON3	69.2629,-141.6809	398
22	Kongakut River	KON2	69.1086,-141.9913	565
23	Kongakut River	KON1	68.9785,-142.1850	604
24	Porcupine River	POR4	66.7918,-144.1121	161
25	Porcupine River	POR3	67.0666,-142.5003	188
26	Porcupine River	POR2	67.2756,-141.6276	219
27	Porcupine River	POR1	67.5275,-139.9232	247
28	Yukon River	YUK10	64.6550,-156.4706	41
29	Yukon River	YUK9	64.8464,-155.1548	58
30	Yukon River	YUK8	65.1891,-152.7477	91
31	Yukon River	YUK2	62.2829,-136.3140	495
32	Yukon River	YUK1	61.0750,-135.2210	664

Table 3.2 Community table of Eurybiae sibirica-Populetum balsamiferae ass. nova

	salicetosum alaxensis var. alaxensis, arctic group																			rositosum acicularis, boreal group												
	riparian relevés											south-facing slope relevés						spring relevés		riparian relevés												
Relevé No.	2	23	12	14	17	16	4	22	1	9	18	19	20	10	21	11	3	15	13	32	29	26	31	8	27	30	7	5	25	28	24	6
Field relevé reference	NOA3	KON1	IVI4	IVI2	CAN2	CAN3	NOA1	KON2	NOA4	DIE1	CAN1	HUL1	HUL2	TOO1	KON3	SAG1	NOA2	IVI1	IVI3	YUK1	YUK9	POR2	YUK2	KOB1	POR1	YUK8	KOB2	KOB4	POR3	YUK10	POR4	KOB3
Number of vascular taxa	32	30	40	38	19	33	24	27	31	28	21	48	28	25	37	26	26	50	32	36	19	34	20	26	36	20	23	32	22	14	16	25
Number of nonvascular taxa	0	0	2	2	6	4	0	0	0	0	3	3	4	6	4	0	0	1	1	0	3	2	1	0	0	1	0	0	0	3	0	5
Total number of taxa	32	30	42	40	25	37	24	27	31	28	24	51	32	31	41	26	26	51	33	36	22	36	21	26	36	21	23	32	22	17	16	30
Association: Eurybiae sibirica-Populetum balsamiferae																																
Populus balsamifera	3	4	4	4	3	4	4	5	3	3	3	3	4	4	4	3	4	4	3	2	3	3	2	4	3	4	4	4	4	3	4	3
Eurybia sibirica	.	1	1	1	2	2	+	2	1	1	2	2	1	1	+	2	1	2	1	1	2	r	.	3	2	1	1	2	1	+	1	.
Hedysarum americanum	1	1	1	1	1	2	r	1	1	1	1	2	+	1	.	+	2	1	.	1	r	1	1	+	2	.	.	2	+	.	1	1
Chamerion angustifolium ssp. angustifolium	.	.	1		r	1	.	.	1	2	.	1	1	3	.	2	1	2	2	+	.	1	.	2	1	r	1	1	+	.	1	1
Equisetum arvense	+	.	1	1	.	.	+	.	r	.	.	2	.	.	.	.	+	1	+	2	2	2	1	4	2	4	1	2	3	+	2	2
Anticlea elegans	2	1	+	+	.	2	.	.	1	.	+	.	2	2	2	2	2	1	1	.	.	+	.	+	1	.	.	1	+	.	.	+
Shepherdia canadensis	2	2	2	2	3	3	1	3	2	2	3	.	r	.	1	.	.	r	.	r	.	2	.	.	3	.	.	.	1	.	+	2
Subassociation: salicetosum alaxensis var. alaxensis, arctic group																																
Senecio lugens	1	1	1	1	r	2	.	1	.	.	.	2	1	1	1	1	1	1	1	.	.	+	.	.	1	.	.	.	.	.	.	.
Salix alaxensis var. alaxensis	1	1	2	1	2	2	2	2	1	1	2	2	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Arctous rubra	2	2	2	3	2	3	1	1	2	2	4	.	.	.	.	r	r	.	+	2	.	+	.	.	+	.	.	1	.	.	.	+
Dasiphora fruticosa	2	+	1	1	.	.	+	.	1	.	.	1	2	2	1	2	2	r	1	+	.	.	.	.	.	.	.	.	.	.	.	.
Festuca altaica	2	2	1	1	.	2	.	.	.	2	1	.	.	2	1	1	.	1	1	.	.	+	.	.	.	.	.	.	.	.	.	.
Anemone parviflora	+	1	1	+	.	1	.	1	1	.	+	1	.	.	2	+	.	1	.	1	.	.	.	.	.	.	.	+	.	.	.	.
Pyrola grandiflora	r	2	1	.	.	.	+	2	+	1	2	.	.	.	1	+	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	.
Delphinium chamissonis	.	.	2	1	2	2	.	r	.	.	.	1	r	.	1	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Betula glandulosa/nana ssp. exilis	.	.	r	+	.	1	+	.	.	2	.	r	.	.	r	1	.	r	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Salix glauca ssp. stipulifera	2	1	.	.	2	1	2	.	.	.	3	.	3	.	.	2	.	1	.	.	.	.	.	.	.	.	.	2	.	.	.	+
Stellaria longipes	.	r	.	+	1	1	.	+	.	1	.	+	.	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gentianella propinqua	.	.	.	+	.	+	.	.	.	1	+	r	r	+	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Calamagrostis purpurascens	1	+	.	.	.	.	.	.	.	.	1	+	2	.	1	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Solidago multiradiata	+	.	1	.	.	.	.	.	.	+	.	.	1	1	1	1	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Aconitum delphinifolium var. delphinifolium	.	.	1	+	.	2	.	.	.	1	.	1	.	1	.	.	.	1	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Polemonium acutiflorum	.	.	2	+	.	1	.	+	.	+	.	1	.	.	.	.	.	2	1	+	.	.	.	.	.	.	.	.	.	.	.	.
Bistorta vivipara	.	.	1	+	.	1	r	.	.	.	+	+	.	.	+	.	.	+	.	r	.	.	.	.	.	.	.	.	.	.	.	.
Salix reticulata	.	.	1	+	.	.	r	.	.	.	.	+	.	.	.	.	.	+	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Lupinus arcticus	1	.	.	.	+	1	.	2	.	.	1	.	.	.	1	2	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Pedicularis capitata	.	r	.	+	.	+	.	.	.	.	r	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Stellaria laeta	.	.	+	.	.	.	1	.	.	.	.	+	+	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Vaccinium uliginosum ssp. microphyllum	1	.	.	r	.	.	+	.	.	2	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Valeriana capitata	.	.	2	+	.	1	.	.	.	.	.	1	.	.	.	.	.	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Chamerion latifolium	.	.	+	+	+	.	.	.	.	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Arnica griscomii ssp. frigida	.	+	.	.	.	.	.	1	.	.	+	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Arnica angustifolia ssp. angustifolia	1	.	+	.	.	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex capillaris	+	.	+	.	.	.	.	.	.	.	.	1	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex krausei	+	.	.	.	.	.	.	+	.	.	.	1	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Festuca rubra ssp. richardsonii	.	.	.	.	.	.	.	.	+	.	1	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Flavocetraria cucullata	.	.	.	.	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Phlox alaskensis	.	.	.	r	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oxytropis jordalii	1	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Botrichium lunaria	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	+	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Juncus castaneus	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cerastium beeringianum	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa arctica	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Saxifraga hirculus	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Leymus innovatus	r	1	2	1	.	.	2	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Peltigera elisabethae	.	.	1	1	1	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dryas integrifolia ssp. integrifolia	1	2	.	r	.	.	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bistorta plumosa	.	r	.	.	.	.	r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lysiella obtusata	+	.	.	.	.	.	r	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sanionia uncinata	.	.	.	.	2	+	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pedicularis verticillata	1	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Festuca rubra	.	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hylocomium splendens	.	.	.	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Abietinella abietina	.	.	.	.	.	2	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Tofieldia pusilla	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pedicularis interior	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex membranacea	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex scirpoidea ssp. scirpoidea	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Leptogium saturninum	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pedicularis lapponica	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rhytidium rugosum	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oxytropis koyukukensis	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Astragalus umbellatus	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Empetrum nigrum	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Salix pulchra	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pedicularis labradorica	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 3.2 (cont.)

	salicetosum alaxensis var. alaxensis, arctic group																			rositosum acicularis, boreal group												
	riparian relevés											south-facing slope relevés						spring relevés		riparian relevés												
Relevé No.	2	23	12	14	17	16	4	22	1	9	18	19	20	10	21	11	3	15	13	32	29	26	31	8	27	30	7	5	25	28	24	6
Field relevé reference	NOA3	KON1	IVI4	IVI2	CAN2	CAN3	NOA1	KON2	NOA4	DIE1	CAN1	HUL1	HUL2	TOO1	KON3	SAG1	NOA2	IVI1	IVI3	YUK1	YUK9	POR2	YUK2	KOB1	POR1	YUK8	KOB2	KOB4	POR3	YUK10	POR4	KOB3
Variant: androsace chamaejasme, south-facing slopes																																
Androsace chamaejasme ssp. andersonii	.	.	.	.	.	.	.	.	.	.	.	r	1	r	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex obtusata	.	.	.	.	.	.	.	.	.	.	.	1	+	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bupleurum arcticum	.	.	.	.	.	.	.	.	.	.	.	.	.	+	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Anemone drummondii	.	.	.	.	.	.	.	.	.	.	.	1	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potentilla nivea x arenosa	.	.	.	.	.	.	.	.	.	.	.	r	.	1	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Saxifraga tricuspidata	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Micranthes reflexa	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cladonia pocillum	.	.	.	.	.	.	.	.	.	.	.	+	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potentilla arenosa ssp. arenosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cystopteris fragilis	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Artemisia kruhsiana ssp. alaskana	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Thamnolia subuliformis	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potentilla nivea	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Flavocetraria nivalis	.	.	.	.	.	.	.	.	.	.	.	+	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cardamine pratensis ssp. angustifolia	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chyrsosplenium tetrandrum	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Draba praealta	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potentilla litoralis	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oxytropis borealis	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Peltigera extenuata	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dryas ajanensis	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Flavocetraria cucullata x nivalis	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Arctous alpina	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex albonigra	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cladonia symphycarpia	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dicranum dispersum	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ochrolechia frigida	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Stellaria calycantha	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Minuartia arctica	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hedysarum boreale ssp. mackenzii	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lecanora epibryon	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Thalictrum alpinum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Peltigera praetextata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Selaginella sibirica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pulsatilla patens ssp. multifida	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Draba cinerea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Variant: cystopteris montanum, adjacent to springs																																
Carex podocarpa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Cystopteris montana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex atrosquama	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Artemisia arctica ssp. arctica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dodecatheon frigidum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Epilobium hornemanii ssp. hornemanii	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Luzula parviflora ssp. parviflora	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Myosotis alpestris ssp. asiatica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Micranthes nelsoniana ssp. nelsoniana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sciuro-hypnum ornellanum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Subassociation: rositosum acicularis, boreal group																																
Rosa acicularis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	2	3	3	1	1	3	.	2	.	2	1
Picea glauca	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	2	2	r	1	1	+	1	.	r	1	.	r	2
Artemisia tilesii	.	.	.	.	.	.	.	1	.	1	.	2	.	.	1	.	.	1	.	.	1	+	+	2	1	1	1	1	1	+	.	.
Galium boreale	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	.	1	+	+	3	1	.	2	2	+	.	1	.
Viburnum edule	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	3	3	+	2	.	3	3	.	1	3	.	1	1	.	.
Salix alaxensis var. longistylis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	2	1	2	1	1	2	.
Pyrola asarifolia var. incarnata	.	.	.	.	2	1	.	.	.	.	.	.	.	.	.	.	.	1	.	1	1	1	+	.	2	r	.	.	.	1	.	.
Alnus incana ssp. tenuifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	3	+	1	.	.	2	.	.	1	2	.	.
Mertensia paniculata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	1	.	2	1	.	2	r	1	.	+	.
Alnus viridis ssp. fruticosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	3	2	.	3	1	.	.	2	.
Swida sericea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	3	.	r	.	.	.	+	1	2	.
Moehringia lateriflora	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	r	+	1	.	1	.	.	.	.	.
Salix bebbiana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	2	1	.	.	+	.	.	.	.	.	.
Tanacetum bipinnatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	2	.	.	.	1
Elymus alaskanus ssp. alaskanus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	1	.	.	.	.	.	.
Betula neoalaskana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	1	.	.	.	.	.	.
Boschniakia rossica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	1	.	.	.	.	.	.	.
Eleagnus commutata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	2	.
Actaea rubra	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	+	.	.	.	.	.	.	.	.	.
Fragaria virginiana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	r	.	.	.	.	.	.	.	.	.
Geocaulon lividum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.
Iris setosa var. setosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	1	.	.	.	.

Table 3.2 (cont.)

	sallcetosum alaxensis var. alaxensis, arctic group																			rositosum acicularis, boreal group												
	riparian relevés											south-facing slope relevés						spring relevés		riparian relevés												
Relevé No.	2	23	12	14	17	16	4	22	1	9	18	19	20	10	21	11	3	15	13	32	29	26	31	8	27	30	7	5	25	28	24	6
Field relevé reference	NOA3	KON1	IVI4	IVI2	CAN2	CAN3	NOA1	KON2	NOA4	DIE1	CAN1	HUL1	HUL2	TOO1	KON3	SAG1	NOA2	IVI1	IVI3	YUK1	YUK9	POR2	YUK2	KOB1	POR1	YUK8	KOB2	KOB4	POR3	YUK10	POR4	KOB3
Monses uniflora	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	+	.	.
Peltigera collina	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.
Limnorchis aquilonis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	r
Rubus idaeus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+	.	.
Sanguisorba officinalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	+	.	.	.	.
Wilhemsia physodes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	1	.	.	.	.	.
Vaccinium caespitosum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
Carex media	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
Oxytropis deflexa ssp. foliolosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Poa glauca	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Silene williamsii	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Taraxacum officinale	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.
Equisetum fluviatile	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Populus tremuloides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
Ledum groenlandicum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.
Listera borealis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.
Delphinium glaucum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Chamaepericlymenum canadense	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.
Epilobium palustre	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.
Trientalis europaea ssp. europaea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Aconogonon alaskanum var. alaskanum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.
Thalictrum sparsiflorum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.
Astragalus alpinus ssp. alpinus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Castilleja caudata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Allium schoenoprasum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Stellaria crassifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
Polemonium pulcherrimum ssp. lindleyi	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Peltigera neckeri	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.
Arctostaphylos uva-ursi	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Polytrichum piliferum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Oxytropis kobukensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Stereocaulon rivulorum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Companions																																
Bromus pumpellianus ssp. arcticus	.	.	1	1	1	1	.	1	1	.	1	1	1	+	+	1	1	.	.	.	.	1	.	.	.	.	.	2	+	.	+	2
Salix arbusculoides	.	1	.	.	1	.	+	1	3	+	.	.	.	.	1	.	3	.	.	2	.	.	.	.	1	+	+	.	+	.	+	.
Equisetum scirpoides	+	+	1	.	.	1	+	+	+	.	+	.	.	.	.	.	.	+	+	1	.	.	.	.	+	.	.	r	.	.	.	.
Salix hastata	.	1	2	3	.	1	.	1	.	1	1	.	.	.	.	.	.	2	.	.	.	.	.	1	1	.	1	+	.	.	.	.
Salix glauca ssp. acutiflora	.	.	3	3	.	.	.	.	2	1	.	.	.	2	1	.	2	.	4	2	.	+	.	.	.	1	.	.	.	.	.	.
Anemone richardsonii	.	.	1	+	.	2	r	+	1	.	.	.	.	.	.	.	.	.	+	.	.	.	.	1	r	.	1	.	.	.	.	.
Orthilia secunda	.	1	r	.	1	2	.	.	.	+	.	.	.	.	.	.	.	.	.	.	1	.	1	1	+	.	.	.	.	.	.	r
Salix niphoclada	1	1	+	.	.	.	.	.	1	.	.	3	.	.	2	.	1	.	.	2	.	.	.	.	.	.	.	1	.	.	.	.
Rubus arcticus	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	2	2	2	.	+	.	1	.	.	2	+	.	.	.	2
Parnassia palustris	1	.	r	+	.	1	.	.	.	.	.	1	.	.	.	.	.	1	.	.	.	.	.	.	.	r	.	1	.	.	.	+
Calamagrostis canadensis	.	.	.	.	.	.	.	.	1	2	.	.	.	.	.	.	.	1	1	.	r	.	+	.	1	2	.	.	.	+	.	.
Linnea borealis ssp. americana	.	.	.	1	.	.	.	.	2	1	.	.	.	.	.	.	.	2	1	1	.	1	.	1	.	.	.	.	+	.	.	.
Castilleja elegans	.	+	.	.	+	1	.	1	.	.	.	.	.	.	1	.	1	1	.	.	.	r	.	.	+	.	.	.	.	.	.	.
Arctagrostis latifolia	.	.	2	+	.	.	.	.	1	.	.	+	.	.	.	.	.	1	1	2	.	+	.	.	.	.	2	.	.	.	.	.
Cnidium cnidiifolium	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	1	.	.	.	.	.	.	.	1	.	.	1	r	.	.	1
Peltigera canina	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	+	.	.	.	.	.	.	.	.	+	.	r
Juniperus communis ssp. nana	.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	.	.	.	2	.	.	.	.	.	.	.	.	.	1	.	.	+
Carex concinna	1	1	.	+	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Poa pratensis ssp. alpigena	.	.	.	.	.	1	r	.	.	.	.	.	.	1	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
Astragalus alpinus ssp.alaskanus	.	.	.	.	.	.	.	4	.	.	.	.	.	.	1	.	.	.	.	.	.	+	.	.	1	.	.	.	.	.	.	.
Peltigera leucophlebia	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	1	.	.	.	.	.	.	r	.	.
Peltigera rufescens	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	+
Ribes triste	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	2	.	.	+	.	.	.	.	.	.	.	.	.
Rhododendron lapponicum ssp. parvifolium	+	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
Astragalus eucosmus	.	+	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.
Salix richardsonii	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	2
Kobresia myosuroides	.	.	.	.	.	.	.	.	.	.	.	+	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Petasites frigidus ssp. frigidus	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	.
Aulacomnium palustre	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	+
Achillea millefolium ssp. borealis	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	+	.	.	.	.	.	.	.
Corallorrhiza trifida	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Calamagrostis neglecta	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Cypripedium passerinum	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
Hierochloe odorata	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Oxytropis varians	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
Petasites frigidus ssp. nivalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	r	.	.	.	.	.	.	.
Vaccinium vitis-idaea ssp. minus	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.

Table 3.3 Mean (± SE) values of select metric characteristics of balsam poplar communities.

	Eurybia sibirica-Populetum balsamiferae	subass. salicetosum alaxensis var. alaxensis	variant androsace chamaejasme	variant cystopteris montanum	subass. rositosum acicularis
N	32	19	6	2	13
Tree characteristics					
Canopy height (m)	9.76±0.75	7.52±0.75	4.79±1.03	9.38±1.78	13.05±0.93
Density of stand (trees/ha)	2223.31±312.35	1841.82±293.24	2806.69±710.96	1899.03±244.33	2780.87±623.09
Basal area of stand (m^2/ha)	198.92±37.15	141.13±17.48	90.14±31.65	178.68±91.81	283.39±84.30
DBH[a] of poplar trees (cm)	14.26±2.50	12.93±0.69	11.97±1.57	14.04±4.07	16.00±2.21
Stand characteristics					
Shrub height (m)	2.46±0.44	1.65±0.13	1.30±0.27	1.75±0.25	3.65±0.45
Sapling cover (%)	17.91±3.17	20.79±2.54	17.50±4.79	17.50±2.50	13.69±2.10
Litter depth (cm)	2.22±0.39	1.51±0.36	2.25±0.89	1.75±0.25	3.27±0.60
Physical characteristics					
Slope angle (°)	11.09±1.96	15.00±4.91	45.00±3.16	3.50±3.50	5.38±4.02
Elevation (m)	328.59±58.09	431.79±40.90	462.00±64.82	377.50±8.50	177.77±54.65
SWI[b]	35.97±6.36	28.48±1.56	32.68±1.52	26.80±0.00	46.90±1.60
Site moisture (1-10)	4.91±0.87	4.58±0.19	3.83±0.31	5.00±0.00	5.38±0.31
Soil moisture (1-10)	3.50±0.62	3.89±0.29	3.67±0.56	4.00±0.00	2.92±0.14
Snow duration (1-10)	4.31±0.76	4.37±0.19	3.83±0.31	4.00±0.00	4.23±0.17

[a] diameter at breast height

[b] summer warmth index

Table 3.4 Balsam poplar occurrences north of treeline in arctic Alaska.

Stand	Locality	Latitude (°N)	Longitude (°W)	Source
1	Kakagrak Hills	67.2700	-163.6700	ALA[a]
2	Eli River	67.4800	-162.9600	ALA
3	Noatak River	67.5600	-162.9600	ALA
4	Noatak River	67.8084	-162.7422	A. Breen
5	Sekuiak Bluff, Noatak River	67.9500	-161.6900	ALA
6	Sisiak Creek, Noatak River	67.8973	-160.5698	A. Breen
7	Utukok River	69.2716	-160.0150	D. Mann
8	Nimiuktuk River	68.1999	-159.9399	A. Balser
9	Nimiuktuk & Noatak Rivers	68.1239	-159.9348	A. Breen
10	Nimiuktuk River	68.2700	-159.9100	ALA
11	Nimiuktuk River	68.3500	-159.8833	A. Breen
12	Noatak River	68.0438	-158.7587	A. Breen
13	Etivluk River	68.5002	-156.5400	CHAPMAN *et al.* 1964
14	Nigikpalvgurururak Creek, Noatak River	67.7051	-155.8914	A. Breen
15	Kugrak Springs	67.6237	-155.6219	A. Balser
16	Noatak River	67.6849	-155.6127	A. Balser
17	Kigalik River	69.3230	-154.6930	D. Mann
18	Oolamnagavik River	68.5002	-154.4781	CHAPMAN *et al.* 1964
19	Easter Creek, Killik River	68.1167	-154.1667	BOCKHEIM *et al.* 2003
20	Ivisak Creek, Killik River	68.3500	-154.1000	ALA
21	Killik River	68.6572	-153.7319	CHAPMAN *et al.* 1964
22	Loon Lake	67.9583	-152.6167	STAENDER & STAENDER 1970
23	Chandler River	68.8300	-152.0000	BOCKHEIM *et al.* 2003
24	Chandler River	68.8008	-151.9969	K. Tape
25	Chandler River	68.8333	-151.9667	A. Breen
26	Chandler & Siksikpuk Rivers	68.8680	-151.9249	K. Tape
27	Siksikpuk River	68.8199	-151.7809	K. Tape
28	Chandler River	69.0603	-151.8749	K. Tape
29	Anaktuvuk River	68.4000	-151.4170	BOCKHEIM *et al.* 2003
30	Anaktuvuk River	69.2007	-151.0164	T. Hollingsworth
31	May Creek	68.6836	-150.2910	M. Sturm

Table 3.4 (cont.)

Stand	Locality	Latitude (°N)	Longitude (°W)	Source
32	Dietrich River	68.0351	-149.6573	A. Breen
33	Itkillik River	68.8989	-150.0855	C. Johnson
34	Itkillik River	68.9310	150.1149	C. Johnson
35	Toolik Lake, Jade Mountain	68.6289	-149.6529	A. Breen
36	Toolik Lake, old camp pad	68.6403	-149.5746	A. Breen
37	Slope Mountain, Oksrukuyik Creek	68.7247	-149.0100	S. Walker
38	Sagavanirktok River	68.7769	-148.8480	A. Breen
39	Sagavanirktok River	68.7500	-148.8300	Bockheim *et al.* 2003
40	Cottonwood Creek, Ivishak River	69.1024	-147.8944	A. Breen
41	Ivishak River	69.0833	-147.8167	Bockheim *et al.* 2003
42	Ivishak River	69.0667	-147.8167	Bockheim *et al.* 2003
43	Ivishak Hot Springs	69.0238	-147.7187	A. Breen
44	Ivishak River	69.0540	-147.7110	A. Breen
45	Ivishak River	69.0476	-147.7095	A. Breen
46	Gilead Creek	69.2000	-147.7000	Bockheim *et al.* 2003
47	Gilead Creek	69.1830	-147.7000	Bockheim *et al.* 2003
48	Ivishak (Echooka) River	69.1000	-147.7000	Bockheim *et al.* 2003
49	Echooka Springs	69.2672	-147.3531	S. Parker
50	Echooka River	69.2500	-147.3500	Bockheim *et al.* 2003
51	Echooka River	69.2300	-147.2170	Bockheim *et al.* 2003
52	Echooka River	69.1300	-147.1700	Bockheim *et al.* 2003
53	Juniper Creek	69.4300	-146.7600	Bockheim *et al.* 2003
54	Kavik River	69.3700	-146.5000	Bockheim *et al.* 2003
55	Porcupine Lake[b]	68.7500	-146.3300	Bockheim *et al.* 2003
56	Canning River	69.4667	-146.2000	Bockheim *et al.* 2003
57	Cache Creek, Canning River	69.4167	-145.9999	A. Breen
58	Ikiakpuk Valley, Cache Creek	69.4160	-145.5000	Bockheim *et al.* 2003
59	Shulbik Springs	69.4500	-146.0500	D. Murray
60	Canning River	69.2833	-146.1000	Bockheim *et al.* 2003
61	Marsh Fork, Canning River	69.2170	-145.9000	Bockheim *et al.* 2003
62	Canning River	69.2419	-145.8886	A. Breen

Table 3.4 (cont.)

Stand	Locality	Latitude (°N)
63	Franklin Creek, Canning River	69.1607
64	Sadlerochit Springs	69.6583
65	Hulahula River	69.4694
66	Hulahula River	69.4674
67	Old Man Creek, Hulahula River	69.3500
68	Egaksrak River[b]	69.2000
69	Kongakut River	68.9785
70	Kongakut River	69.1086
71	Kongakut River	69.2629

[a] Herbarium of the University of Alaska Museum of the North

[b] Identified from color-infrared photos and not ground-truthed

Longitude (°W)	Source
-145.7605	A. Breen
-144.3999	D. Murray
-144.3705	A. Breen
-144.3665	A. Breen
-144.2700	BOCKHEIM *et al.* 2003
-142.2500	BOCKHEIM *et al.* 2003
-142.1850	A. Breen
-141.9913	A. Breen
-141.6809	A. Breen

CHAPTER FOUR: NUCLEOTIDE DIVERSITY AMONG NATURAL POPULATIONS OF A NORTH AMERICAN POPLAR (*Populus balsamifera* L., Salicaceae)[1]

4.1 Summary

- Poplars (*Populus* spp.) comprise an important component of circumpolar boreal forest ecosystems and are the model species for tree genomics. In this study, we surveyed genetic variation and population differentiation in three nuclear genes among populations of balsam poplar (*Populus balsamifera*) in North America.
- We examined nucleotide sequence variation of *Adh1* and *G3pdh*, two well-studied nuclear loci in plants, and *ABI1B*, a locus coincident with timing of seasonal dormancy in QTL studies of hybrid poplars. We compare estimates of baseline population genetic parameters of these loci with studies of other poplar species, particularly European aspen (*P. tremula*).
- Average pairwise nucleotide diversity ($\pi_{tot} = 0.00216$-0.00353) was equivalent to *P. trichocarpa*, but markedly less than in *P. tremula*. Elevated levels of population structure were observed in *ABI1B* between the northern and southern regions ($F_{CT} = 0.184, p < 0.001$) and among populations ($F_{ST} = 0.256, p < 0.001$).
- These results suggest geographic or taxonomic factors are important for understanding patterns of variation throughout the genus *Populus*. Our findings have potential to aid in the design of sampling regimes for conservation and breeding stock

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[1] Breen, A. L., Glenn, E., Yeager, A. & Olson, M. S. (2009) Nucleotide diversity among natural populations of a North American poplar (*Populus balsamifera* L., Salicaceae). *New Phytologist* 182: 763-773.

- as well as contribute to historical inferences that shaped genetic diversity of boreal plant species.

4.2 Introduction

The North America boreal forest spans the continent, extending more than 10° of latitude from central Labrador westward through Canada to interior Alaska. Climatological gradients across the region largely reflect latitude with temperature and net radiation decreasing to the north (Elliott-Fisk 2000). Periods of major climate fluctuations, including alternating glacial and interglacial cycles, predominate in the history of the boreal forest. During the late-Quaternary and previous glaciations, the boreal region was covered with glacial ice. Boreal forest organisms were largely displaced south of their current limits in North America during glaciation events and migrated northward when the climate warmed and glaciers receded (Juday *et al.* 2005). Genetic diversity in key boreal forest organisms may reveal signals of historical demography and adaptation to climate, thus providing a context for understanding species responses to future climate change and development of conservation strategies.

The genus *Populus* (aspen, cottonwood and poplar; collectively referred to as poplars) comprise an important, and sometimes dominant, component of circumpolar boreal forest ecosystems. Poplars also are firmly established as the model species for tree genomics, with the recent publication of the full genome of western black cottonwood, *Populus trichocarpa* (Tuskan *et al.* 2006). The general picture of nucleotide variation in poplar is underdeveloped and at present is based almost exclusively on European aspen, *Populus tremula* (Ingvarsson 2005a, 2005b, 2008; Ingvarsson *et al.* 2006; Garcia & Ingvarsson 2007); the single exception is a study of nucleotide variation in *P. trichocarpa* across its coastal range in northwestern North America (Gilchrist *et al.* 2006).

Poplars are long-lived trees characterized by a dioecious breeding system, wind dispersal of pollen and seeds, clonality, and often continental-scale distribution. As a result, poplars are potentially comprised of interbreeding populations of immense size. These life history traits typify a plant expected to exhibit abundant genetic variation and little population differentiation (Hamrik & Godt 1996; Brunner *et al.* 2004). Undoubtedly, other aspects of individual poplar species' biology such as hybridization, introgression, migration and demographic history also influence genetic diversity and effective population size. Recent estimates of population genetic parameters in *P. tremula* indicate relatively high levels of genetic variation (77 loci; $\pi_{sil} = 0.0120$; Ingvarsson 2008) compared to coniferous trees (mean π_{sil} ranges from 0.0038 in *Cryptomeria japonica* to 0.0064 in *Pinus taeda*; Kado *et al.* 2003, Brown *et al.* 2004). This species-wide level of silent polymorphism is equivalent to that found in other outcrossing plant species including *Arabidopsis lyrata* ssp. *petrea* (mean $\pi_{sil} = 0.029$; Wright *et al.* 2003) and *A. halleri* ($\pi_{sil} = 0.015$; Ramos-Onsins *et al.* 2004).

The influence of geographic or taxonomic factors on patterns of variation throughout the genus *Populus* is an open question. *Populus tremula* exhibits levels of nucleotide variation over 5-fold greater than that found in *P. trichocarpa* (mean $\pi_{sil} = 0.0029$; Gilchrist *et al.* 2006), a North American poplar. Moreover, moderate population differentiation exists in *P. tremula* (for 11 loci F_{ST} ranges from 0.040-0.214; Ingvarsson

2005a, 2005b). In contrast, isozyme and microsatellite surveys in North American poplars have found little to no population differentiation (*P. balsamifera*: for 8 isozyme loci F_{ST} ranges from 0.008-0.023, Farmer *et al.* 1988; *P. deltoides*: mean F_{ST} for 22 isozyme loci = 0.064 (range not reported), Marty 1984; *P. trichocarpa:* mean F_{ST} for 12 isozyme loci = 0.063 (range not reported), Weber & Stettler 1981; *P. tremuloides*: for 10 isozyme loci F_{ST} ranges from -0.006-0.061, Lund *et al.* 1992; for 16 microsatellite loci F_{ST} ranges from 0.006-0.045, Cole 2005; mean F_{ST} for 15 isozyme loci = 0.068 (range not reported), Hyun *et al.* 1987; and mean F_{ST} for 4 microsatellite loci = 0.032 (range not reported), Wyman *et al.* 2003). Although these estimates for North American poplars are approximately one-half less than estimates for *P. tremula*, several of the studies were conducted across small geographic scales relative to the entire range of the study species and may not capture a species-wide estimate of diversity or structure.

Comprehensive knowledge of the levels of genetic variation and population structure is crucial for informed decisions concerning breeding stocks, conservation, and responses to future environmental change. The disparity in nucleotide diversity and population structure estimates among different poplar species suggests genetic parameters of a single poplar species, such as *P. tremula*, may not be generalized across all poplars. Here we report the results of a genetic variation and population differentiation survey of three nuclear genes sampled in balsam poplar (*Populus balsamifera*) at the northern- and southern-most limits of its distribution in North America (from ~40-70° N; Table 4.1 and Fig. 4.1). We surveyed two well-studied nuclear loci in plants, *Adh1* and *G3pdh*, and a gene coincident with timing of seasonal dormancy in QTL studies of hybrid poplars, *ABI1B* (Frewen *et al.* 2000). We expected that of these loci *ABI1B* would be most likely to exhibit differences among northern and southern populations because it putatively controls the timing of bud set, a trait linked to dormancy adaptation across latitude (Chen *et al.* 2002). Moreover, Garcia and Ingvarsson (2007) recently reported an excess of nonsynonymous site diversity and extensive haplotype structure at the *ABI1B* locus in *P. tremula* suggestive of balancing selection. We compare our results with expectations from previous poplar studies.

4.3 Materials and methods

4.3.1 Study system and tissue collection

Balsam poplar is among the most widely distributed species of *Populus* in North America (Little 1971), ranging from Newfoundland northwest to Alaska (Fig. 4.1). Despite past recognition of *Populus balsamifera* and *Populus trichocarpa* as infraspecific taxa of *P. balsamifera* (*P. balsamifera* = *P. balsamifera* subsp. *balsamifera* L. and *P. trichocarpa* = *P. balsamifera* subsp. *trichocarpa* (Torr. & A. Gray) Hultén), the two taxa are regarded as separate species within the literature today (Eckenwalder 2010) and we will follow that precedent here.

Leaf or bud tissues for genetic analyses were collected from 5 to 18 individuals from each of eight populations of *P. balsamifera* (Table 4.1). Of the eight study populations, four were from the northern- and four from the southern-most limits of balsam poplar's distribution in North America (Fig. 4.1). This design was chosen in attempt to estimate the upper bound for diversity and population structure for this species.

We also sampled a single population of western black cottonwood (*Populus trichocarpa*) and eastern cottonwood (*Populus deltoides*) to determine whether diversity was influenced by introgression from these closely related species at the margins of *P. balsamifera*'s range (Table 4.1, Fig. 4.1; Hamzeh & Dayanandan 2004). Because poplars are clonal, within each population we sampled trees separated by at least 15 m to limit sampling multiple ramets from a single genet. Nucleotide genotype determinations indicated this distance was sufficient for collecting from genetically different individuals. Specimens were stored at -80C until DNA extraction at the University of Alaska Fairbanks, USA.

Three loci were studied. These included portions of the *Alcohol dehydrogenase 1* (*Adh1*), *Glyceraldehyde 3-phosphate dehydrogenase* (*G3pdh*), and *Abscisic Acid Insensitivity 1B* (*ABI1B)* genes. The *Adh1* enzyme allows plants to compensate for low oxygen stress, whereas the *G3pdh* enzyme plays an important role in glycolysis and glyconeogenesis. *ABI1B* is involved in transduction of abscisic acid response signals and is putatively associated with seasonal leaf dormancy in poplar (Frewen *et al.* 2000). These loci are well studied in other plant species, including European aspen, allowing us to compare nucleotide diversity among poplar species (Ingvarsson 2005; Garcia & Ingvarsson 2007).

4.3.2 DNA extraction, PCR and sequencing

We extracted DNA from frozen leaf or bud tissue with DNeasy Plant Extraction kits (Qiagen, Valencia, CA, USA). PCR primers were designed to amplify ~600 bp segments of the *Adh1* and *G3pdh* loci using published sequences of *P. tremula* in the

EMBL/Genbank database (accession nos. AJ580717 and AJ843581). To amplify *ABI1B*, we designed primers from the genomic sequence of *P. trichocarpa* obtained from the Bradshaw lab at the University of Washington, USA (Frewen *et al.* 2000). Primers for each locus were: 1) *Adh1* (5'-ATA AGT TAC AAC CAT CAG CGA TTA GTG-3') and (5'-GTG AAT ACA CCG TCT GCC ATA TTG-3'), 2) *G3pdh* (5'-TGC AGC GTG AAA CAC AAC MAT T-3') and (5'ATG GGC TAC TTA TTT AAC AAT CAT-3') and 3) *ABI1B* (5'-GGC CTG AGT GAT GGA AGT AT-3') and (5'-CGT CTT ATG ATT ATG AAC AT-3').

We generated bidirectional sequence data from PCR fragments of *Adh1*, *G3pdh* and *ABI1B*. Loci were amplified using TaKaRa *Ex Taq* polymerase (Takara Bio Inc., Madison, WI, USA) and column purified (Qiagen, Valencia CA, USA). PCR products were sequenced using the above primers and BigDye Terminator Cycle Sequencing chemistry (v 3.1; Applied Biosystems, Foster City, CA, USA) on an ABI3100 Genetic Analyzer.

4.3.3 Analyses

Aligner (v. 2.0.5, CodonCode Corporation, Dedham, MA, USA) was used for curating and trimming sequences based on Phred quality scores. Sequence ends were trimmed until the average quality value was Phred > 25 in a window of 10 bases. We aligned bidirectional sequences for each individual separately and visually inspected the chromatograms using Sequencher (v. 4.7, Gene Codes, Ann Arbor, MI, USA). Heterozygous sites were scored using the 'call secondary peaks' function in Sequencher with the minimum lower peak height set at > 60% and manually confirmed (Weckx *et al.*

2005). If the bidirectional reads for a single individual differed, the final call was either made visually or was based on the higher quality chromatogram (Phred > 30) which was almost always homozygous. Although this method was among the least likely to introduce unknown bias, we are cognizant that, if anything, it may have slightly underestimated the frequency of singletons and uncommon alleles in our samples. Nonetheless, our curatorial methods were consistent across the loci presented herein. A polymorphic insertion/deletion (indel) in the middle of the *G3pdh* region resulted in high quality (Phred > 30) chromatogram in one direction up to the indel region and lower quality thereafter for some heterozygous individuals. For these few individuals, we changed the base calls of the indel region to missing data and base calls in the regions flanking the indel were based on Phred > 30 scores from one directional sequence.

Levels of genetic variation were estimated as average per site pairwise nucleotide diversity (π; Nei & Gojobori 1986) and as the relationship between segregating sites and alleles sampled (θ_W; Watterson 1975). To determine the extent of linkage disequilibrium across each locus, we plotted r^2 values against pairwise distances between polymorphic sites and calculated the nonlinear quadratic regression slope using JMP (v. 7; SAS Institute, Cary, NC, USA; Hill & Robertson 1968). To test whether the folded site frequency spectrum was consistent with expectations derived from neutral evolution, we estimated Tajima's *D* (Tajima 1989) and Fu and Li's *F** (Fu & Li 1993) separately for each population. Estimates of genetic diversity, neutrality and linkage disequilibrium were calculated using DnaSP software (v. 4.50.3, Rozas *et al.* 2003). We obtained the associated one-tailed p-values for Tajima's *D* and Fu and Li's *F** by computing 10,000 coalescent simulations based on θ from the observed data and assuming free recombination in DnaSP (Hudson 1990). Because we performed multiple tests for neutrality across eight populations, we applied a standard Bonferroni correction to levels of significance for each locus (8 tests/neutrality statistic, Bonferroni critical value $\alpha = 0.006$; Rice 1989).

We examined population structure of *P. balsamifera* by calculating an analysis of molecular variance (AMOVA) implemented in the Arlequin software package (v. 3.11, Excoffier *et al.* 1992; Schneider *et al.* 1997). This method partitioned the genetic variance among northern and southern regions (F_{CT}), among populations within those regions (F_{ST}) and within populations (F_{SC}). Significance levels were determined using uncorrected pairwise differences between haplotypes through 1000 random permutation replicates.

Using PHASE (v. 2.1.1; Stephens *et al.* 2001; Stephens & Donnelly 2003), we calculated ρ, the recombination parameter, and inferred the haplotype phase for heterozygous alleles to present a geographical display of patterns of variation for each locus (see Figure 4.1). We used the default model (-MR0), which is the general model for recombination rate variation. Separate PHASE runs for each locus were performed with a burn-in-period of 100 followed by 10,000 iterations to ensure convergence of haplotype estimation. Over 97% of haplotypes were determined at a confidence probability of $\geq$ 95%. The remaining haplotypes contained only a single polymorphic site of uncertain phase. Rho was estimated for each locus as the median of the results for the posterior distribution of the recombination parameter across 10,000 data sets generated by coalescence (Crawford *et al.* 2004). All sequences were deposited in the EMBL/GenBank nucleotide sequence database (accession nos. FJ581048-FJ581417).

We compared average per site pairwise nucleotide diversity (π) between our North American collections of poplar (*P. balsamifera*, *P. deltoides*, *P. trichocarpa*) and three Eurasian *Populus* species (*P. alba*, *P. nigra*, *P. tremula*) using samples available from EMBL/GenBank. For *Adh1* we were able to compare diversity among *P. balsamifera*, *P. deltoides*, *P. trichocarpa* (data presented herein), *P. tremula* (accession nos. AJ842873-AJ842906; Ingvarsson 2005), *P. nigra* (accession nos. AJ580714-AJ580723; unpublished) and *P. alba* (accession nos. AJ580702-AJ580713; unpublished). For *G3pdh* and *ABI1B*, however, we were able to compare only *P. balsamifera*, *P. deltoides*, *P. trichocarpa* and *P. tremula* (*G3pdh*: accession nos. AJ843576-AJ843623, Ingvarsson 2005; *ABI1B*: accession nos. AM690392-AM690435; Garcia and Ingvarsson 2007) because data for the other two Eurasian poplar species were not available. Sequence data were obtained from EMBL/Genbank, aligned in Sequencher and trimmed to the length of our partial sequence prior to analysis.

4.4 Results

4.4.1 Nucleotide diversity in P. balsamifera

We sequenced regions of *Adh1*, *G3pdh* and *ABI1B* from 5-18 individuals within eight populations of *P. balsamifera* for a total sample of 102-105 trees and 204-210 alleles per locus. For each individual, we aligned a total of 1,827 bp. Overall, this included 858 bases from coding regions and 969 bases from introns and untranslated regions. The average sequence length was 609 bp and included both coding and noncoding sites for each locus (Table 4.2). The complete coding regions were obtained for exons II-IV, exons V-V11 and exon III for *Adh1*, *G3pdh* and *ABI1B*, respectively.

We observed from 5 to 8 segregating sites (SNPs) per locus; values of θ_W ranged from 0.00150 in *Adh1* to 0.00205 in both *G3pdh* and *ABI1B* (Table 4.3). We observed only synonomous SNPs in coding regions for *Adh1*. In contrast, only nonsynonymous SNPs were observed in coding regions of *G3pdh* and *ABI1B*. A total of 3 singletons were detected across all loci. Our estimates of nucleotide diversity for *P. trichocarpa* (θ_W ranged from 0.00131-0.401) and *P. deltoides* (θ_W ranged from 0.00160-0.00245) were similar to those observed in *P. balsamifera*.

A decline in LD as pairwise nucleotide distance increased, represented by the relationship between r^2 and distance in base pairs between polymorphic sites, was not apparent for any of the loci, most likely because the average distance between sites was < 250 bp (quadratic regression r^2 ranged from 0.02-0.24, $p > 0.35$ for all regions). Estimates of the recombination parameter generated from PHASE varied by 3 orders of magnitude among loci ($\rho = 1.89 \times 10^{-3}$, *Adh1*; 6.03×10^{-4}, *G3pdh*; 4.48×10^{-6}, *ABI1B*). The recombination rate (r) relative to mutation (μ) estimated as ρ/θ_W ($= 4N_er/4N_e\mu = r/\mu$) varied from 1.26 in *Adh1* to 0.294 in *G3pdh* and 0.0022 in *ABI1B*. Based on a heuristic comparison of the average ρ/θ-ratio in *P. tremula* ($\rho/\theta \approx 1$; Ingvarsson 2008), the only locus that appears to be an outlier is *ABI1B*.

Haplotype diversity (H_d) was similar across all loci in *P. balsamifera*, ranging from 0.630 in *Adh1* to 0.730 in *G3pdh* and 0.734 in *ABI1B* (Fig. 4.1, Table 4.3). *Populus* *balsamifera* and *P. trichocarpa* shared no haplotypes with *P. deltoides*. In contrast, *P. balsamifera* and *P. trichocarpa* shared haplotypes for all three loci. Of eight *Adh1* haplotypes observed in *P. balsamifera* and *P. trichocarpa*, one was found in both species. Two of eleven *G3pdh* haplotypes and five of ten *ABI1B* haplotypes also were shared. The most common *ABI1B* variant in *P. trichocarpa* (12/30 alleles), however, was unique to that taxa.

Levels of genetic variation as estimated by S, θ_W, π and H_d in *G3pdh* and *ABI1B* were greater for populations in the north than the south (Table 4.3). This trend also was observed in *Adh1* for π, but not for S, θ_W, and H_d. Based on Tajima's *D* and Fu & Li's *F**, we observed evidence for departure from neutral evolution in a few populations for *G3pdh* and *ABI1B* although Tajima's *D* was significant only in the Alaskan Chena River population for *ABI1B* after Bonferroni correction (Table 4.4; $D = 1.881, p = 0.002$; Bonferroni critical value $\propto = 0.006$). For *Adh1*, both Tajima's *D* (range 1.242-2.495; $p = 0.001\text{-}0.052$) and Fu & Li's *F** (range 0.646-1.715, $p = 0.003\text{-}0.277$) were elevated in most populations. For *P. trichocarpa* and *P. deltoides*, we observed evidence for non-neutral evolution only in *Adh1* for *P. trichocarpa* (Table 4.4; $F^* = 1.337, p = 0.033$).

Because positive values of Tajima's *D* and Fu & Li's *F** can be generated by sampling across paralogs of a duplicated gene, we examined this possibility for *Adh1* in *P. balsamifera*. *Populus trichocarpa* is an ancient polyploid (Tuskan *et al.* 2006); so we performed BLAST searches for *Adh* duplicates within the assembled genome of *P. trichocarpa*, available at http://genome.jgi-psf.org/ (Tuskan *et al.* 2006). This search yielded only one copy of *Adh1*. Sampling across paralogs also is expected to yield a phylogenetic tree with two distinct clades and long internal branches. To address this possibility, we constructed a neighbor-joining tree using PAUP* assuming a Jukes-Cantor model of evolution (v. 4 Beta, Swofford 2002). The topology we observed (not shown) lacked deep coalescence and long internal branches, which is inconsistent with patterns expected from sampling across paralogs. Finally, if we had sampled across paralogs we would have expected most polymorphic sites to be heterozygous for nearly all individuals, however, this pattern was not detected. Our analyses, combined with the observations of low genetic diversity and high recombination, collectively suggest we did not sample paralogs from an ancient gene duplication event.

4.4.2 Population differentiation in P. balsamifera

Over 18% of the genetic variation in *ABI1B* was attributed to regional differentiation between the northern and southern populations of balsam poplar (F_{CT} = 0.184, $p \leq 0.00$; Table 4.5; Fig 4.1). In comparison, regional grouping accounted for < 1% of genetic variation for both *Adh1* and *G3pdh* (Table 4.5; Fig. 4.1). Among-population differentiation also was significant for both *G3pdh* and *ABI1B*, but not for *Adh1*. For all three loci, the majority of genetic variance could be found within populations, accounting for 74.39% of the variation in *ABI1B*, 87.85% of the variation in *G3pdh,* and 98.24% of the variation in *Adh1*.

4.4.3 Nucleotide diversity across Populus

A comparison of nucleotide diversity (π_{tot}) for three loci sampled in North American and European poplars showed that estimates of diversity are consistently greater in European poplars (Fig. 4.2). For *Adh1*, diversity was consistently elevated in the European poplars (π_{tot} ranges from 0.00628-0.01031) compared to the North American poplars (π_{tot} ranges from 0.00106-0.00483). The highest estimate of nucleotide diversity was observed in *P. tremula* for *G3pdh* ($\pi_{tot} = 0.01454$, SE ±0.08804), but was dramatically lower for North American poplar species (π_{tot} ranges from 0.00150-0.00258). Total nucleotide diversity was also elevated for *ABI1B* in *P. tremula* compared to the three North American species, but the differences were not as dramatic as for the other two loci examined.

4.5 Discussion

In this first report of nucleotide diversity in *Populus balsamifera*, we chose to sample populations from the northern- and southern-most limit of balsam poplar's range to maximize our ability to identify unique nucleotide variants and population structure at the regional scale. The reader should recognize that this sampling design may contribute to an upward bias in our estimates of population structure (F_{ST}) and diversity, and may be thought of as an upper bound for the sampled loci. Nonetheless, our results exhibited strikingly high regional population structure in *ABI1B* and markedly low nucleotide variation compared to the same loci sampled in Eurasian poplar species. These patterns challenge the view of low population structure and high variation in poplar species. Our findings have potential to aid in the design of sampling regimes for conservation and breeding stock as well as to contribute to historical inferences that shaped genetic diversity of boreal plant species throughout the Quaternary.

Poplars are well known for hybridizing across species. Although we observed no shared haplotypes between *P. balsamifera* and *P. deltoides*, we did find shared haplotypes between *P. balsamifera* and our Alaskan population of *P. trichocarpa*. Of the 20 segregating sites observed in *P. balsamifera*, 13 were also polymorphic in *P. trichocarpa*. Because these species are closely related (see methods), it is unclear whether this pattern should be considered ongoing hybridization or incomplete lineage sorting, but the reality is likely somewhere in between. We found no geographic basis to shared haplotypes or segregating sites between these species (Fig. 4.1) suggesting a greater contribution of ancestral polymorphism to the observed patterns of similarity.

4.5.1 Nucleotide diversity in P. balsamifera

Average pairwise sequence diversity in *P. balsamifera* was substantially lower than reported for *P. tremula* (Ingvarsson 2005a, 2008), yet similar to that observed in other tree species including *P. trichocarpa* (Gilchrist *et al.* 2006) and various gymnosperms (Dvornyk *et al.* 2002; García-Gil *et al.* 2003; Kado *et al.* 2003; Brown *et al.* 2004; Heuertz *et al.* 2006). Nucleotide diversity in *P. tremula* (mean $\pi_{tot} = 0.0111$; mean $\pi_{sil} = 0.0160$, Ingvarsson 2005a and $\pi_{tot} = 0.0042$; mean $\pi_{sil} = 0.0120$; Ingvarsson 2008) is approximately two- to five-fold greater than we observed in *P. balsamifera* (mean $\pi_{tot} = 0.0025$; mean $\pi_{sil} = 0.0033$), which is equivalent to *P. trichocarpa* (mean $\pi_{tot} = 0.0018$; mean $\pi_{sil} = 0.0029$; Gilchrist *et al.* 2006). To further illustrate this disparity, because θ is roughly proportional to heterozygosity we can estimate that two randomly chosen balsam poplar sequences vary on average in ~1 of 526 bases (i.e., 1/0.0019 is

~526; Table 4.3). In contrast, two European aspen sequences vary on average from in ~1 of 60 bp (Ingvarsson 2005a) to in ~1 of 208 bp (Ingvarsson 2008).

The lower nucleotide diversity in *P. balsamifera* compared to *P. tremula* is striking and we suggest that it reflects a lower effective population size in *P. balsamifera* that may have been caused by one or more historical population size bottlenecks (see below). Similar to other plant species at high latitudes in North America, *P. balsamifera* likely experienced repeated population size expansions and contractions in response to the advance and retreat of ice sheets during the Quaternary (2 Ma; Hewitt 2004). Prior to the last glacial maximum (ca. 28-18 Ka) dated macrofossils show boreal woody plants, including *Populus* species, were common at high latitudes in North America (*ca.* 60-30 Ka; Anderson & Lozhkin 2001). Boreal tree and shrub taxa are not again widespread in this record until 12-9 Ka (Anderson & Brubaker 1994). The palynological record suggests poplar became a consistent component of pollen assemblages in the boreal region within the high latitude ice age refugium of eastern Beringia, including Alaska, before the widespread rise of boreal trees and shrubs (*ca.* 16 Ka; Brubaker *et al.* 2005), but it is unclear whether poplar colonized via long-distance dispersal following the last glacial maximum or survived *in situ*. The elevated levels of nucleotide diversity we observed at the northern-most limit of *P. balsamifera*'s range may reflect its persistence within this refugium. This hypothesis is supported by the observed pattern of nucleotide frequency spectra that is suggestive of a recent population contraction, following a bottleneck event, that has not yet reached the recovery phase (in our data Tajima's $D > 0$ and Fu and Li's $F^* > 0$). We caution, however, that neither of these patterns is definitive and a proper test of these hypotheses will require a statistical phylogeographic approach that is beyond the scope of the current data set.

We currently have no explanation for the elevated rates of Tajima's *D* and Fu and Li's *F** for *Adh1* compared to *G3pdh.* Whereas it appears unlikely we sampled paralogs of *Adh* in *P. balsamifera*, gene duplication of *Adh* loci is well documented in plants (Chiang *et al.* 2003) and a duplication event may be a characteristic of *P. balsamifera* that is not shared with *P. trichocarpa.* Therefore, we are hesitant to fully dismiss this possibility without further investigation. Interestingly, our estimates of Tajima's *D* for *Adh1* in *P. trichocarpa* or *P. deltoides* do not show departure from neutrality. Furthermore, a significantly negative Tajima's *D* for *Adh1* was observed in *P. tremula* (*D* = -1.816). Of course, it also is possible that the elevated numbers of medium frequency alleles, which are characterized by positive values of these summary statistics, are generated by balancing selection maintaining two or more *Adh1* alleles at medium frequencies within populations. These hypotheses can be discriminated by further characterizing genomic variation in balsam poplar and specifically studying the functional importance of naturally occurring *Adh1* variants.

4.5.2 Comparative nucleotide diversity among Populus species

The difference in levels of nucleotide diversity we observed between poplars native to the North American and Eurasia may be due to the continents' distinct geological histories. A similar pattern in levels of nucleotide polymorphism is observed between the North American *Arabidopsis lyrata* subsp. *lyrata* (mean $\pi_{sil} = 0.0039$) and the European *Arabidopsis lyrata* subsp. *petraea* (mean $\pi_{sil} = 0.0285$) (Wright *et al.* 2003).

These authors suggested this disparity was the result of either a population bottleneck in North American populations or recent admixture of diverged European populations. These hypotheses also may explain the disparity we observed between European and North American poplars. Additionally, *P. balsamifera* occurs in section *Tacamahaca*, a clade distinct from *P. tremula* (section *Populus*) (Hamzeh & Dayanandan 2004). We cannot, therefore, dismiss the possibility that the differences observed in nucleotide diversity estimates in the two *Populus* species are due to phylogenetic constraint. An explicit test of the hypothesis of the effect of continental history on nucleotide diversity would require comparison of diversity in *P. tremula* with its sister species in North America, *P. tremuloides*. Moreover, it would be of great interest to address differences in other sister species that occur in boreal regions of North America and Eurasia to determine whether common population genetic patterns might trace to differences in continental climatic and geographic factors that have influenced population histories.

4.5.3 Population differentiation in P. balsamifera

The level of population differentiation we observed in *P. balsamifera* is higher than previous estimates of population structure in North America poplars. We observed moderate and significant population structure for two of the three loci surveyed in balsam poplar (*Adh1*: $F_{ST} = 0.018, p \leq 0.212$; *G3pdh*: $F_{ST} = 0.121, p \leq 0.001$; *ABI1B*: $F_{ST} = 0.256, p \leq 0.001$). These estimates are similar to those reported for five loci of *P. tremula* across a similar latitudinal transect (F_{ST} ranges from 0.040-0.161; Ingvarsson 2005a). The upper estimates of population structure we observed in *P. balsamifera* contrast with those reported previously for isozyme and microsatellite studies of North American poplars

(Weber & Stettler 1981; Marty 1984; Hyun *et al.* 1987; Farmer *et al.* 1988; Lund *et al.* 1992; Wyman *et al.* 2003; Cole 2005). These estimates of mean F_{ST} range from 0.003 in *P. tremuloides* (Lund *et al.* 1992) to 0.064 in *P. deltoides* (Marty 1984). Because the geographic extent of the samples in *P. balsamifera* and *P. tremula* are comparable, the low levels of population structure observed previously in poplar likely result from earlier studies' geographically restricted sampling designs.

We observed the highest F_{ST} yet reported for poplar at the *ABI1B* locus in *P. balsamifera* ($F_{ST} = 0.256, p \leq 0.001$; compare to $F_{ST} = 0.214$ in the defense gene *TI5*, Ingvarsson 2005b). Diversity in this gene was also significantly structured between northern and southern regions ($F_{CT} = 0.184$ $p \leq 0.001$). Previous and current work demonstrates balsam poplar displays a striking genetically-determined latitudinal cline in the timing of seasonal dormancy including bud break, cessation of growth and bud set (Pauley & Perry 1954; S. Silim & W. Schroeder, pers. comm.). The elevated level of population differentiation in balsam poplar appears to be the result of two replacement sites: the first at amino acid 331 with isoleucine and threonine alleles, and the second at amino acid 408 with alanine or threonine alleles. When considering all replacement sites in the data set, which includes only sites in *ABI1B* and *G3pdh*, significant population differentiation is observed only between geographic regions for amino acid changing substitutions in *ABI1B* (Table 4.6; aa 331, $F_{CT} = 0.433$, $p \leq 0.034$; aa 408 $F_{CT} = 0.401$, $p \leq 0.022$). Moreover, the frequency of nonsynonymous substitutions differs between regions for both *ABI1B* replacement sites (Kruskal-Wallace Rank Sum Test; aa 331, $\chi^2 = 4.0833, p < 0.05$; aa 408, $\chi^2 = 7.000, p < 0.01$), but no differences were apparent for the frequency of nonsynonsymous substitutions between regions for *G3pdh* (for all tests $p >$ 0.75).

A comparison between *ABI1B* nucleotide sequences of *Populus* species revealed these two amino acid changing substitutions are also polymorphic in our Alaskan population of *P. trichocarpa*. The alleles more common in northern *P. balsamifera* (isoleucine allele; aa 331 and threonine allele; aa 408) were observed in ~15% of the *P. trichocarpa* individuals sampled from coastal Alaska. These *ABI1B* replacement sites are fixed in our sample of *P. deltoides* and in *P. tremula* (Garcia & Ingvarsson 2007; EMBL/Genbank AM690392-AM690435). Interestingly, extensive haplotype structure also was reported at the *ABI1B* locus in *P. tremula*, which the authors suggest may be the result of balancing selection (Garcia & Ingvarsson 2007). *ABI1B* appears to be an interesting candidate for additional detailed studies regarding the functional importance of natural variation given QTL studies and multiple studies of nucleotide diversity identified curious patterns of association.

In conclusion, data presented herein demonstrates the need for thorough studies of population diversity and structure in boreal forest trees. These estimates are necessary for educated conservation decisions and identification of diversity for forestry breeding programs. Poplars are commercially exploited worldwide and are predicted to play a key role in bio-fuel production and CO_2 bio-sequestration programs (Bradshaw *et al.* 2000). We show that although the majority of diversity can be accounted for by sampling within populations, genotypes and alleles occur at different frequencies across the range of balsam poplar. To fully characterize genetic variants adapted to the wide-ranging environments of subarctic and boreal North America, selection of genotypes must extend beyond a single population, or even a single region. Moreover, with impending climate change and subsequent northern migration of plant species it is essential that we develop baseline estimates of population genetic diversity for economically important plant species such as poplar, and particularly *P. balsamifera*, which ranges the furthest north of any *Populus* species in the world.

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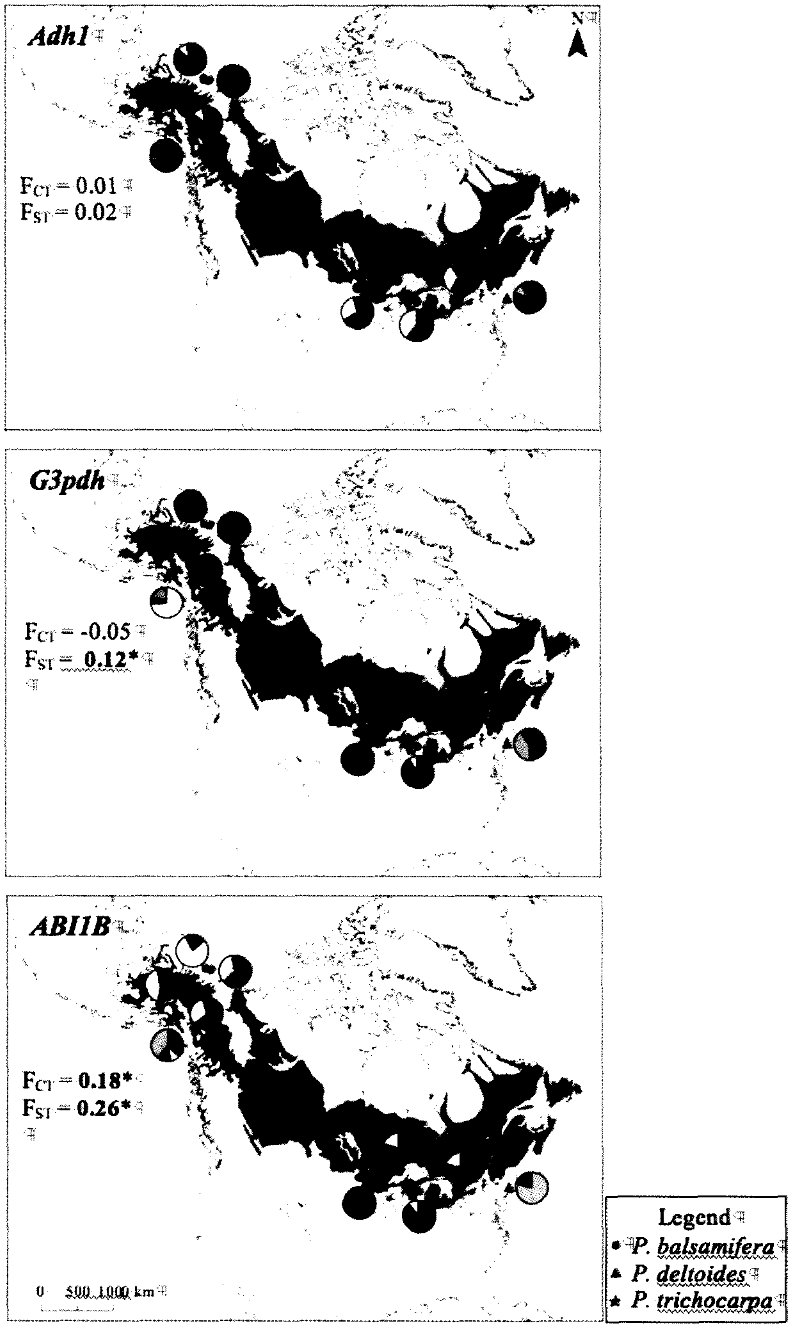


Figure 4.1 Distribution of *Adh1*, *G3pdh* and *ABI1B* haplotypes observed in *Populus balsamifera*, *Populus trichocarpa* and *Populus deltoides*. Pie charts indicate frequency of haplotypes within each population and unique alleles are indicated by different colors. The range of *P. balsamifera* in North America is shown in green (U.S. Geological Survey 2006). Significant variance components between northern and southern regions (F_{CT}) and among populations within regions (F_{ST}) are indicated in bold and denoted with an asterisk ($P \leq 0.001$).

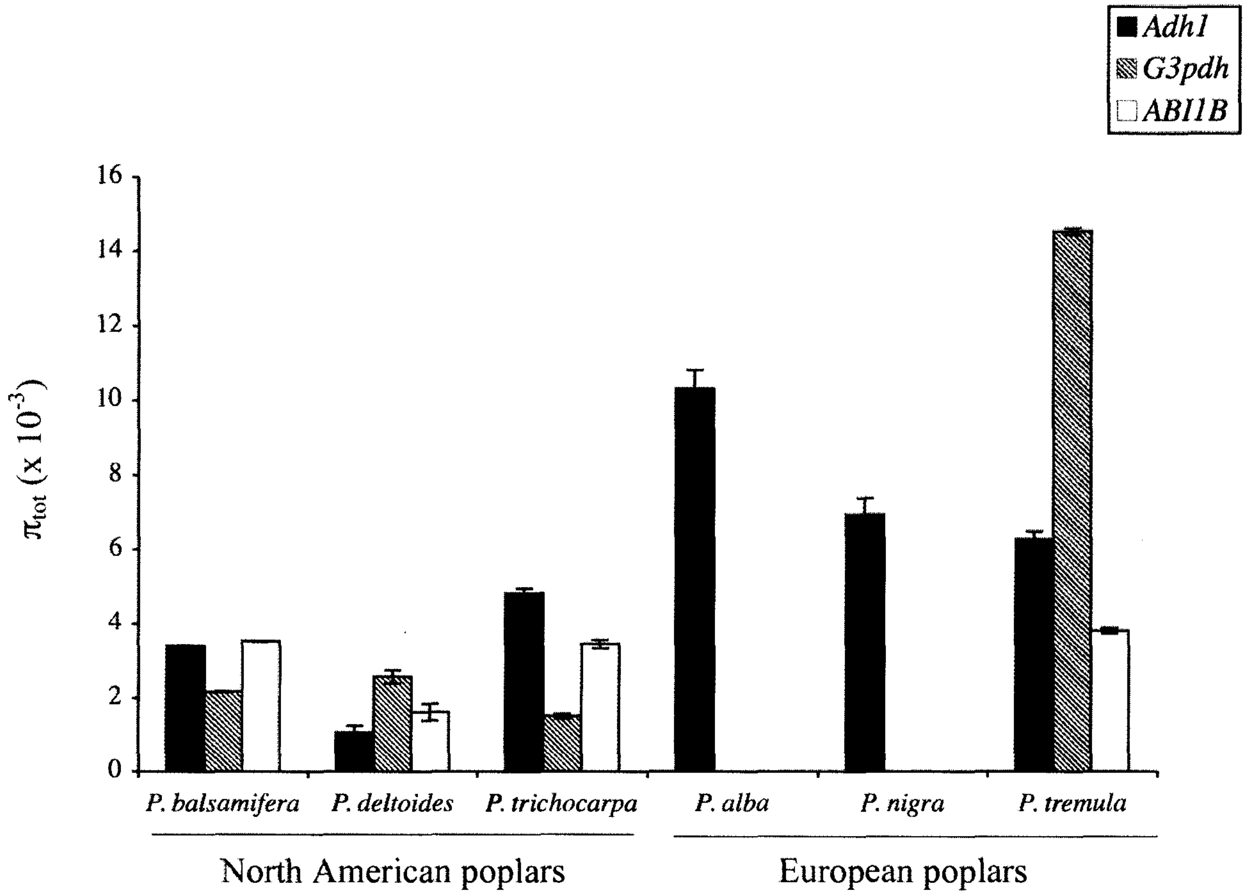


Figure 4.2 Total average pairwise nucleotide diversity (π_{tot}) by locus for *Populus* species native to North America and Europe. Histograms indicate parameter estimates and brackets denote associated standard errors.

Table 4.1 Sample sizes and locations of the study populations of North American *Populus*.

Species	Population	State or Province	Latitude (°N)	Longitude (°W)	No. of individuals		
					Adh1	*G3pdh*	*ABI1B*
P. balsamifera	Cache Creek	Alaska	69.41	145.88	14	16	15
	Cottonwood Creek	Alaska	69.10	147.89	15	15	15
	Yukon River bridge	Alaska	65.88	149.72	15	16	15
	Chena River	Alaska	65.07	146.08	17	17	15
	Grand Portage	Minnesota	47.98	89.66	5	5	5
	Grand Forks	Minnesota	47.93	97.02	15	16	18
	Ridges State Park	Wisconsin	45.07	87.11	5	5	5
	Guelph Lake	Ontario	43.60	80.26	16	15	15
P. deltoides	Hubbard State Park	Connecticut	41.55	72.83	5	5	5
P. trichocarpa	Valdez	Alaska	61.13	146.35	15	15	15

Table 4.2 Loci studied and length of regions analyzed in *Populus balsamifera*.

					Coding[b]	
Locus	Alleles	Total[a]	Coding	Noncoding[a]	Nonsynonymous	Synonymous
Adh1	204	567	225	342	169.17	55.83
G3pdh	210	599	324	275	245.95	78.05
ABI1B	206	661	309	352	231.11	77.89
Total		1827	858	969	646.23	211.77

[a] Including indels.

[b] The total number of synonymous and nonsynonymous sites were computed as in Nei and Gojobori (1986)

Table 4.3 Estimates of nucleotide diversity in North American *Populus*.

						Polymorphism (x 10^{-3})				
Species	Locus	Region	Alleles	*S*	H_d	θ_W	π_{tot}	π_{sil}	π_{syn}	π_{nonsyn}
P. balsamifera	*Adh1*	North	122	4	0.574	1.31	3.48	4.96	8.94	0.00
		South	82	5	0.669	1.77	3.27	4.67	9.02	0.00
		Total	204	5	0.630	1.50	3.41	4.87	8.93	0.00
		SD			0.022	0.07	0.07			
	G3pdh	North	128	6	0.735	1.91	2.26	1.58	0.00	3.20
		South	82	4	0.717	1.39	2.01	1.89	0.00	2.20
		Total	210	7	0.730	2.05	2.16	1.70	0.00	2.81
		SD			0.017	0.77	0.08			
	ABI1B	North	120	8	0.696	2.26	3.67	3.35	0.00	4.28
		South	86	5	0.614	1.51	2.38	2.41	0.00	2.33
		Total	206	8	0.734	2.05	3.53	3.26	0.00	4.03
P. deltoides	*Adh1*		10		0.012	0.73	0.08			
	G3pdh		10	4	0.644	2.45	2.58	2.84	2.56	2.26
	ABI1B		10	3	0.356	1.60	1.61	2.49	9.13	0.00
P. trichocarpa	*Adh1*		30	9	0.655	4.01	4.83	6.88	4.28	0.00
	G3pdh		30	3	0.432	1.31	1.50	2.24	0.00	0.52
	ABI1B		30	10	0.779	3.82	3.46	4.35	2.39	1.84

Table 4.4 Estimates of neutrality observed in North American *Populus* species. One-tailed p-values for Tajima's D and Fu and Li's F^* calculated by coalescent simulation are in parentheses. Significant neutrality estimates are in bold type ($P \leq 0.050$). Estimates that were significant after correction for multiple inferences are indicated with an asterisk ($\alpha = 0.006$).

			D_{Tajima}			$F^*_{\text{Fu\&Li}}$		
Species	Region	Population	*Adh1*	*G3pdh*	*ABI1B*	*Adh1*	*G3pdh*	*ABI1B*
P. balsamifera	North	Cache Creek	**2.266***	0.214	**1.707**	**1.639***	0.279	0.952
			(0.001)	(0.360)	(0.010)	(0.009)	(0.424)	(0.113)
		Cottonwood Creek	**2.495***	0.061	0.334	**1.715***	0.804	1.108
			(0.001)	(0.458)	(0.334)	(0.003)	(0.262)	(0.099)
		Yukon River bridge	**2.109***	**1.530**	1.213	**1.584***	**1.289**	0.781
			(0.001)	(0.039)	(0.053)	(0.003)	(0.042)	(0.178)
		Chena River	**2.491***	0.383	**1.881***	**1.712***	-0.119	1.013
			(0.001)	(0.286)	(0.002)	(0.005)	(0.582)	(0.096)
	South	Grand Portage	**1.953***	0.096	**1.471**	**1.582***	0.174	**1.450**
			(0.001)	(0.424)	(0.019)	(0.004)	(0.426)	(0.021)
		Grand Forks	1.242	**1.573**	1.114	0.646	1.303	1.234
			(0.052)	(0.026)	(0.080)	(0.277)	(0.055)	(0.085)
		Ridges State Park	**1.471**	**1.438**	-0.329	**1.450**	1.255	0.450
			(0.014)	(0.042)	(0.646)	(0.022)	(0.067)	(0.322)
		Guelph Lake	**2.154***	1.190	0.755	**1.597***	1.180	1.197
			(0.003)	(0.082)	(0.159)	(0.009)	(0.089)	(0.080)
P. deltoides		Hubbard State Park	-1.562	0.204	0.021	-1.934	-0.231	0.982
			(0.986)	(0.379)	(0.475)	(0.986)	(0.599)	(0.216)
P. trichocarpa		Valdez	0.638	0.333	-0.300	**1.337**	0.894	0.595
			(0.163)	(0.338)	(0.673)	(0.033)	(0.208)	(0.226)

Table 4.5 Tests of population genetic subdivision in haplotypes of *Adh1*, *G3pdh* and *ABI1B* across eight populations of *Populus balsamifera*. For each locus, the AMOVA was partitioned at three levels: between northern and southern regions, among populations within regions and within populations. Significant genetic variance components in bold type and indicated with an asterisk are significant at $P \leq 0.05$.

Source of variation	DF	SS	Variance components	Percent Variation	Fixation indices	$P \leq$
1) *Adh1*						
a) Between north & south	1	1.92	0.0069	0.71	$F_{CT} = 0.007$	0.264
b) Among populations	6	7.24	0.0102	1.05	$F_{ST} = 0.018$	0.212
c) Within populations	196	187.31	0.9557	98.24	$F_{SC} = 0.011$	0.137
2) *G3pdh*						
a) Between north & south	1	0.64	-0.0286[a]	-4.59	$F_{CT} = -0.046$	0.919
b) Among populations	6	19.18	**0.1045**	16.74	$F_{ST} = 0.121$	**0.001***
c) Within populations	202	110.82	**0.5486**	87.85	$F_{SC} = 0.160$	**0.001***
3) *ABI1B*						
a) Between north & south	1	27.65	**0.2395**	18.43	$F_{CT} = 0.184$	**0.001***
b) Among populations	6	19.62	**0.0933**	7.18	$F_{ST} = 0.256$	**0.001***
c) Within populations	198	191.39	0.9666	74.39	$F_{SC} = 0.088$	**0.032***

[a] A slightly negative parameter estimate can occur if the parameter value is zero.

Table 4.6 Fixation indices for amino acid changing substitutions in *G3pdh* and *ABI1B* partitioned within populations (F_{SC}), among populations within regions (F_{ST}) at the northern- and southern-most limits of *Populus balsamifera*'s range and between regions (F_{CT}). Numbering of amino acids is based on the full protein sequence. Fixation indices in bold type and indicated with an asterisk are significant at $P \leq 0.05$.

		Among regions			Among populations within regions			Within populations		
Locus	Amino acid	Percent Variation	F_{CT}	$p \leq$	Percent Variation	F_{SC}	$p \leq$	Percent Variation	F_{ST}	$p \leq$
G3pdh	226	-1.00	-0.010	0.849	9.42	**0.093**	**0.018***	91.58	**0.084**	**0.007***
	242	-31.63	-0.316	0.906	59.79	**0.454**	**0.001***	71.84	**0.282**	**0.001***
	252	0.57	0.006	0.470	8.47	**0.085**	**0.024***	90.96	**0.090**	**0.016***
	288	-10.87	-0.109	0.767	62.42	**0.563**	**0.001***	48.46	**0.515**	**0.001***
ABI1B	331	44.34	**0.443**	**0.034***	18.03	**0.324**	**0.001***	37.63	**0.623**	**0.001***
	408	40.59	**0.401**	**0.022***	11.83	**0.199**	**0.001***	47.57	**0.524**	**0.001***

CHAPTER FIVE: CONCLUSIONS

My dissertation research examined historical biogeography, floristic diversity and nucleotide variation in *Populus balsamifera*, or balsam poplar. The following major conclusions were reached:

5.1 Historical biogeography

Molecular analyses have great potential to contribute toward understanding long-standing paleoecological questions such as the role of Beringia as a refugium, specifically for boreal trees. Balsam poplar exhibits a pattern of chloroplast nucleotide diversity consistent with the presence of a single, large, widespread population south of the continental ice sheets through the Late Quaternary. The cpDNA genealogy is shallow and lacks distinct phylogroups that would be indicative of long-term isolation during the Late Quaternary. We observed a loss of allelic richness along a northern route of colonization indicative of balsam poplar being a recent migrant to Alaska and northern Canada. If we accept dates provided by the pollen record, the presence of balsam poplar within Beringia 18,000 yr BP might be best explained by long-distance dispersal of present-day genotypes from the south across the massive Laurentide and Cordilleran ice sheets since an ice-free migration corridor did not open until *c.* 14,000-12,000 calibrated yr BP. This result does not prove the absence of balsam poplar in Beringia, but if it was present, populations were probably small and overwhelmed by migration of genotypes from the south with climate warming and glacial retreat.

5.2 Floristic diversity

Five balsam poplar communities on the Arctic Slope and interior Alaska and Yukon Territory were described resulting in classification of one association, two subassociations and two variants. Despite the wide range of the association from the boreal forest north to the Arctic Slope, the well-developed woody and herbaceous layers share many faithful taxa within the community. Arctic and boreal balsam poplar communities are differentiated into distinct subassociations. The arctic communities are further differentiated by habitat into a south-facing slope variant and a spring variant. A similar pattern was not observed in the boreal forest where balsam poplar occurs predominantly on floodplains.

The principle environmental factors resulting in the characteristic vegetation types of the described communities are latitude, elevation, SWI, tree canopy and shrub height, stand basal area, litter depth, slope angle and aspect, and various cover estimates. Ordination axes correspond to a complex productivity gradient which reflects the south-to-north transition from boreal forest to arctic tundra in northern Alaska and to a complex gradient in slope and aspect apparent among the arctic communities.

In all communities, species richness is driven by herbaceous and woody species, which make up 85% of the total species. Arctic communities are dominated by arctic-alpine taxa (47-55%), while boreal communities are dominated by boreal taxa (37%). All communities are dominated by species with circumpolar or circumboreal ranges.

A strong linkage between summer warmth index (SWI), the sum of mean monthly temperatures > 0° C, and the presence of balsam poplar was observed (SWI > 25 for

~80% of the stands) was observed. Alteration of temperature regimes caused by global climate change will likely lead to an increase in the abundance and distribution of balsam poplar on the Arctic Slope of Alaska.

5.3 Nucleotide variation

We observed substantial differences in levels of nucleotide diversity between poplars native to the North American and Eurasia. Average pairwise sequence diversity in *Populus balsamifera* was substantially lower than reported for *P. tremula*, yet similar to that observed in other tree species including *P. trichocarpa* and various gymnosperms. The lower nucleotide diversity in *P. balsamifera* compared to *P. tremula* is striking and we suggest that it reflects a lower effective population size in *P. balsamifera* that may have been caused by one or more historical population size bottlenecks. Similar to other plant species at high latitudes in North America, *P. balsamifera* likely experienced repeated population size expansions and contractions in response to the advance and retreat of ice sheets during the Quaternary.

Levels of population differentiation observed in *P. balsamifera* are higher than previous estimates of population structure in North America poplars. We observed moderate and significant population structure for two of the three loci surveyed in balsam poplar (*Adh1* and *G3pdh*). In the third locus, we observed the highest F_{ST} yet reported for poplar at the *ABI1B* locus in *P. balsamifera*. Diversity in this gene was also significantly structured between northern and southern regions. Previous and current work demonstrates balsam poplar displays a striking genetically-determined latitudinal cline in the timing of seasonal dormancy including bud break, cessation of growth and bud set. *ABI1B* appears to be an interesting candidate for additional detailed studies regarding the functional importance of natural variation given QTL studies and multiple studies of nucleotide diversity identified curious patterns of association.

We show that although the majority of diversity can be accounted for by sampling within populations, genotypes and alleles occur at different frequencies across the range of balsam poplar. To fully characterize genetic variants adapted to the wide-ranging environments of subarctic and boreal North America, selection of genotypes must extend beyond a single population, or even a single region. Moreover, with impending climate change and subsequent northern migration of plant species it is essential that we develop baseline estimates of population genetic diversity for economically important plant species such as poplar, and particularly *P. balsamifera*, which ranges the furthest north of any *Populus* species in the world.

5.4 Synthesis

> *".. there is no more positive guide to the past occupation of any area by a particular species [than the discovery of fossils]. Nevertheless, we may garner a great deal of information from... genecological studies of well chosen species.."*
>
> (Baker 1959)

My dissertation research investigated the ecology and evolution of *Populus balsamifera* at the northern range limit of its distribution in North America. Balsam poplar comprises treeline in northern Alaska occurring in small, isolated groves that are virtual oases where the forest meets the tundra in arctic Alaska. The overarching theme of my dissertation, from historical biogeography to floristic diversity and nucleotide variation in balsam polar, is the study of this species' unique geographic distribution. To better understand the processes leading to balsam poplar's extant range, I also utilized the fossil record to place range expansions and contractions within a historical context.

The primary question I aspired to answer when I began my dissertation research was: "What is the origin of balsam poplar groves in arctic Alaska?" At that time, I believed the alternatives were simple. The origin of balsam poplar populations on the Arctic Slope was either: a) persistence from the Last Interglacial when forests extended further north to the coastal plain, or b) recent migration from south of the Brooks Range. I have since learned the answer is far from simple. Although my research has contributed toward our understanding of the origin of arctic balsam poplar populations, the definitive answer is still elusive. Rather than a choice between persistence vs. recent migration, the answer is more complex. Balsam poplar has a dynamic history in arctic Alaska.

The paleoecological record, both pollen and macrofossils, supports the hypothesis of long-term persistence of balsam poplar *in situ* within Beringia since the Last Interglacial. Interestingly, my cpDNA survey of genetic diversity in balsam poplar did not show a similar pattern in support of a northern refugium. I do believe balsam poplar did persist within Beringia. The refugium likely consisted of small, isolated populations of balsam poplar analogous to groves that occur in the Arctic today. These small populations likely experienced a loss of diversity, or a population bottleneck, when balsam poplar's range contracted as the climate cooled prior to the onset of the Last

Glacial. Furthermore, a massive northern migration of balsam poplar following the most recent deglaciation likely swamped and homogenized any standing genetic variation that persisted within Beringia.

Arctic balsam poplar groves, however, are unique as was revealed by my studies of floristic diversity and nucleotide variation. Balsam poplar groves differ floristically from those in the boreal forest south of the Brooks Range. Sequence variation in *ABI1B*, a candidate gene for seasonal dormancy traits in balsam poplar, also differs between populations of balsam poplar at the northern- and southern-most margins of its range. I believe this work has established a starting point toward understanding the ecological and evolutionary processes that occur at the northern range limit of balsam poplar's distribution.

5.5 Literature cited

Baker, H. G. 1959. The contribution of autecological and genecological studies to our knowledge of past migrations of plants. *American Naturalist* **93**: 255-272.

APPENDIX

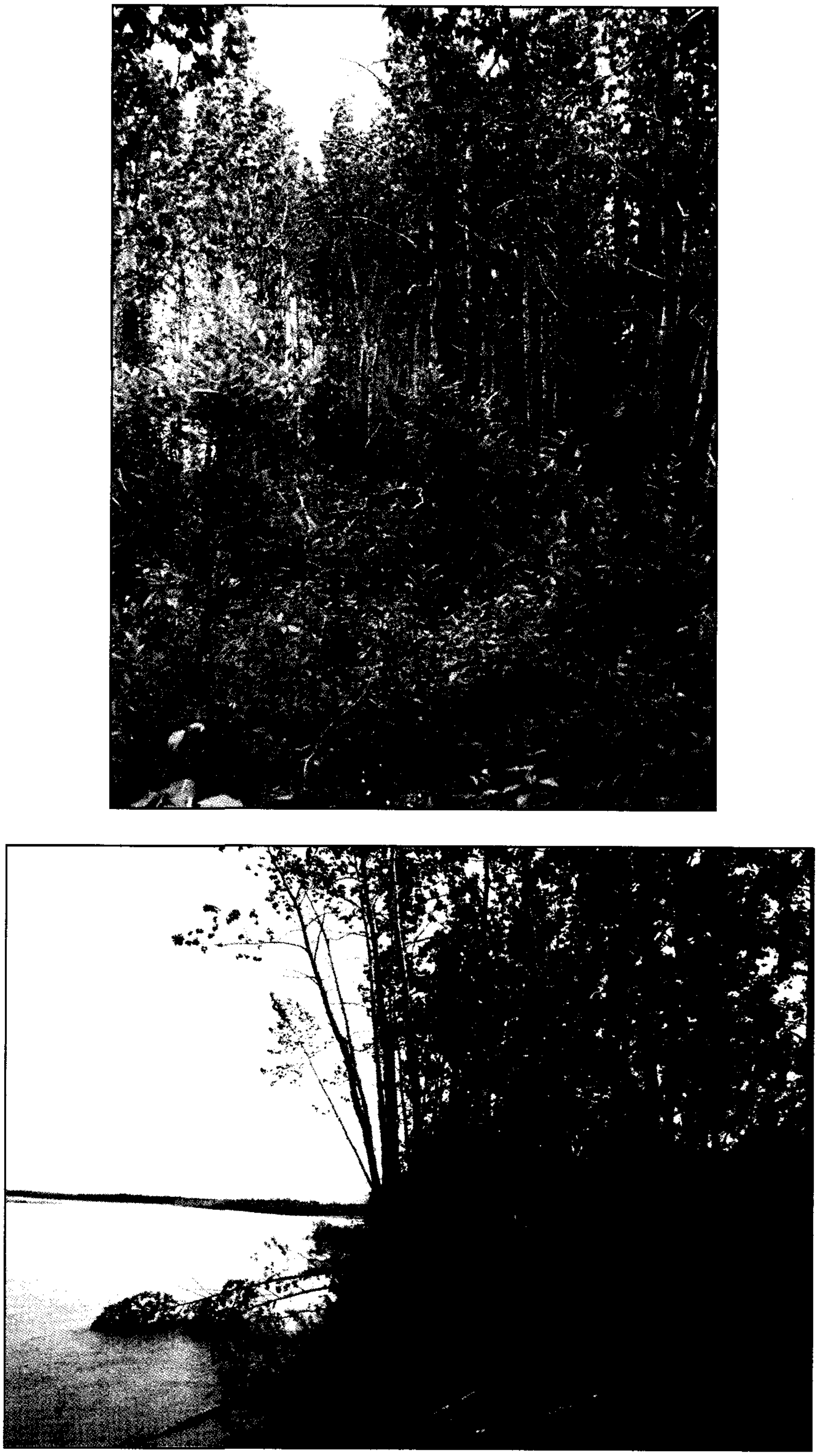


Figure A.1 Noatak River (Relevé 1, NOA4) balsam poplar grove. July 4, 2005.

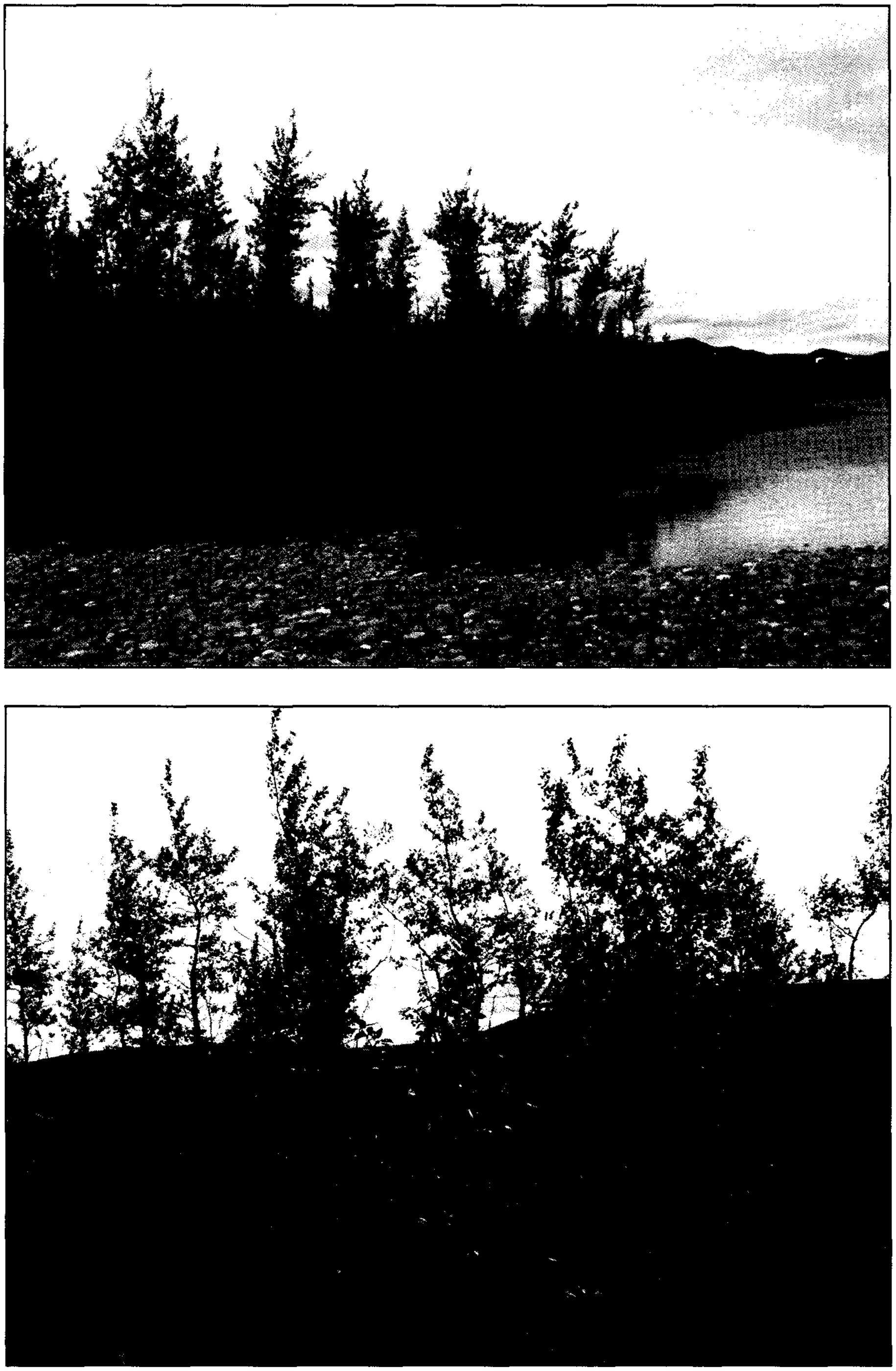
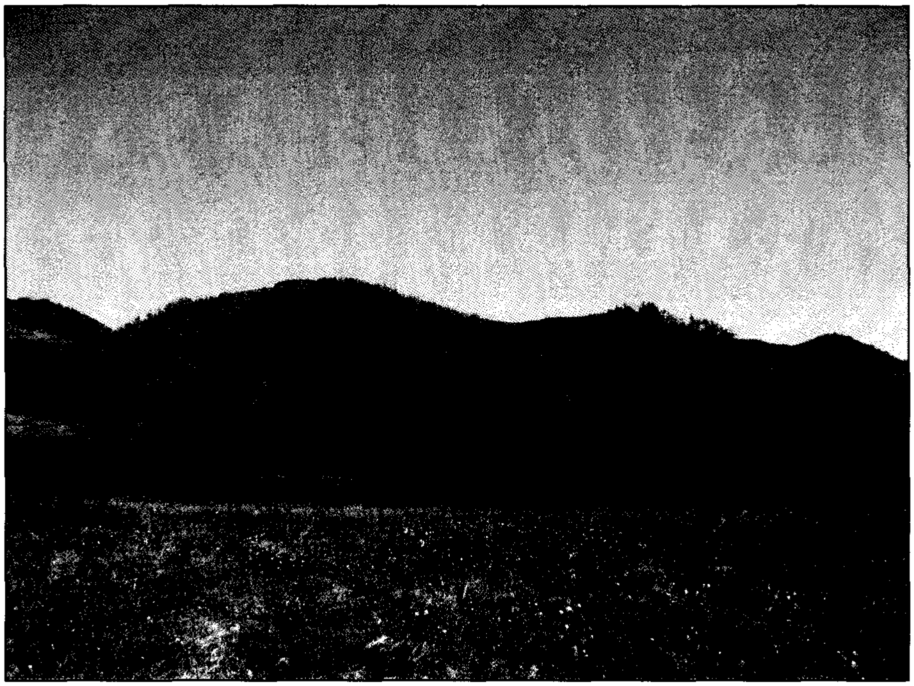


Figure A.2 Noatak River (Relevé 2, NOA3) balsam poplar grove. July 1, 2005.



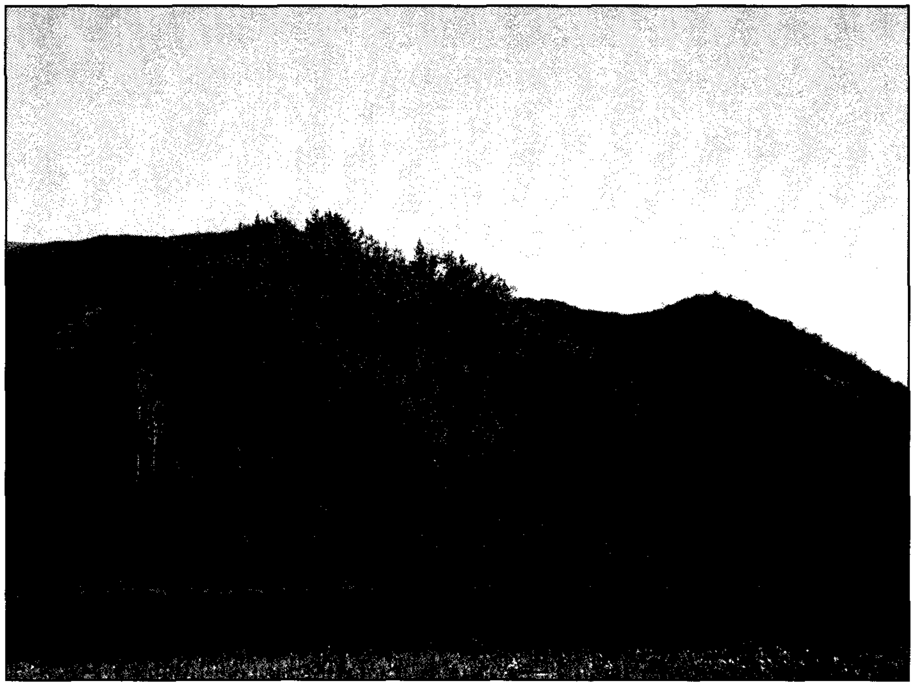


Figure A.3.1 Noatak River (Relevé 3, NOA2) balsam poplar grove. June 28, 2005.

Figure A.3.2 Noatak River (Relevé 3, NOA2) balsam poplar grove. June 28, 2005.

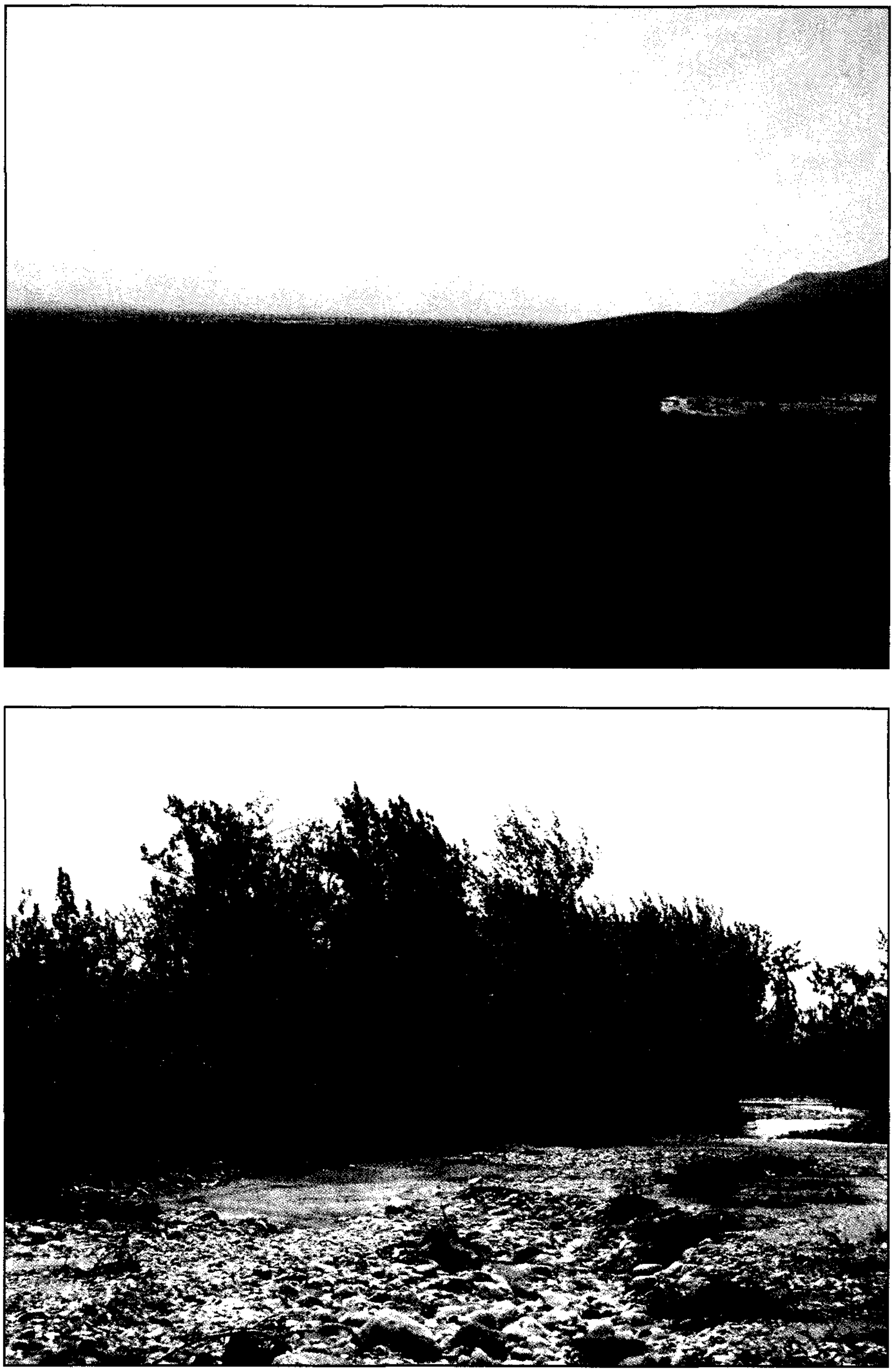


Figure A.4.1 Noatak River (Relevé 4, NOA1) balsam poplar grove. June 21, 2005.

Figure A.4.2 Noatak River (Relevé 4, NOA1) balsam poplar grove. June 21, 2005.

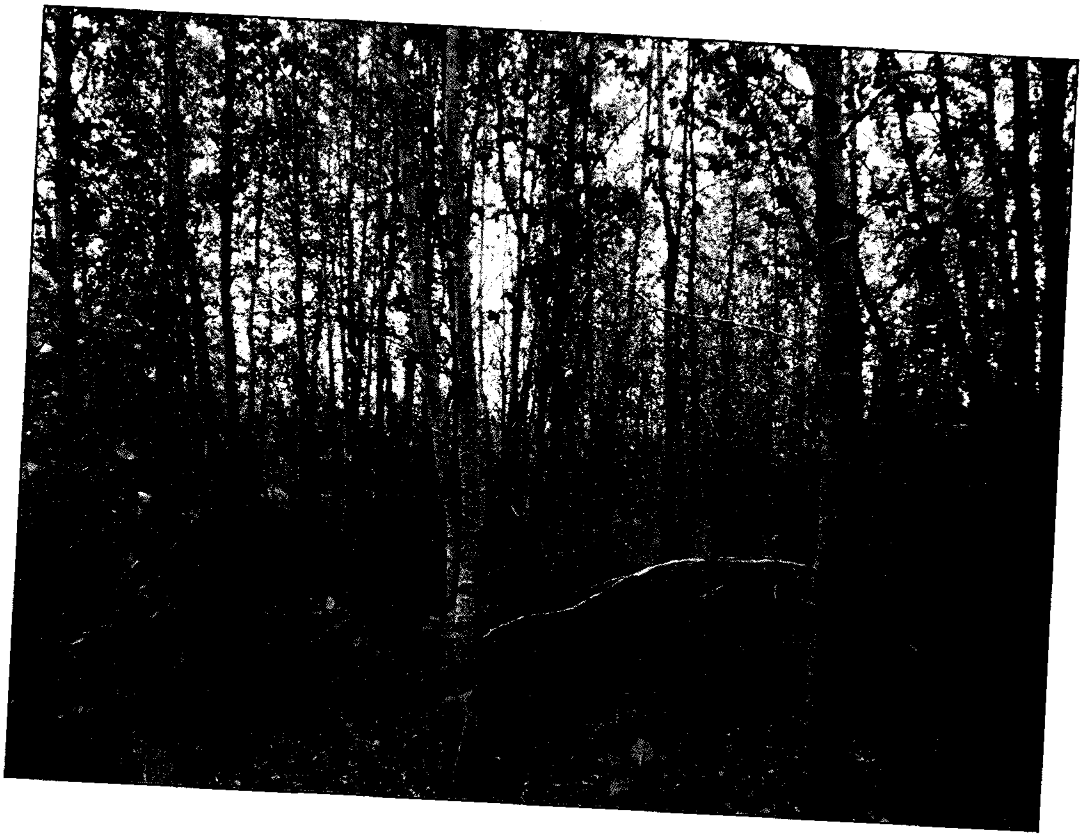


Figure A.5 Kobuk River (Relevé 5, KOB4) balsam poplar grove. June 29, 2003.

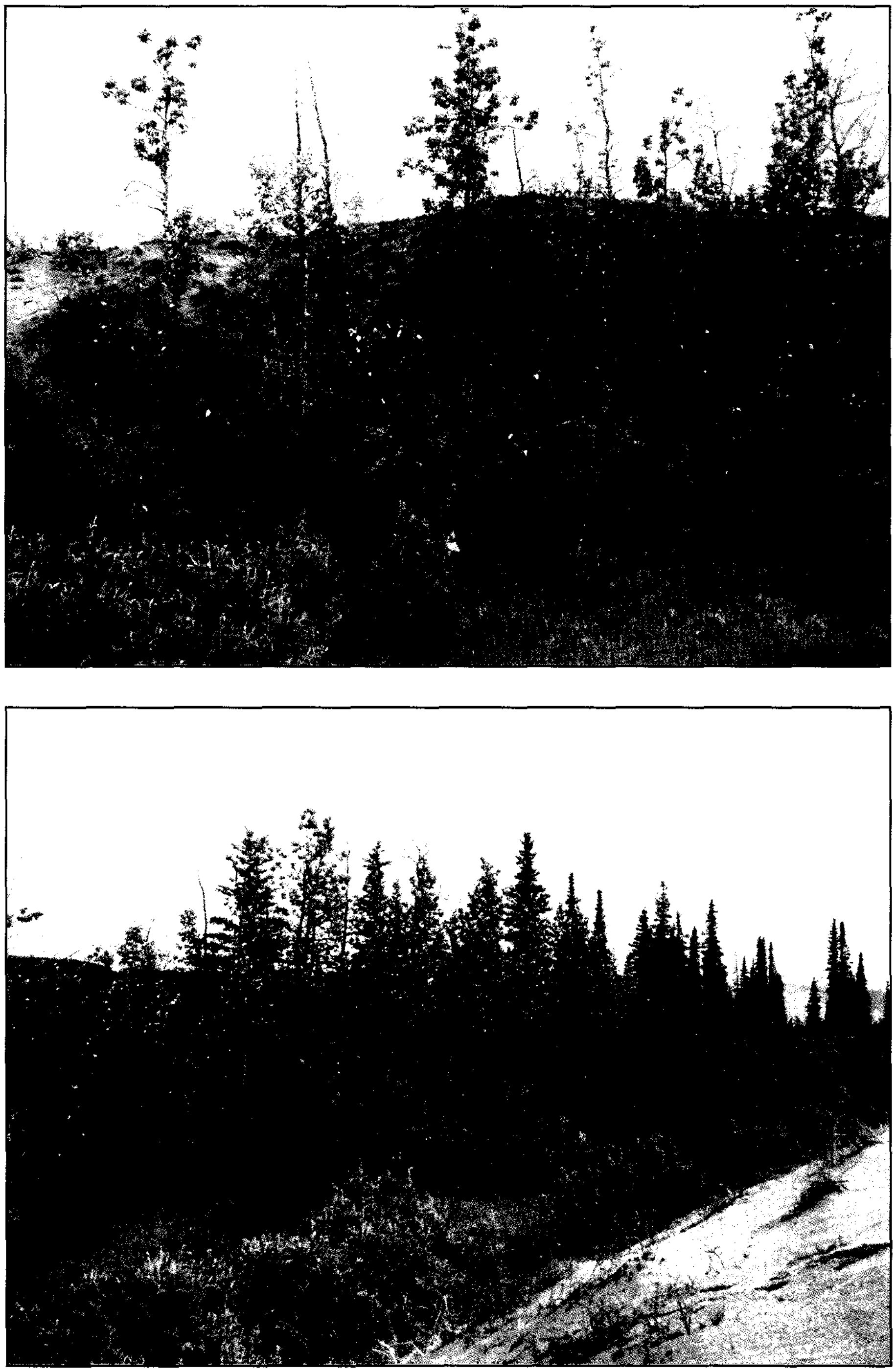


Figure A.6 Kobuk River (Relevé 6, KOB3) balsam poplar grove. June 23, 2003.

Figure A.7 Kobuk River (Relevé 7, KOB2) balsam poplar grove. June 23, 2003.

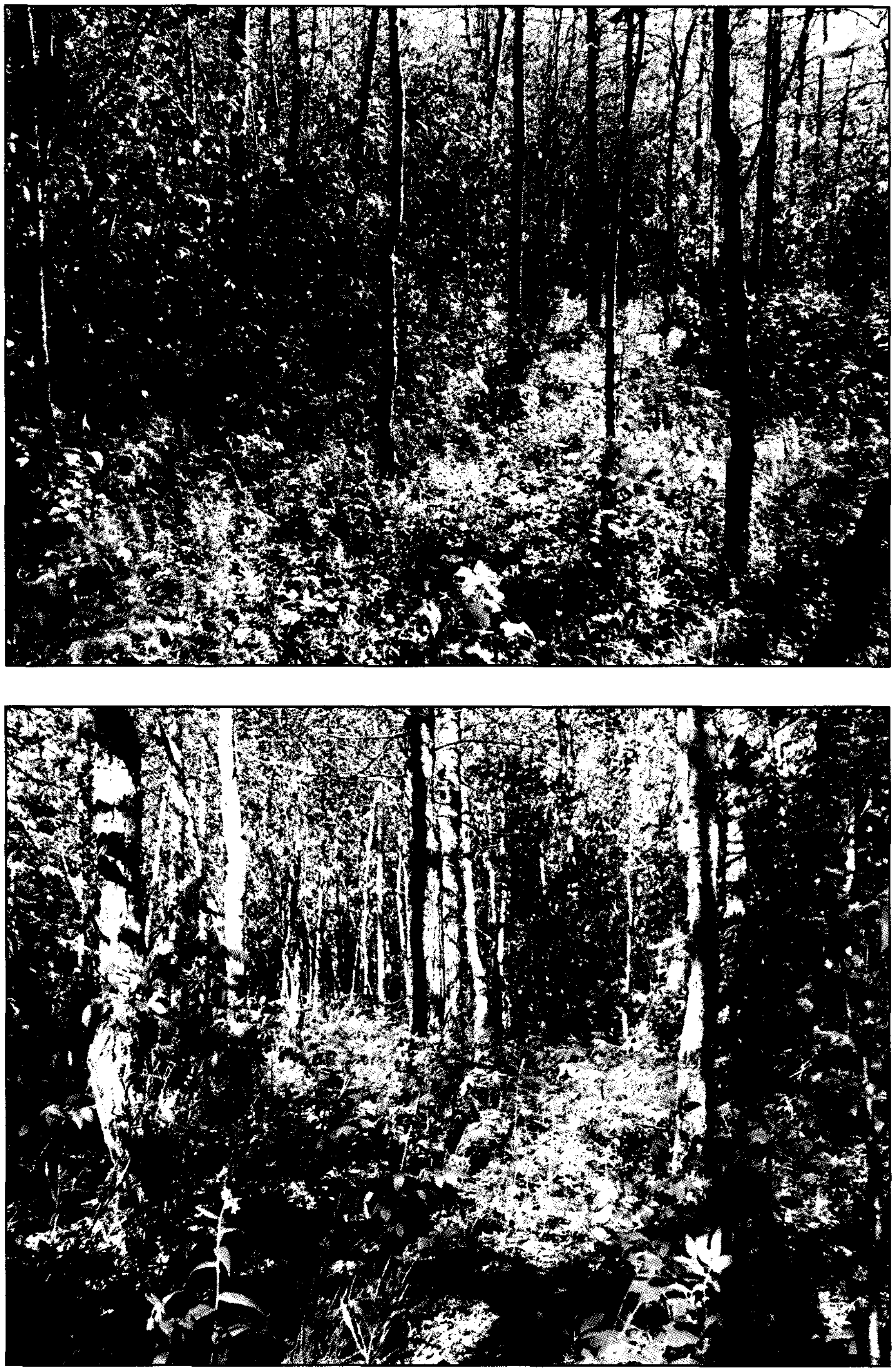


Figure A.8 Kobuk River (Relevé 8, KOB1) balsam poplar grove. June 23, 2003.

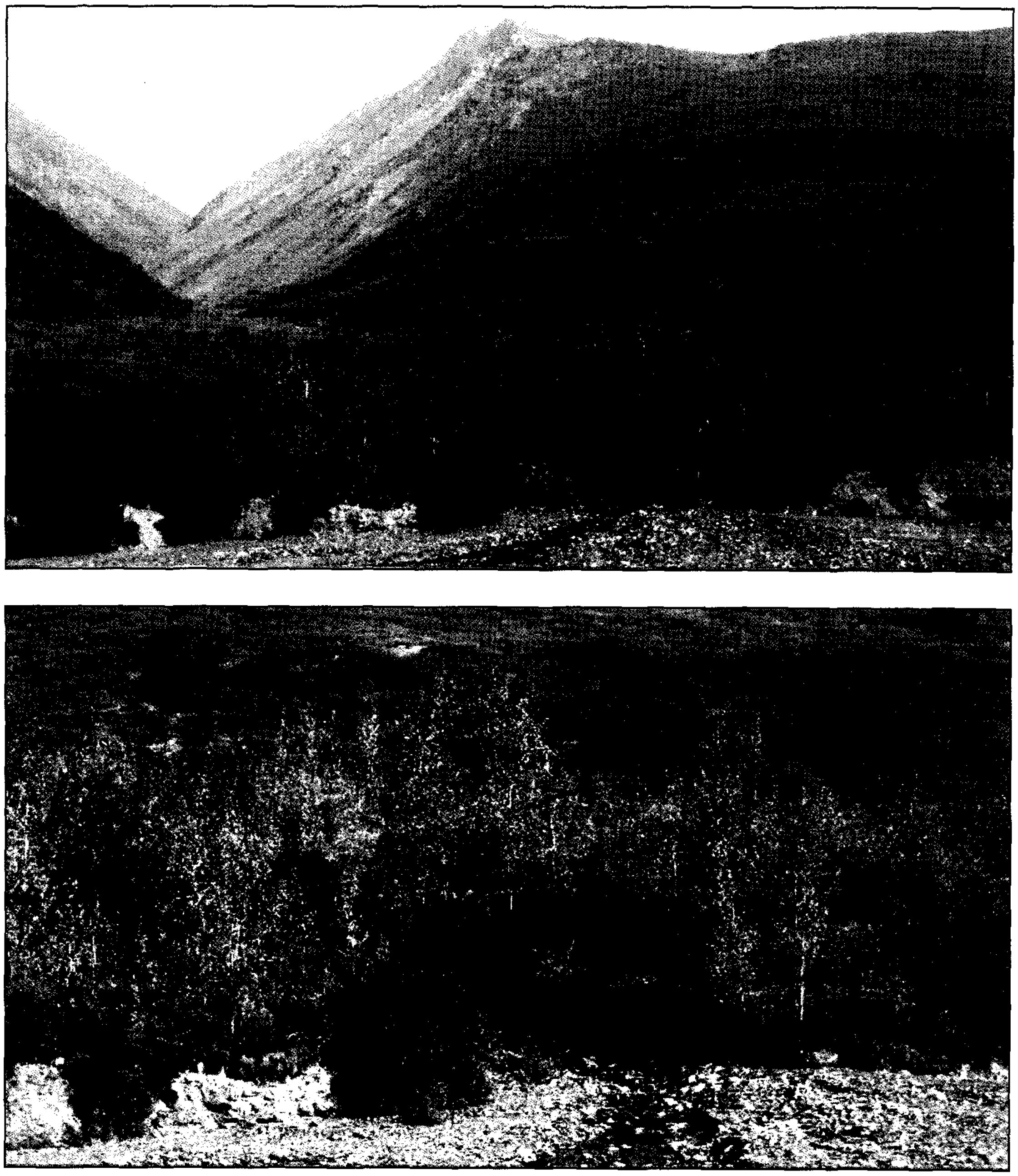


Figure A.9 Dietrich River (Relevé 9, DIE1) balsam poplar grove. August 5, 2009.

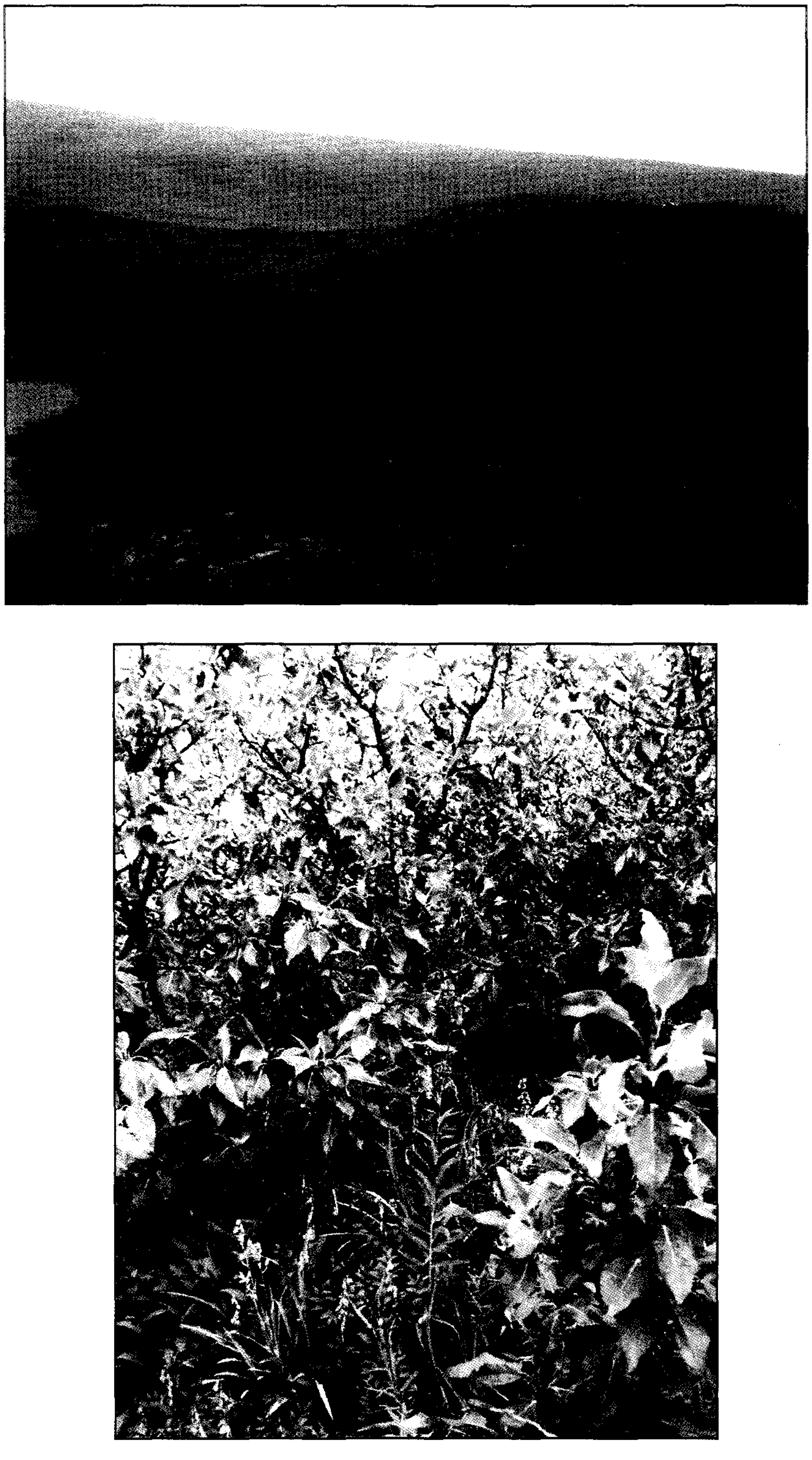


Figure A.10 Toolik Lake (Relevé 10, TOO1) balsam poplar grove. Top: August 4, 2009; Bottom: July 10, 2003.

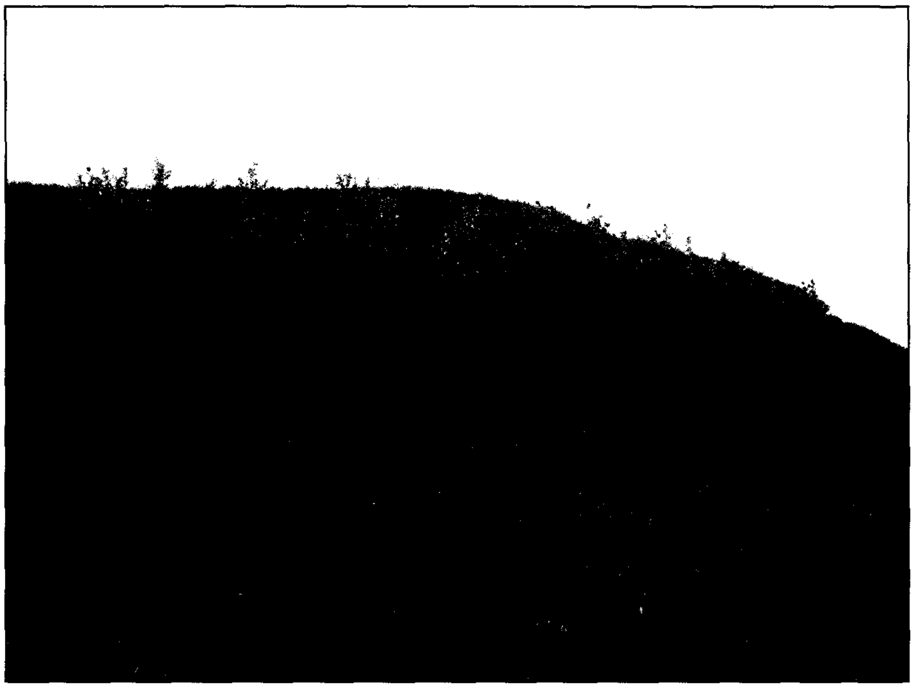


Figure A.11 Sagavanirktok River (Relevé 11, SAG1) balsam poplar grove. August 3, 2009.

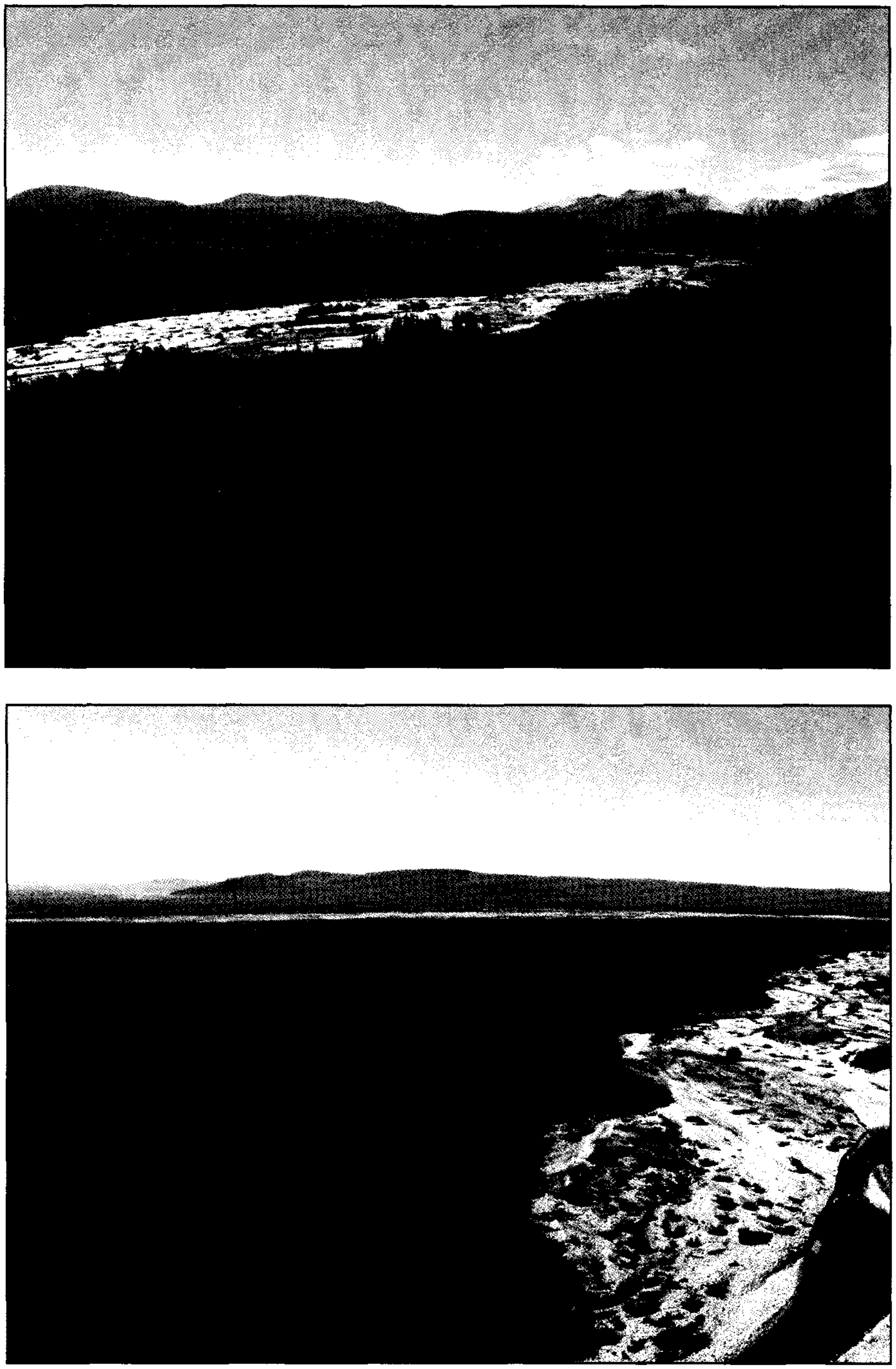


Figure A.12.1 Ivishak River (Relevé 12, IVI4) balsam poplar grove. August 18, 2004; photo credit top & bottom: Torsten Sachs.

Figure A.12.2 Ivishak River (Relevé 12, IVI4) balsam poplar grove. Top: July 2, 2004; bottom: April 1, 2007.

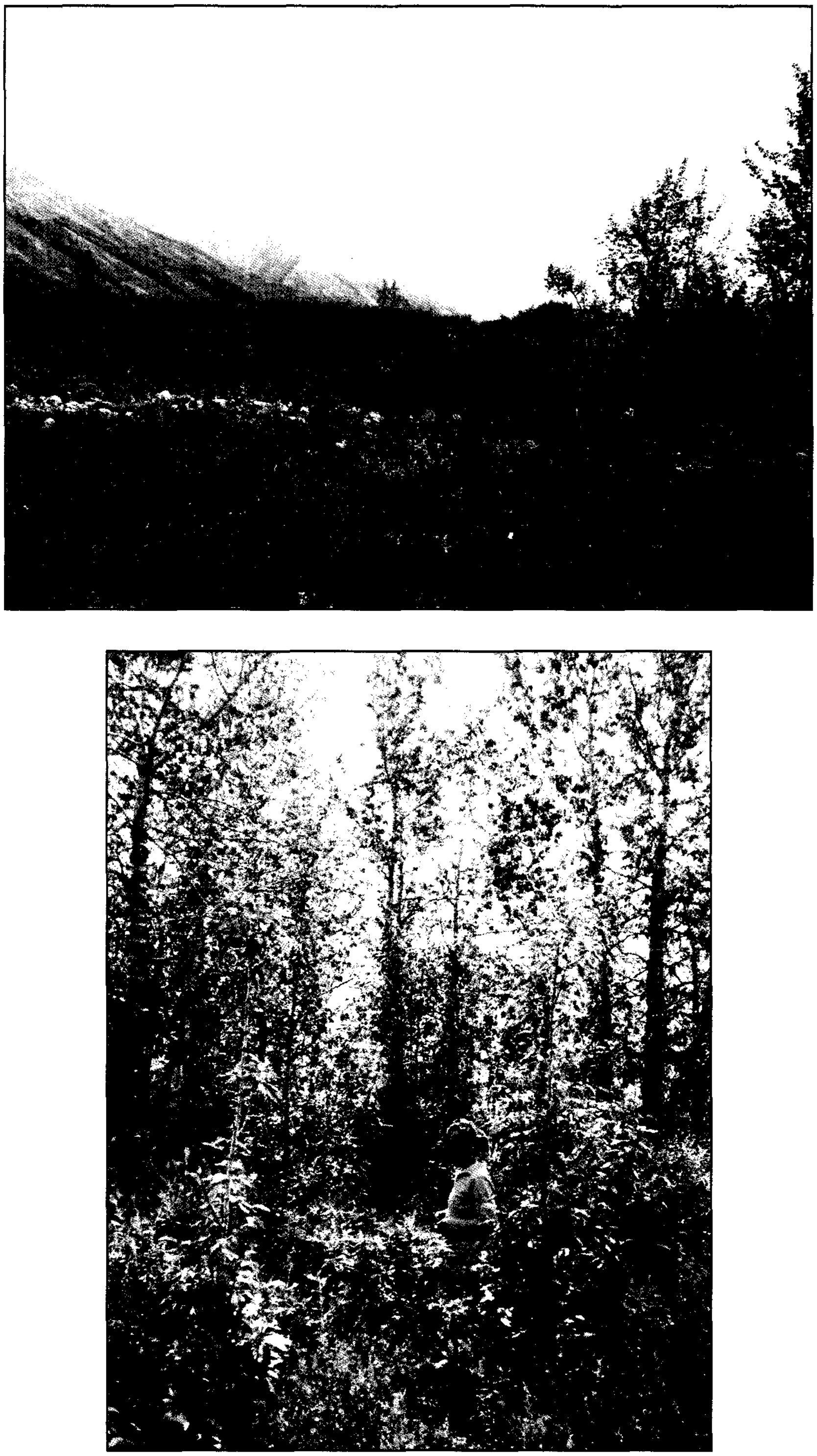


Figure A.13 Ivishak River (Relevé 13, IVI3) balsam poplar grove. July 1, 2004.

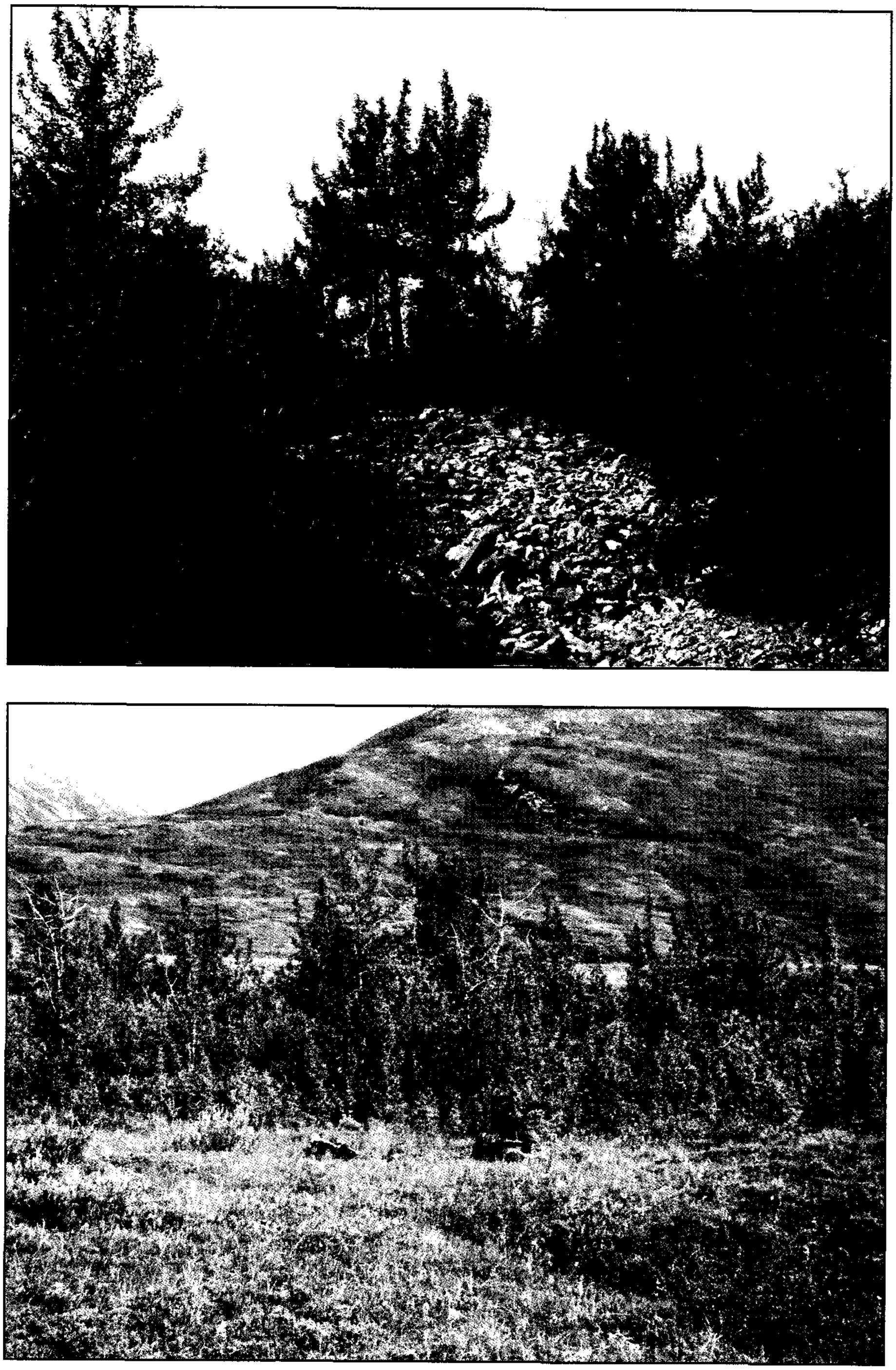


Figure A.14 Ivishak River (Relevé 14, IVI2) balsam poplar grove. July 1, 2004.

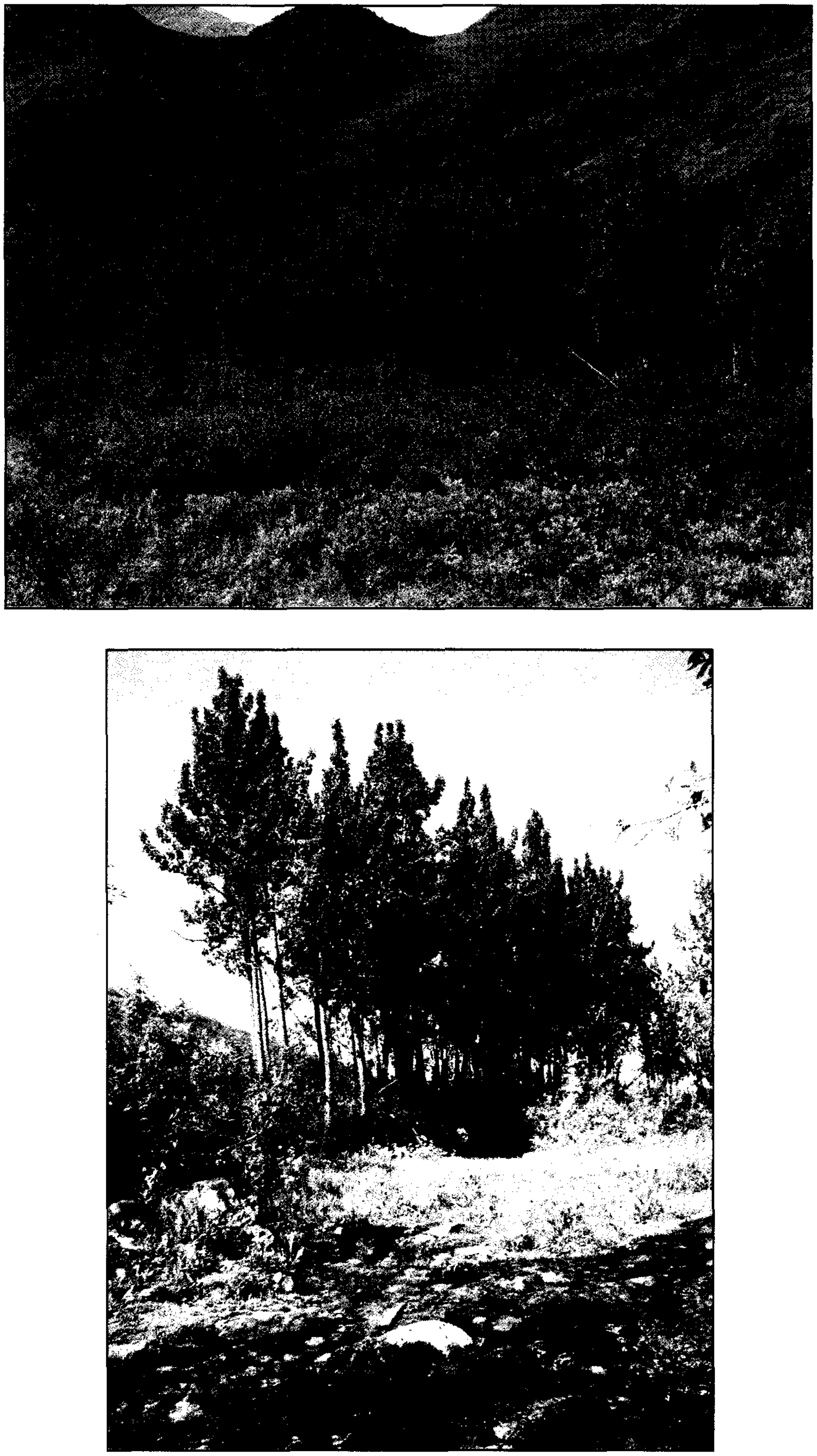


Figure A.15.1 Ivishak River (Relevé 15, IVI1) balsam poplar grove. Top: August 1, 2002; Bottom: June 30, 2004.

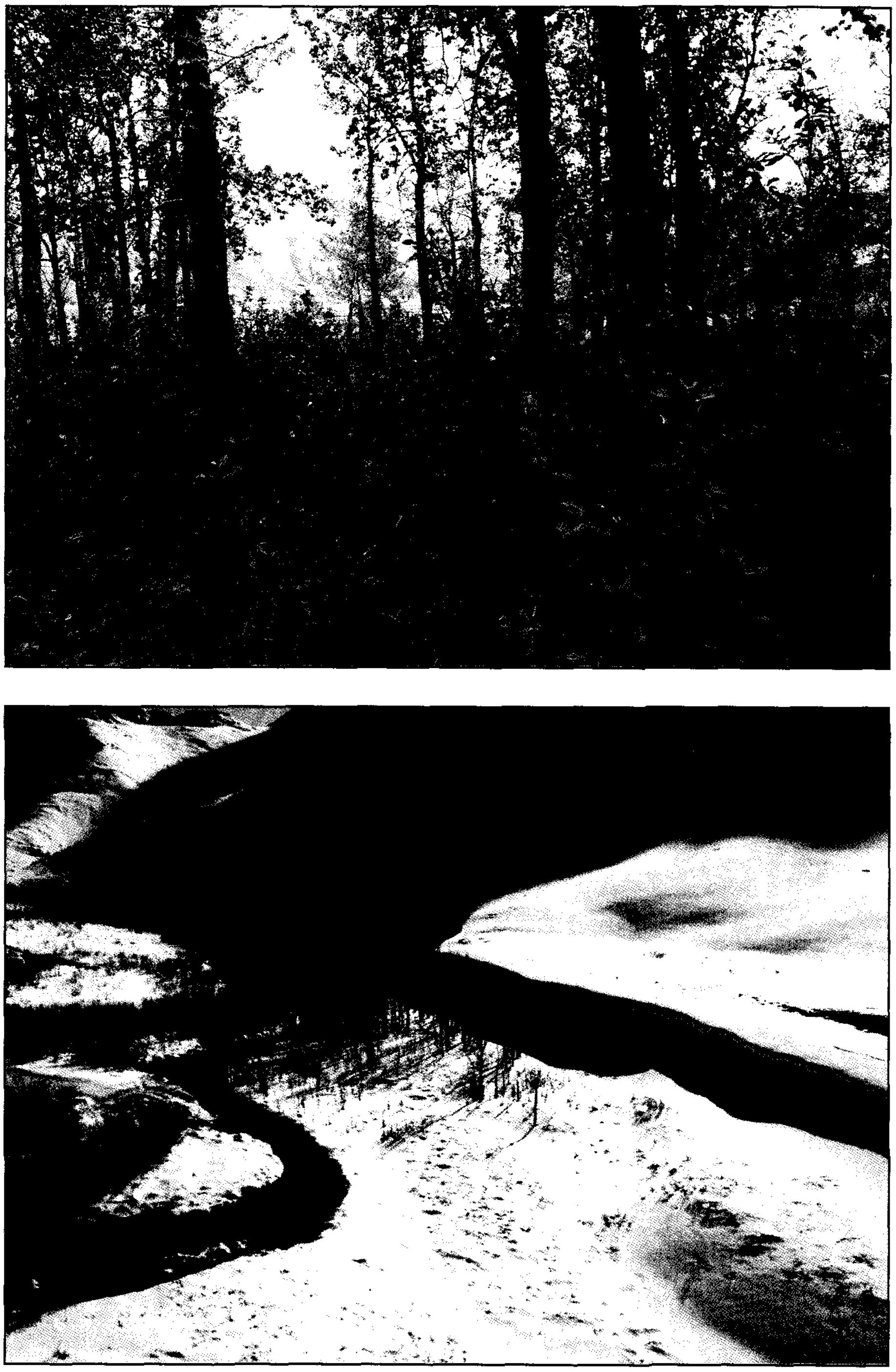


Figure A.15.2 Ivishak River (Relevé 15, IVI1) balsam poplar grove. Top: August 1, 2002; Bottom: April 1, 2007.

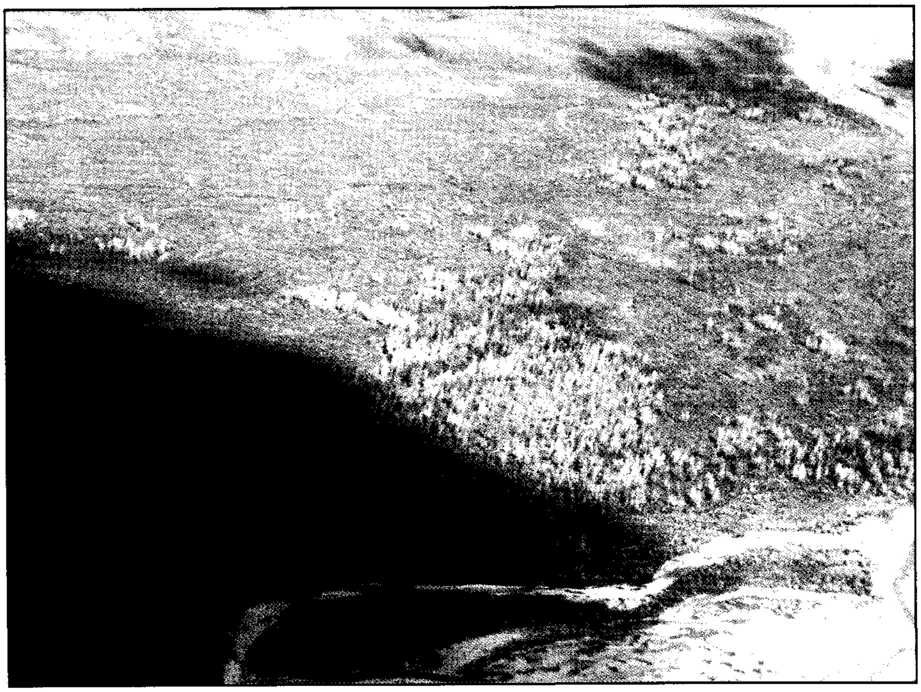
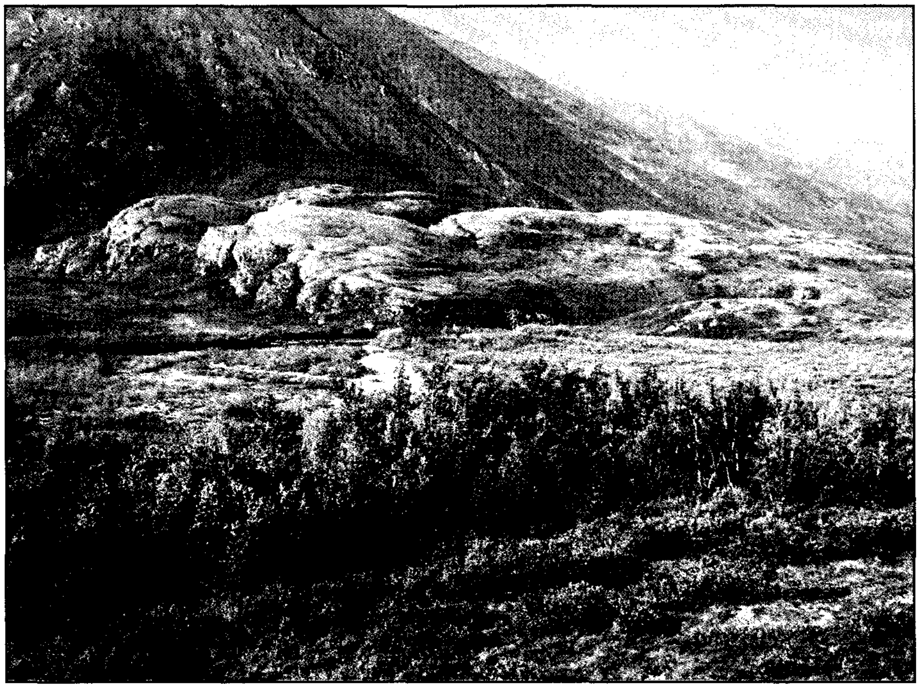


Figure A.16 Canning River (Relevé 16, CAN3) balsam poplar grove. Top: July 18, 2003; bottom: July 24, 2003.

Figure A.17 Canning River (Relevé 17, CAN2) balsam poplar grove. July 22, 2003.



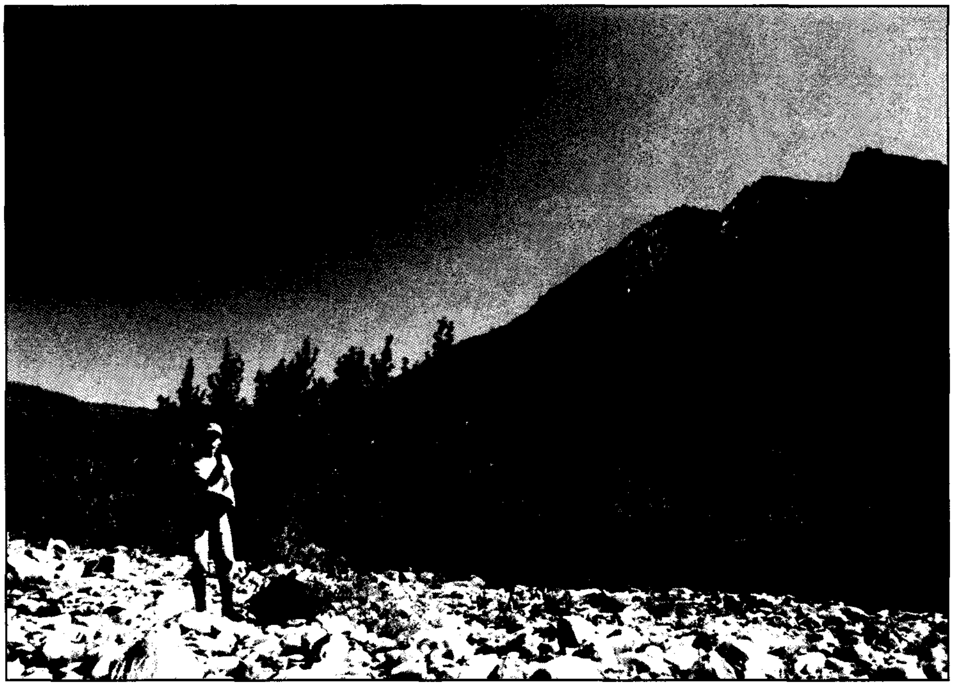


Figure A.18 Canning River (Relevé 18, CAN1) balsam poplar grove. July 20, 2003; photo credit bottom: Robert Breen.

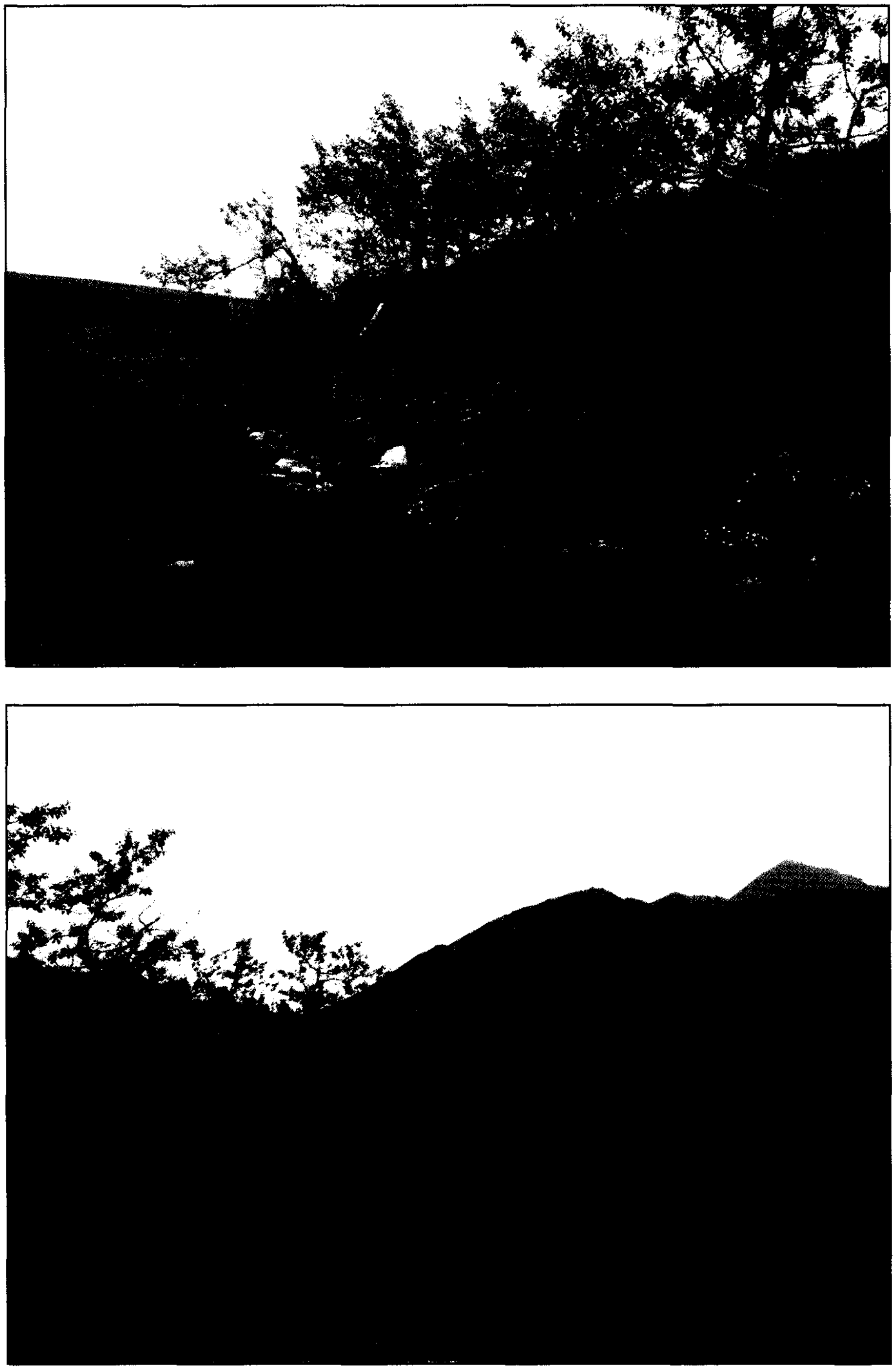
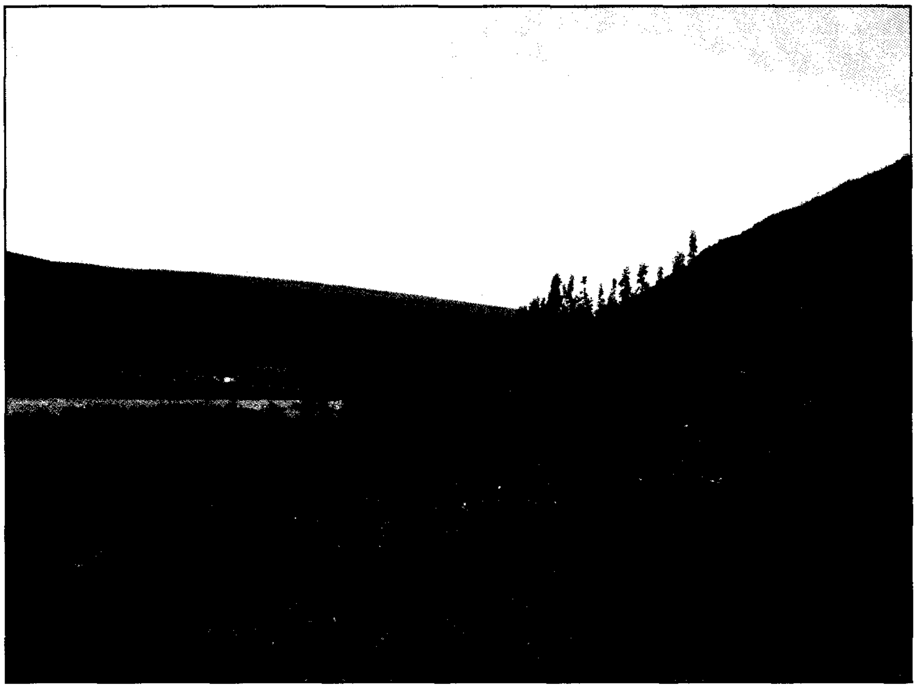


Figure A.19 Hulahula River (Relevé 19, HUL1) balsam poplar grove. June 24, 2004.



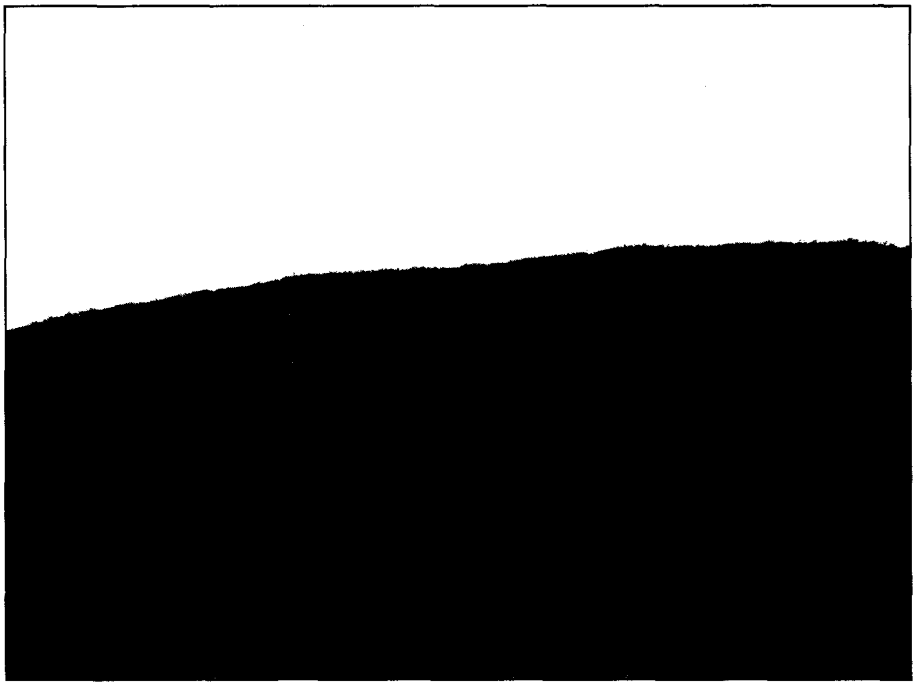


Figure A.20 Hulahula River (Relevé 20, HUL2) balsam poplar grove. June 25, 2004.

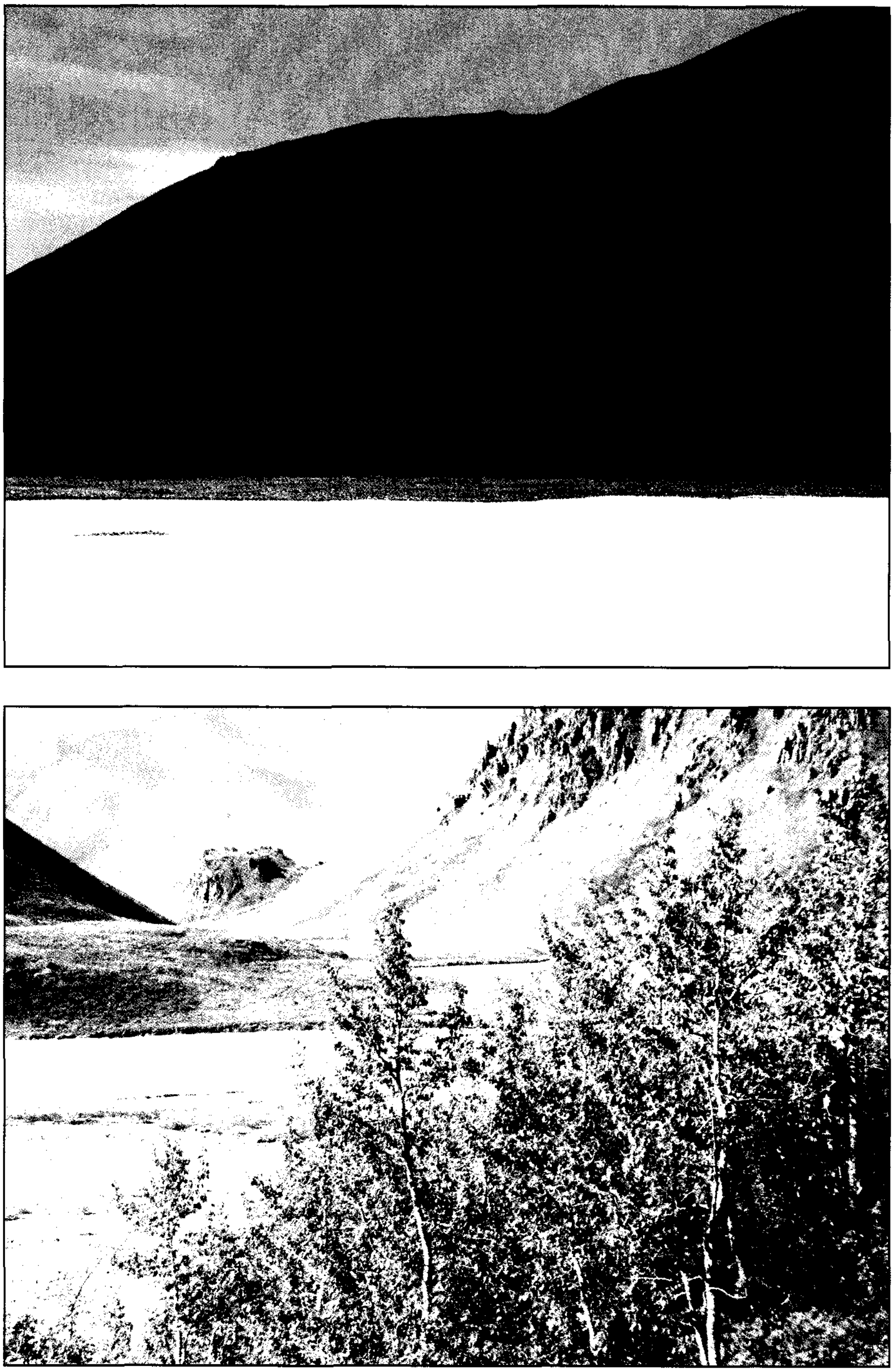


Figure A.21.1 Kongakut River (Relevé 21, KON3) balsam poplar grove. June 20, 2004.

Figure A.21.2 Kongakut River (Relevé 21, KON3) balsam poplar grove. June 20, 2004.

Figure A.22.1 Kongakut River (Relevé 22, KON2) balsam poplar grove. June 18, 2004.

Figure A.22.2 Kongakut River (Relevé 22, KON2) balsam poplar grove. June 18, 2004.

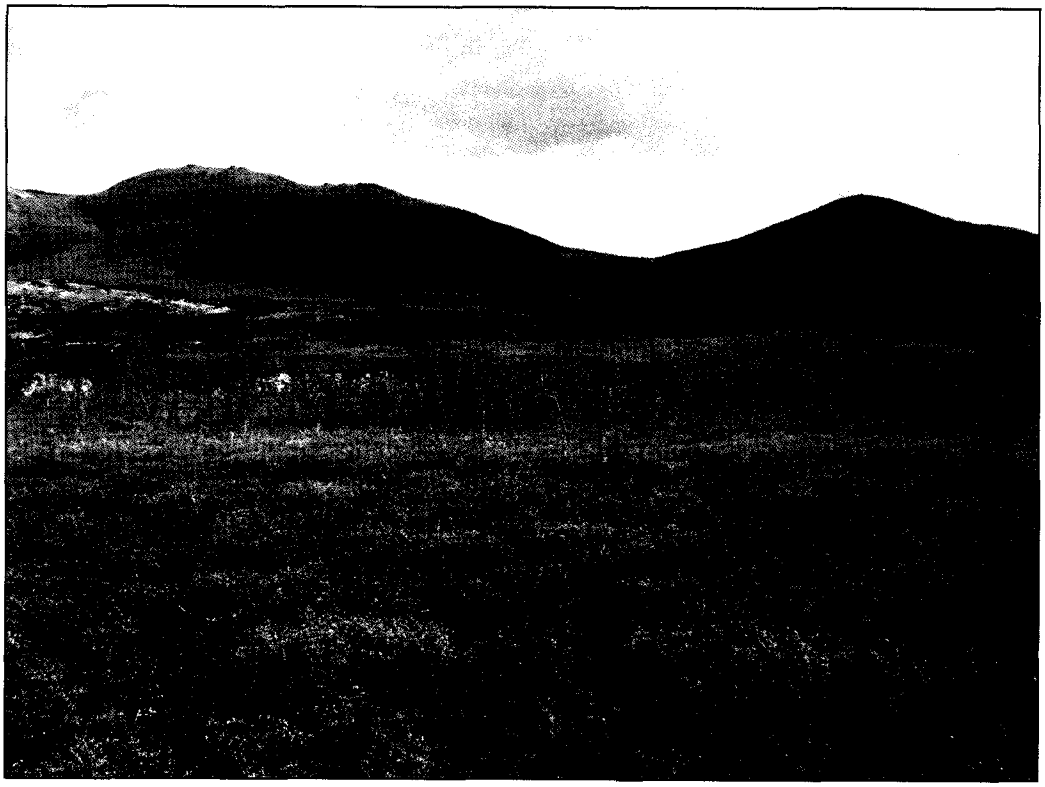


Figure A.23 Kongakut River (Relevé 23, KON1) balsam poplar grove. June 16, 2004.

Figure A.24 Porcupine River (Relevé 24, POR4) balsam poplar grove. August 18, 2003.

Figure A.25 Porcupine River (Relevé 25, POR3) balsam poplar grove. August 13, 2003.

Figure A.26 Porcupine River (Relevé 26, POR2) balsam poplar grove. August 10, 2003.

Figure A.27 Porcupine River (Relevé 27, POR1) balsam poplar grove. August 7, 2003.

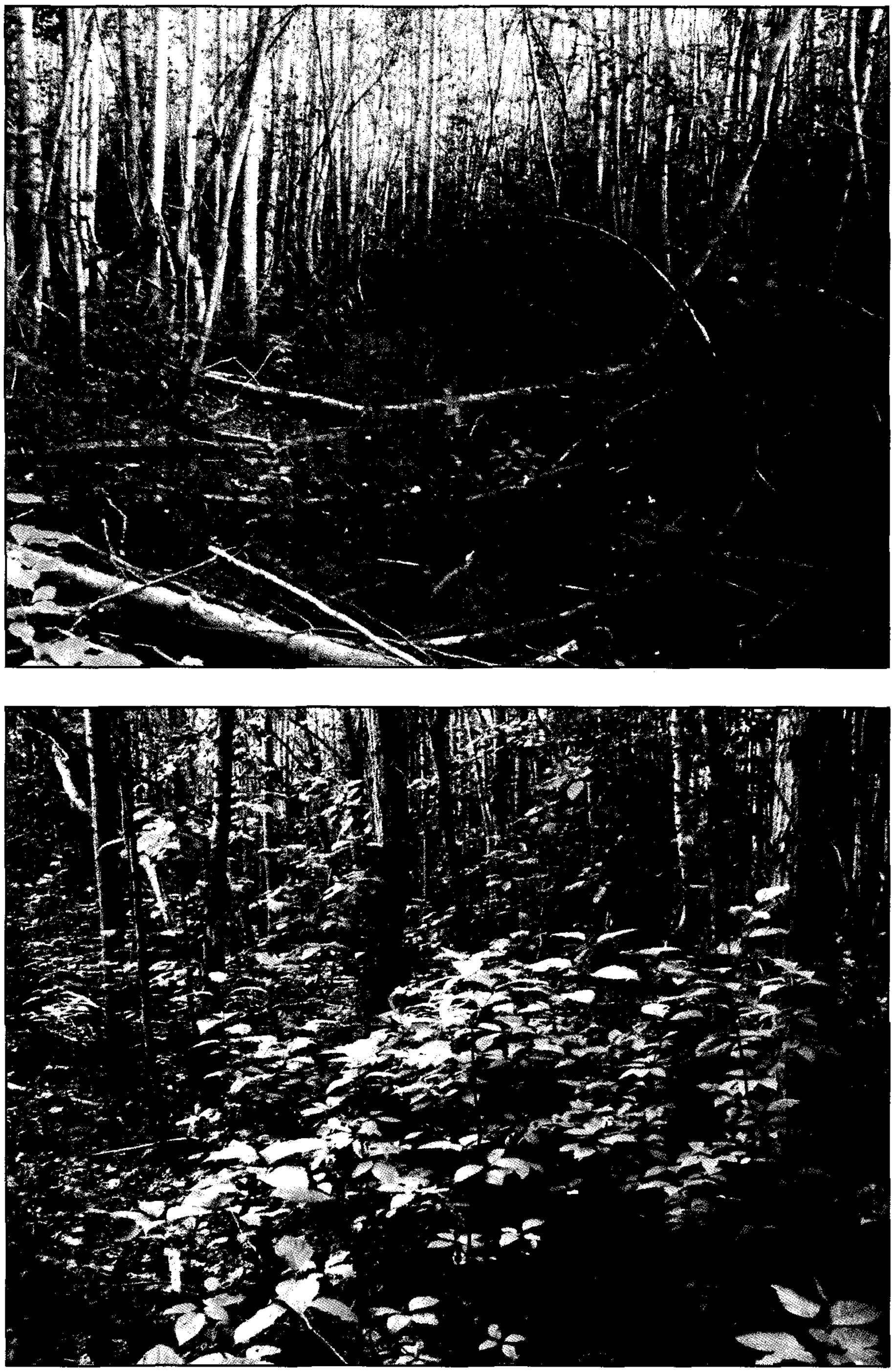


Figure A.28 Yukon River (Relevé 28, YUK10) balsam poplar grove. August 17, 2004.

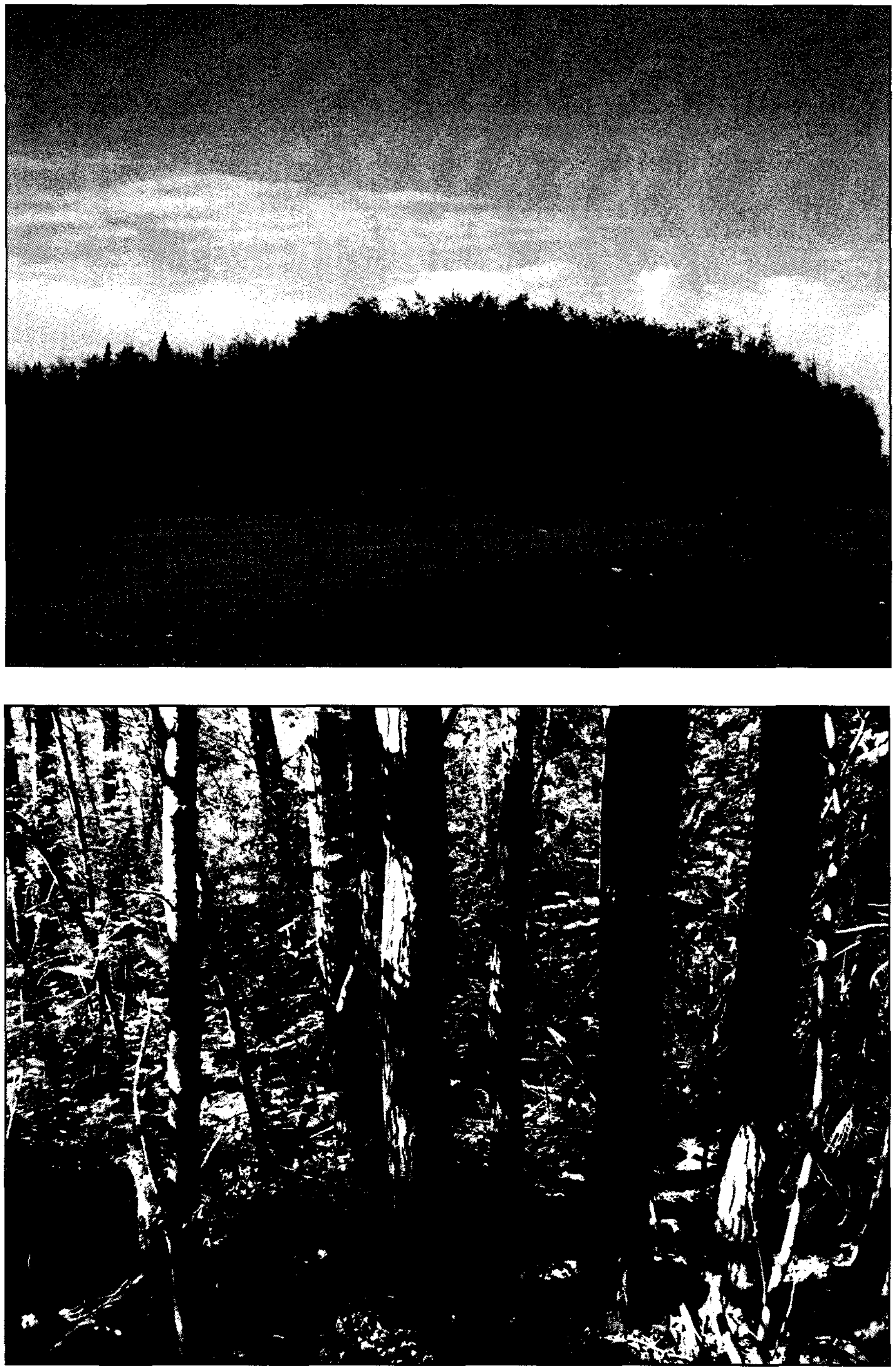


Figure A.29.1 Yukon River (Relevé 29, YUK9) balsam poplar grove. August 15, 2004.

Figure A.29.2 Yukon River (Relevé 29, YUK9) balsam poplar grove. August 15, 2004.

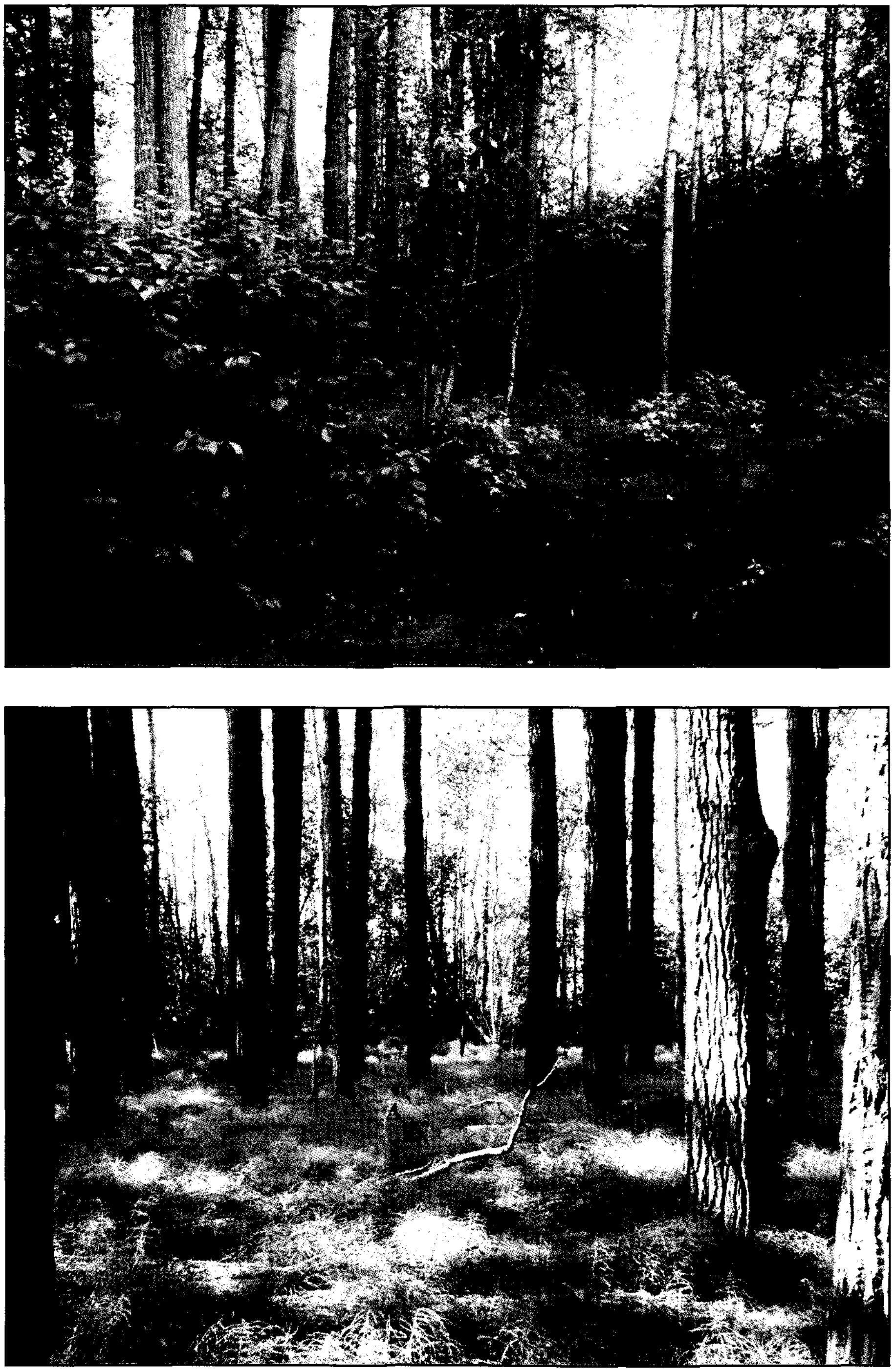


Figure A.30 Yukon River (Relevé 30, YUK8) balsam poplar grove. August 10, 2004.

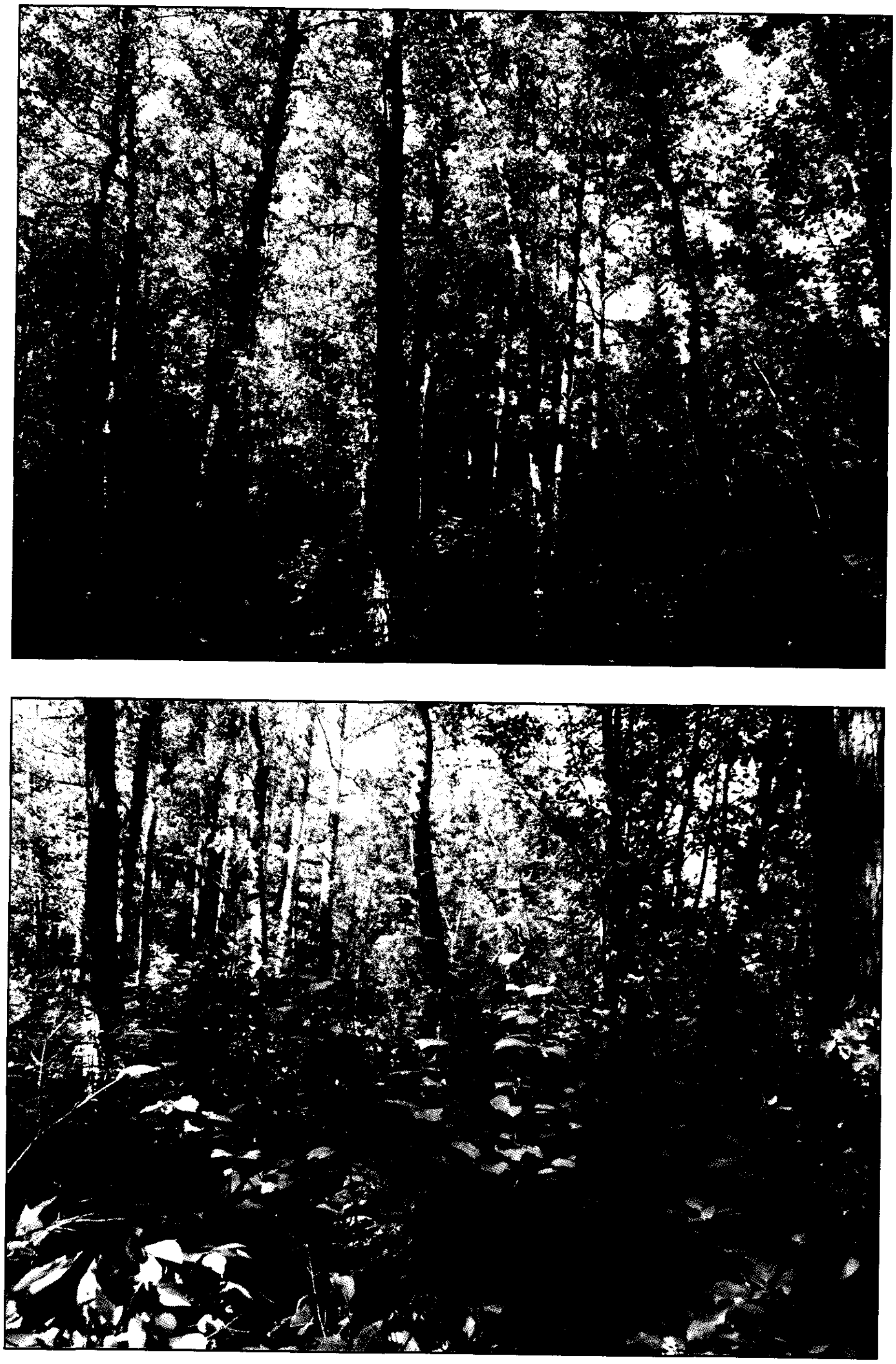


Figure A.31 Yukon River (Relevé 31, YUK2) balsam poplar grove. July 23, 2004.

Figure A.32 Yukon River (Relevé 32, YUK1) balsam poplar grove. July 22, 2004.

Table A.1 Complete species list and vascular plant floristic classifications for *Populus balsamifera* relevés.

Accepted species name and authority	Species name listed in Hultén (1968)	Regional unit	Geographic range
Trees			
Betula neoalaskana Sarg.	Betula papyrifera	boreal	Beringia
Picea glauca (Moench) Voss	Picea glauca	boreal	N. America
Populus balsamifera L.	Populus balsamifera subsp. balsamifera	boreal	N. America
Populus tremuloides Michx.	Populus tremuloides	boreal	N. America
Shrubs			
Alnus incana (L.) Moench. subsp. tenuifolia (Nutt.) Breitung	Alnus incana subsp. tenuifolia	boreal	N. America
Alnus viridis (Chaix) DC. subsp. fruticosa (Rupr.) Nyman	Alnus crispa subsp. crispa	boreal	N. America, Asia
Arctostaphylos uva-ursi (L.) Spreng.	Arctostaphylos uva-ursi var. uva-ursi	boreal-alpine	Circumpolar/boreal
Arctous alpina (L.) Nied.	Arctostaphylos alpina	arctic-alpine	Circumpolar/boreal
Arctous rubra (Rehder & E.H.Wilson) Nakai & Koidz.	Arctostaphylos rubra	arctic-alpine	N. America, Asia
Betula glandulosa Michx./Betula nana L. subsp. exilis (Sukaczev) Hultén	Betula glandulosa/nana subsp. exilis	arctic-alpine	N. America, Asia
Chamaepericlymenum canadense (L.) Graebn.	Cornus canadensis	boreal	N. America, Asia
Dasiphora fruticosa (L.) Rydb.	Potentilla fruticosa	arctic-boreal	Circumpolar/boreal
Dryas ajanensis Juz.	Dryas octopetala subsp. octopetala	arctic-alpine	N. America, Asia
Dryas integrifolia Vahl subsp. integrifolia	Dryas integrifolia subsp. integrifolia	arctic-alpine	N. America, Asia
Eleagnus commutata Bernh.[1]	Eleagnus commutata	boreal	N. America
Empetrum nigrum L.	Empetrum nigrum	arctic-boreal	Circumpolar/boreal
Juniperus communis L. subsp. nana (Willd.) Syme	Juniperus communis subsp. nana	boreal	N. America, Asia, Europe
Ledum groenlandicum Oeder	Ledum palustre subsp. groenlandicum	boreal	N. America
Linnaea borealis L. subsp. americana (J.Forbes) Hultén	Linnea borealis subsp. americana	arctic-boreal	N. America
Rhododendron lapponicum (L.) Wahlenb. subsp. parvifolium (Adams) Malyschev	Rhododendron lapponicum	arctic-alpine	N. America, Asia
Ribes triste Pall.	Ribes triste	boreal	N. America, Asia
Rosa acicularis Lindl.	Rosa acicularis	boreal	Circumpolar/boreal
Rubus arcticus L.	Rubus arcticus	boreal	N. America, Asia, Europe
Rubus idaeus L.	Rubus idaeus	boreal	Circumpolar/boreal
Salix alaxensis (Andersson) Coville var. alaxensis	Salix alaxensis subsp. alaxensis	arctic	N. America, Asia
Salix alaxensis (Andersson) Coville var. longistylis (Rydberg) C. K. Schneider	Salix alaxensis subsp. longistylis	boreal	N. America
Salix arbusculoides Andersson	Salix arbusculoides	arctic-boreal	N. America
Salix bebbiana Sarg.	Salix depressa subsp. rostrata	boreal	Circumpolar/boreal
Salix glauca L. subsp. acutifolia (Hook.) Hultén	Salix glauca subsp. acutifolia	arctic-alpine	N. America
Salix glauca L. subsp. stipulifera (Flod. ex Häyrén) Hiitonen	Salix glauca	arctic-alpine	N. America, Asia
Salix hastata L.	Salix hastata	arctic-alpine	N. America, Asia, Europe
Salix niphoclada Rydb.	Salix niphoclada	arctic-alpine	Beringia
Salix pulchra Cham.	Salix pulchra	arctic-alpine	N. America, Asia
Salix reticulata L.	Salix reticulata	arctic-alpine	Circumpolar/boreal
Salix richardsonii Hook.	Salix lanata subsp. richardsonii	arctic	N. America, Asia
Shepherdia canadensis (L.) Nutt.	Shepherdia canadensis	boreal	N. America
Swida sericea (L.) Holub	Cornus stolonifera	boreal-alpine	N. America
Vaccinium cespitosum Michx.	Vaccinium caespitosum	boreal	N. America
Vaccinium uliginosum L. subsp. microphyllum (Lange) Tolm.	Vaccinium uliginosum subsp. alpinum	arctic-boreal	Circumpolar/boreal
Vaccinium vitis-idaea L. subsp. minus (Lodd.) Hultén	Vaccinium vitis-idaea subsp. minus	arctic-boreal	Circumpolar/boreal
Viburnum edule (Michx.) Raf.	Viburnum edule	boreal	N. America, Asia
Forbs			
Oxytropis deflexa (Pall.) DC. subsp. foliolosa (Hook.) Cody	Oxytropis deflexa var. foliolosa	arctic-alpine	N. America
Achillea millefolium L. subsp. borealis (Bong.) Breitung	Achillea borealis	arctic-boreal	N. America, Asia
Aconitum delphinifolium DC. var. delphinifolium	Aconitum delphinifolium subsp. delphinifolium	arctic-boreal	N. America, Asia
Aconogonon alaskanum (Wight ex Hultén) Soják var. alaskanum	Polygonum alaskanum	boreal	Beringia
Actaea rubra (Aiton) Willd.	Actaea rubra subsp. rubra	boreal	N. America
Allium schoenoprasum L. s. lat.	Allium schoenoprasum var. sibiricum	arctic-alpine	Circumpolar/boreal
Androsace chamaejasme Wulfen subsp. andersonii (Hultén) Hultén	Androsace chamaejasme subsp. lehmanniana	arctic-alpine	N. America, Asia
Anemone drummondii S.Watson	Anemone drummondii	boreal	N. America, Asia
Anemone parviflora Michx.	Anemone parviflora	arctic-alpine	N. America, Asia
Anemone richardsonii Hook.	Anemone richardsonii	arctic-boreal	N. America, Asia
Anticlea elegans (Pursh) Rydb.	Zygadenus elegans	arctic-boreal	N. America
Arnica angustifolia Vahl subsp. angustifolia s. lat.	Arnica alpina subsp. angustifolia	arctic-alpine	Circumpolar/boreal
Arnica griscomii Fernald subsp. frigida (C.A.Mey. ex Iljin) S.J.Wolf	Arnica frigida	arctic-alpine	Beringia
Artemisia arctica Less. subsp. arctica	Artemisia arctica subsp. arctica	arctic-alpine	N. America
Artemisia kruhsiana Besser subsp. alaskana (Rydb.) D.F.Murray & Elven comb. et stat. n	Artemisia alaskana	arctic	Beringia
Artemisia tilesii Ledeb.	Artemisia tilesii	arctic-alpine	N. America, Asia. Europe
Astragalus alpinus L. subsp. alaskanus Hultén	Astragalus alpinus subsp. alaskanus	boreal	Beringia
Astragalus alpinus L. subsp. alpinus	Astragalus alpinus subsp. alpinus	arctic-boreal	Circumpolar/boreal
Astragalus eucosmus B.L.Rob.	Astragalus eucosmus subsp. sealei	arctic	N. America
Astragalus umbellatus	Astragalus umbellatus	arctic	N. America, Asia
Bistorta plumosa (Small) Greene	Polygonum bistorta subsp. plumosum	arctic-alpine	N. America, Asia
Bistorta vivipara (L.) Delarbre	Polygonum viviparum	arctic-alpine	Circumpolar/boreal
Boschniakia rossica (Cham. & Schltdl.) B.Fedtsch.	Boschniakia rossica	boreal-alpine	N. America, Asia
Bupleurum arcticum (Regel) Krasnob.	Bupleurum triradiatum subsp. arcticum	arctic-alpine	Beringia
Cardamine pratensis L. subsp. angustifolia (Hook.) O.E.Schulz	Cardamine pratensis subsp. angustifolia	arctic	Circumpolar/boreal
Castilleja caudata (Pennell) Rebrist.	Castilleja caudata	arctic	N. America, Asia
Castilleja elegans Ostenf.	Castilleja elegans	arctic-alpine	N. America
Cerastium beeringianum Cham. & Schltdl.	Cerastium beeringianum	arctic-alpine	Circumpolar/boreal
Chamerion angustifolium (L.) Holub subsp. angustifolium	Epilobium angustifolium subsp. angustifolium	boreal	Circumpolar/boreal
Chamerion latifolium (L.) Holub	Epilobium latifolium	arctic-alpine	Circumpolar/boreal
Chrysosplenium tetrandrum (N.Lund ex Malmgren) Th.Fr.	Chyrsosplenium tetrandrum	arctic	Circumpolar/boreal
Cnidium cnidiifolium (Turcz.) Schischk.	Cnidium cnidiifolium	arctic	N. America, Asia
Corallorhiza trifida Châtel.	Corallorrhiza trifida	arctic-boreal	Circumpolar/boreal
Cypripedium passerinum Richardson	Cypripedium passerinum	arctic-boreal	N. America
Delphinium chamissonis Pritz. Ex Walp.	Delphinium brachycentrum	arctic-alpine	N. America, Asia
Delphinium glaucum S.Watson	Delphinium glaucum	arctic-boreal	N. America
Dodecatheon frigidum Cham. & Schltdl.	Dodecatheon frigidum	arctic	Beringia
Draba cinerea Adams	Draba cinerea	arctic-alpine	Circumpolar/boreal
Draba praealta Greene	Draba praealta	boreal	N. America
Epilobium hornemannii Rchb.	Epilobium hornemanii	arctic-boreal	N. America, Asia. Europe
Epilobium palustre L.	Epilobium palustre	boreal	Circumpolar/boreal
Eurybia sibirica (L.) G.L.Nesom	Aster sibiricus	arctic-alpine	N. America, Asia. Europe
Fragaria virginiana Mill.	Fragaria virginiana	boreal	N. America
Galium boreale L. s. lat.	Galium boreale	boreal	Circumpolar/boreal
Gentianella propinqua (Richardson) J.M.Gillett	Gentiana propinqua	arctic-alpine	N. America
Geocaulon lividum (Richardson) Fernald	Geocaulon lividum	boreal	N. America
Hedysarum americanum (Michx.) Britton	Hedysarum alpinum subsp. americanum	arctic-alpine	N. America
Hedysarum boreale Nutt. subsp. mackenzii (Richardson) S.L.Welsh	Hedysarum mackenzii	arctic-boreal	N. America
Iris setosa Pall. ex Link var. setosa	Iris setosa subsp. setosa	boreal	N. America, Asia
Limnorchis aequilonis (Sheviak) Rebrist. & Elven	Platanthera hyperborea	boreal	N. America
Listera borealis Morong	Listera borealis	arctic-boreal	N. America

Table A.1 (cont.)

Accepted species name and authority	Species name listed in Hultén (1968)	Regional unit	Geographic range
Forbs (cont.)			
Lupinus arcticus S.Watson	Lupinus arcticus	arctic	N. America
Lysiella obtusata (Banks ex Pursh) Rydb.	Platanthera obtusata	boreal	N. America
Mertensia paniculata (Aiton) G.Don	Mertensia paniculata var. paniculata	boreal	N. America
Micranthes nelsoniana (D.Don) Small subsp. nelsoniana	Saxifraga punctata subsp. nelsoniana	arctic-alpine	N. America, Asia
Micranthes reflexa (Hook.) Small	Saxifraga reflexa	arctic-alpine	Beringia
Minuartia arctica (Steven ex Ser.) Graebn.	Minuartia arctica	arctic-alpine	N. America, Asia
Moehringia lateriflora (L.) Fenzl	Moehringia lateriflora	arctic-boreal	N. America, Asia. Europe
Moneses uniflora (L.) A.Gray	Monses uniflora	boreal	Circumpolar/boreal
Myosotis alpestris F.W.Schmidt subsp. asiatica Vestergr.	Myosotis alpestris subsp. asiatica	arctic-alpine	N. America, Asia. Europe
Orthilia secunda (L.) House	Pyrola secunda	boreal	Circumpolar/boreal
Oxytropis borealis DC.	Oxytropis borealis	arctic	Beringia
Oxytropis jordalii A.E.Porsild	Oxytropis campestris subsp. jordalii	arctic-alpine	Beringia
Oxytropis kobukensis S.L.Welsh[1]	Oxytropis kobukensis	arctic	Beringia
Oxytropis koyukukensis A.E.Porsild	Oxytropis koyukukensis	arctic	Beringia
Oxytropis varians (Rydb.) K.Schum.	Oxytropis campestris subsp. gracilis	arctic-alpine	N. America
Parnassia palustris L.	Parnassia palustris subsp. neogaea	arctic-boreal	Circumpolar/boreal
Pedicularis capitata Adams	Pedicularis capitata	arctic-alpine	N. America, Asia
Pedicularis interior (Hultén) Molau & D.F.Murray	Pedicularis sudetica subsp. interior	arctic-alpine	N. America, Asia
Pedicularis labradorica Wirsing	Pedicularis labradorica	arctic-boreal	N. America, Asia
Pedicularis lapponica L.	Pedicularis lapponica	arctic-alpine	Circumpolar/boreal
Pedicularis verticillata L.	Pedicularis verticillata	arctic-boreal	N. America, Asia. Europe
Petasites frigidus (L.) Fr. subsp. frigidus	Petasites frigidus var. frigidus	arctic-boreal	N. America, Asia. Europe
Petasites frigidus (L.) Fr. subsp. nivalis (Greene) Cody	Petasites frigidus var. nivalis	arctic-alpine	N. America, Asia
Phlox alaskensis Jordal	Phlox sibirica	arctic	Beringia
Polemonium acutiflorum Willd.	Polemonium acutiflorum	arctic-alpine	N. America, Asia. Europe
Polemonium pulcherrimum Hook. subsp. lindleyi (Wherry) V.E.Grant	Polemonium pulcherrimum	boreal-alpine	N. America
Potentilla arenosa (Turcz.) Juz. subsp. arenosa	Potentilla hookeriana subsp. hookeriana	arctic-alpine	Circumpolar/boreal
Potentilla litoralis Rydb.	Potentilla virgulata	alpine	N. America
Potentilla nivea L.	Potentilla nivea	arctic-alpine	N. America, Asia
Potentilla nivea x arenosa	Potentilla nivea x hookeriana	arctic-alpine	N. America
Pulsatilla patens (L.) Mill. subsp. multifida (Pritz.) Zamelis	Pulsatilla patens subsp. multifida	boreal	N. America, Asia
Pyrola asarifolia Michx. subsp. incarnata (DC.) E.Murray	Pyrola asarifolia var. purpurea	arctic-boreal	N. America, Asia
Pyrola grandiflora Radius	Pyrola grandiflora	arctic-boreal	Circumpolar/boreal
Sanguisorba officinalis L.	Sanguisorba officinalis	arctic-boreal	N. America, Asia. Europe
Saxifraga hirculus L.	Saxifraga hirculus	arctic-boreal	Circumpolar/boreal
Saxifraga tricuspidata Rottb.	Saxifraga tricuspidata	arctic-alpine	N. America
Senecio lugens Richardson	Senecio lugens	alpine	N. America
Silene williamsii Britton[1]	Silene menziesii subsp. williamsii	boreal	Beringia
Solidago multiradiata Aiton	Solidago multiradiata	arctic-alpine	N. America
Stellaria calycantha (Ledeb.) Bong.	Stellaria calycantha subsp. calycantha	boreal	N. America, Asia
Stellaria crassifolia Ehrh.	Stellaria crassifolia	arctic-boreal	Circumpolar/boreal
Stellaria laeta Richardson	Stellaria laeta	arctic-alpine	N. America
Stellaria longipes Goldie s. lat.	Stellaria edwardsii	arctic	Circumpolar/boreal
Stellaria longipes Goldie s. lat.	Stellaria longipes	arctic	Circumpolar/boreal
Stellaria longipes Goldie s. lat.	Stellaria monantha	arctic	Circumpolar/boreal
Tanacetum bipinnatum (L.) Sch.Bip.	Chrysanthemum bipinnatum subsp. bipinnatum	arctic	N. America, Asia. Europe
Taraxacum officinale F.H. Wigg.[1]	Taraxacum officinale	boreal	Circumpolar/boreal
Thalictrum alpinum L.	Thalictrum alpinum	arctic-alpine	N. America, Asia. Europe
Thalictrum sparsiflorum Turcz. ex Fisch. & C.A.Mey.	Thalictrum sparsiflorum	boreal	N. America, Asia
Tofieldia pusilla (Michx.) Pers.	Tofieldia pusilla	arctic-alpine	Circumpolar/boreal
Trientalis europaea L. subsp. europaea	Trientalis europaea subsp. europaea	boreal	N. America, Asia. Europe
Valeriana capitata Pall. ex Link in Spreng.	Valeriana capitata	arctic-alpine	N. America, Asia. Europe
Wilhelmsia physodes (Fisch. ex Ser.) McNeill	Wilhemsia physodes	arctic	N. America, Asia
Graminoids			
Arctagrostis latifolia (R.Br.) Griseb.	Arctagrostis latifolia	arctic-alpine	Circumpolar/boreal
Bromopsis pumpelliana (Scribn.) Holub subsp. arctica (Shear) Á.Löve & D.Löve	Bromus pumpellianus var. arcticus	arctic-alpine	Beringia
Calamagrostis canadensis (Michx.) P.Beauv.	Calamagrostis canadensis	arctic-boreal	Circumpolar/boreal
Calamagrostis neglecta (Ehrh.) P.Gaertn., B.Mey. & Scherb.	Calamagrostis neglecta	arctic-boreal	Circumpolar/boreal
Calamagrostis purpurascens R.Br.	Calamagrostis purpurascens	arctic-alpine	N. America, Asia
Carex albonigra Mack.	Carex albonigra	alpine	N. America
Carex atrosquama Mack.	Carex atrata subsp. atrosquama	alpine	N. America
Carex capillaris L.	Carex capillaris	arctic-boreal	Circumpolar/boreal
Carex concinna R.Br.	Carex concinna	boreal	N. America
Carex krausei Boeck.	Carex krausei	arctic-alpine	Circumpolar/boreal
Carex media R.Br. ex Richardson	Carex media	arctic-boreal	Circumpolar/boreal
Carex membranacea Hook.	Carex membranacea	arctic-boreal	N. America, Asia
Carex obtusata Lilj.	Carex obtusata	arctic-alpine	Circumpolar/boreal
Carex podocarpa R.Br. ex Richardson	Carex podocarpa	arctic-alpine	N. America, Asia
Carex scirpoidea Michx. subsp. scirpoidea	Carex scirpoidea	arctic-alpine	N. America
Elymus alaskanus (Scribn. & Merr.) Á.Löve subsp. alaskanus	Agropyron boreale subsp. alaskanum	arctic-alpine	Beringia
Festuca altaica Trin.	Festuca altaica	arctic-alpine	N. America, Asia
Festuca rubra L.	Festuca rubra	arctic-boreal	Circumpolar/boreal
Festuca rubra L. subsp. richardsonii (Hook.) Hultén	Festuca rubra subsp. richardsonii	arctic-boreal	Circumpolar/boreal
Hierochloe odorata (L.) Wahlenb.	Hierochloe odorata	arctic-boreal	Circumpolar/boreal
Juncus castaneus Sm.	Juncus castaneus	arctic-alpine	Circumpolar/boreal
Kobresia myosuroides (Vill.) Fiori	Kobresia myosuroides	arctic-alpine	Circumpolar/boreal
Leymus innovatus (Beal) Pilg.	Elymus innovatus	arctic-alpine	N. America
Luzula parviflora (Ehrh.) Desv. subsp. parviflora	Luzula parviflora subsp. parviflora	arctic-alpine	Circumpolar/boreal
Poa arctica R.Br.	Poa arctica	arctic-alpine	Circumpolar/boreal
Poa glauca Vahl	Poa glauca	arctic-alpine	Circumpolar/boreal
Poa pratensis L. subsp. alpigena (Lindm.) Hiitonen	Poa alpigena	arctic-boreal	Circumpolar/boreal
Ferns & fern allies			
Botrychium lunaria (L.) Sw.	Botrichium lunaria	arctic-boreal	Circumpolar/boreal
Cystopteris fragilis (L.) Bernh.	Cystopteris fragilis	arctic-alpine	
Cystopteris montana (Lam.) Desv.	Cystopteris montana	boreal	Circumpolar/boreal
Equisetum arvense L.	Equisetum arvense	arctic-boreal	Circumpolar/boreal
Equisetum fluviatile L.	Equisetum fluviatile	boreal	Circumpolar/boreal
Equisetum scirpoides Michx.	Equisetum scirpoides	arctic-boreal	Circumpolar/boreal
Selaginella sibirica (Milde) Hieron.	Selaginella sibirica	boreal-alpine	N. America, Asia
Bryophytes			
Abietinella abietina (Hedw.) M. Fleisch.			
Aulacomnium palustre (Hedw.) Schwägr.			
Dicranum dispersum Engelmark			
Hylocomium splendens (Hedw.) Schimp.			

Table A.1 (cont.)

Accepted species name and authority	Species name listed in Hultén (1968)	Regional unit	Geographic range
Bryophytes (cont.)			
Polytrichum piliferum Hedw.			
Rhytidium rugosum (Ehrh. ex Hedw.) Kindb.			
Sanionia uncinata (Hedw.) Loeske			
Sciuro-hypnum ornellanum (Molendo) Ignatov & Huttunen			
Lichens			
Cladonia pocillum (Ach.) Grognot			
Cladonia symphycarpia (Flörke) Fr.			
Flavocetraria cucullata (Bellardi) Kärnefelt & Thell			
Flavocetraria cucullata x nivalis			
Flavocetraria nivalis (L.) Kärnefelt & Thell			
Lecanora epibryon (Ach.) Ach.			
Leptogium saturninum (Dickson) Nyl.			
Ochrolechia frigida (Sw.) Lynge			
Peltigera canina (L.) Willd.			
Peltigera collina (Ach.) Schrader			
Peltigera elisabethae Gyelnik			
Peltigera elisabethae Gyelnik			
Peltigera extenuata (Vainio) Lojka			
Peltigera leucophlebia (Nyl.) Gyelnik			
Peltigera neckeri Hepp ex Müll. Arg.			
Peltigera praetextata (Flörke ex Sommerf.) Zopf			
Peltigera rufescens (Weiss) Humb.			
Stereocaulon rivulorum H. Magn.			
Thamnolia subuliformis (Ehrh.) Culb.			

Table A.2 Selected site variables for *Populus balsamifera* relevés.

	salicetosum alaxensis var. alaxensis, arctic group																															
	riparian relevés											south-facing slope relevés						spring relevés		riparian relevés												
Relevé No.	2	23	12	14	17	16	4	22	1	9	18	19	20	10	21	11	3	15	13	32	29	26	31	8	27	30	7	5	25	28	24	6
Field relevé reference	NOA3	KON1	IVI4	IVI2	CAN2	CAN3	NOA1	KON2	NOA4	DIE1	CAN1	HUL1	HUL2	TOO1	KON3	SAG1	NOA2	IVI1	IVI3	YUK1	YUK9	POR2	YUK2	KOB1	POR1	YUK8	KOB2	KOB4	POR3	YUK10	POR4	KOB3
Tree characteristics																																
Canopy height (m)	7.02	6.60	11.26	6.53	8.57	12.82	10.42	13.66	7.88	5.65	4.91	6.46	1.91	1.69	6.43	4.59	7.66	11.16	7.60	11.34	18.70	6.86	15.06	13.84	12.64	15.83	11.66	9.98	14.12	16.57	14.72	8.28
Basal area of stand (m2/ha)	120.63	117.69	193.88	208.75	249.56	241.63	185.23	138.12	110.54	153.32	63.97	139.96	15.70	11.60	70.88	214.59	88.09	270.49	86.87	120.65	788.49	123.75	621.10	139.08	116.50	983.31	126.53	61.84	174.34	192.52	106.61	129.35
Density of stand (trees/ha)	402.68	703.28	1415.08	1040.58	2475.19	779.71	1290.83	1238.38	2350.78	504.89	2154.97	1295.55	1585.00	2051.00	3173.97	2662.75	6071.85	1654.70	2143.35	2267.57	1103.44	8984.52	701.72	1076.45	4756.24	1202.85	1946.37	1302.11	4082.92	3044.00	3444.99	2238.17
Evidence for reproduction	4	3	1	1	4	4	1	1	1	1	1	4	4	4	1	4	3	3	4	1	1	1	1	1	1	1	1	1	1	1	1	1
Stand characteristics																																
Shrub height (m)	1.5	1.5	2	2	2	3	2	1.5	2	1.5	1	1.5	0.3	1	2	1	2	2	1.5	3	2	4	2	6	5	2.5	5	2	5	5	5	1
Forb/graminoid height (m)	0.1	0.25	0.5	0.3	0.3	0.4	0.1	0.5	0.5	0.5	0.2	0.5	0.2	0.3	0.3	0.4	0.4	0.5	0.4	0.3	0.25	0.75	0.1	0.5	0.5	0.5	0.4	0.3	0.5	0.1	0.4	0.2
Litter depth (cm)	0.00	0.50	1.00	1.50	0.50	0.10	0.00	3.00	0.00	3.00	2.00	1.70	2.50	0.10	3.00	6.00	0.30	1.50	2.00	2.00	8.00	2.50	4.50	0.30	3.00	4.00	4.00	3.00	1.00	5.00	5.00	0.20
% trees	40	55	65	50	45	50	50	70	40	30	40	45	60	50	60	40	70	60	55	40	50	45	30	70	50	70	60	50	60	45	55	40
% saplings	20	30	10	20	40	45	10	20	15	15	30	10	40	20	15	10	10	20	15	25	18	20	5	30	10	15	10	10	5	10	10	10
% deciduous shrubs	40	35	50	70	40	25	20	60	30	30	35	50	25	15	30	25	40	50	55	50	50	30	75	60	30	30	50	20	35	40	40	25
% evergreen shrubs	1	30	5	5	0	0	1	20	1	10	0	5	2	5	10	1	1	5	1	5	5	5	1	1	0	1	0	0	1	2	0	10
% forbs	10	25	25	20	25	30	2	30	20	10	20	60	30	30	40	20	10	60	25	15	7	20	10	20	25	10	25	70	15	5	10	15
% graminoids	7	10	20	5	10	10	1	10	10	20	15	25	10	15	15	15	5	20	5	10	1	3	1	15	5	15	20	20	3	1	2	20
% horsetails	2	1	3	1	0	1	1	2	1	0	1	5	0	0	0	0	1	5	5	25	40	15	5	30	10	65	5	5	50	5	20	20
% bryophytes	20	5	7	5	25	15	1	15	1	15	30	20	10	7	20	10	1	10	2	7	6	1	3	0	2	5	0	0	1	5	1	10
% lichens	5	1	2	1	5	1	1	1	1	10	0	5	3	3	5	2	1	1	1	1	1	1	1	0	1	1	0	0	1	2	1	10
% rock	1	1	0	0	1	1	50	1	0	5	0	5	25	5	5	0	1	0	0	2	0	1	0	0	1	0	0	1	0	0	0	1
% bare soil	1	1	5	1	0	5	30	5	15	2	0	5	5	0	10	0	1	1	1	10	0	1	10	0	1	0	0	2	10	5	1	10
% water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	2
% dead	5	20	20	25	15	20	5	25	2	10	10	15	20	10	20	5	5	15	10	20	35	10	35	20	15	20	10	20	20	60	25	15
% standing dead	5	5	10	10	20	15	2	5	10	10	10	5	1	5	7	5	2	5	5	15	10	5	10	10	15	25	7	15	20	20	15	10
% total dead	12	8	12	13	35	20	5	10	15	5	15	7	2	6	10	7	5	7	7	17	15	15	20	10	20	26	10	20	25	25	25	15
Site characteristics																																
Topographic position	3	2	5	5	5	5	5	1	3	3	5	1	2	2	1	2	2	5	3	5	5	5	5	4	5	5	4	4	5	5	5	2
Landform	10	3	3	11	6	11	3	3	3	11	11	6	10	1	6	11	10	11	1	6	7	10	11	5	3	7	3	7	3	7	3	12
Parent Material	5	5	5	3	5	5	5	3	3	5	5	6	6	6	5	5	5	5	5	3	5	5	3	5	5	5	5	5	3	5	3	14
Exposure	3	2	3	2	1	1	2	2	2	1	2	2	2	2	1	1	2	1	3	1	1	3	1	2	2	1	2	2	2	1	2	2
Stability	2	2	2	2	2	2	4	2	4	4	2	2	3	3	2	2	2	2	2	4	2	2	4	2	2	2	2	2	4	4	2	2
Site moisture	5	5	5	5	5	5	3	6	5	5	5	4	3	4	5	4	3	5	5	5	7	5	6	5	5	7	5	5	5	7	5	3
Soil moisture	4	4	6	3	3	6	2	3	3	5	5	3	4	6	4	3	2	4	4	3	2	3	3	3	4	3	3	3	3	3	3	2
Estimated snow duration	4	5	4	4	6	6	5	5	4	4	5	4	3	4	5	4	3	4	4	4	4	4	4	4	4	4	5	3	5	4	5	5
SWI	37.3	29.1	27.5	26.8	22.2	24.9	22.1	25.0	42.5	15.4	18.7	33.1	33.1	32.9	25.9	33.8	37.3	26.8	26.8	39.0	48.5	50.3	46.7	43.4	48.1	50.3	44.2	42.4	53.7	43.9	59.6	39.6
Slope gradient (%)	0	0	0	5	3	0	0	0	0	0	0	35	50	40	40	50	55	0	7	0	0	20	0	0	0	0	0	0	0	0	0	50
Aspect	1	1	1	3	3	1	1	1	1	1	1	3	3	3	3	3	3	1	3	1	1	4	1	1	1	1	1	1	1	1	1	3
Releve area (m^2)	3	2	2	2	1	3	3	3	3	2	1	2	1	1	2	2	2	2	1	3	2	2	3	2	2	2	2	3	3	3	2	2
Moose disturbance	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	1	1	2	2	1	2	1	1	1	1	1	1	1	1	1
Moose disturbance degree	2	4	4	1	2	4	4	4	4	3	3	2	0	0	0	0	0	2	3	0	0	2	0	2	2	2	3	3	1	2	3	3