SHORT COMMUNICATION

Colonization of vegetation-rich moraines and inference of multiple sources of colonization in the High Arctic for *Salix arctica*

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Abstract Vegetation-rich patches in the High Arctic may serve as a significant source for vegetation reconstruction in the climate changes. Diversity and colonization, however, of such potential source populations in the High Arctic has rarely been studied. We examined chloroplast sequence variation in *Salix arctica*, a key species in the Canadian High Arctic, from four adjacent glacial moraines of differing ages on Ellesmere Island, Canada, as well as two other populations located at the center and southern end of the species' range. The estimated ages of the moraines varied from 35,000 to 250 years old. The older moraine populations showed higher within-population genetic variation compared with the other moraine populations, which is generally attributed to differences in establishment age associated with plant densities among moraines. The moraines with smaller plant density had lower genetic diversity and had no private haplotypes, indicating the local population size and genetic diversity may not be recovered within a few thousand years. This suggests seed dispersal at a local scale may be limited even in species with high velocity of seed dispersal, and that High Arctic vegetation-rich patches may serve as significant source populations for sustaining local genetic diversity. In addition, the three regions we observed comprised an evolutionarily distinct lineage and significant population differentiation. This implies multiple sources for the colonization during the most recent deglaciation, resulting in the current wide distribution. Local as well as range-wide processes of colonization would be essential to understand vegetation responses in High Arctic to the environmental changes.

Keywords Genetic diversity · Loss of genetic diversity · Phylogeography · Polar oasis · Refugia

Introduction

Climate changes induce species range shifts (Davis and Shaw 2001), with possible loss of genetic diversity caused by range dynamics occurring in species with genetically structured populations (Alsos et al. 2009). Identifying levels of genetic variation within and among populations of key species in an ecosystem is essential for the prediction and assessment of potential risks and consequences of diversity loss.

The High Arctic is dominated by polar desert (Freedman et al. 1994), and its vegetation is a fundamental component initiating and sustaining the arctic ecosystem. Vegetation-rich microsites in the polar desert, known as "polar oases" (Muc et al. 1994), are usually geographically very limited but may serve as crucial sources of arctic biota (Bliss and Gold 1994) for further colonization and vegetation development. Moraines, which are formed during glacial retreat, sometimes provide habitats for well-developed vegetation

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in the Canadian High Arctic. A survey of recently developed moraines consisting of rocky soils suggests *Salix arctica* Pall. is one of only a few pioneer plant species in the High Arctic that can invade the rocky and unstable substrate environments of young moraines (Mori et al. 2008). This species also continues to dominate in later successional stages. High velocity of seed dispersal of *Salix* with fluffy seeds (Horn et al. 2001) may contribute to this feature of *S. arctica*. Such plant invasion can modify soil structure, which allows further invasion of other species and vegetation establishment (Chapin et al. 1994). Thus, *S. arctica* is a key species for vegetation development in the Canadian High Arctic.

The multiple retreats of Arklio Glacier on Ellesmere Island have led to the formation of moraines of differing ages (King 1981). The developmental periods of the oldest and youngest moraine are estimated to have occurred 35,000–25,000 and 400–250 years ago, respectively (Okitsu et al. 2004). These age estimates are tentative because the conjectured northern limit of the last glacier is located at the northern edge of the island (Dyke et al. 2002), but soil structure on the oldest moraine suggests a fairly long period of time has passed since establishment of the now well-developed vegetation (Mori et al. 2008). The moraine age had a substantial effect on vegetation succession and also population size of *S. arctica* (Okitsu et al. 2004; Mori et al. 2008). Colonization processes may result in substantially reduced genetic diversity in younger populations caused by founder effects. Such effects may influence rates of colonization and recovery of population size at a local scale. Long-distance dispersal was often featured in High Arctic plants, but the way in which genetic variation in source populations affects colonization at local scales is poorly understood. The different establishment ages of moraines can provide an opportunity to investigate the colonization processes of a species and to roughly estimate the time for population size and genetic diversity to recover in arctic environments on a local scale.

In this study, our goal was to elucidate colonization and genetic diversity among four moraines formed at different ages (ranging from 35,000 to 250 years ago). With a few southern populations, we also investigate genetic diversity in populations located in southern areas within the species' range and the potential sources of colonization.

Materials and methods

Sampling locations

The High Arctic oasis in this study is located within the proglacial field of the southern front of the Arklio Glacier in the Krieger Mountains near Oobloyah Bay, Ellesmere Island, Canada (80°00–52′N, 82°49–51′W). Leaf samples were collected from each of four moraines whose flora has been previously studied (Larkin et al. 2007; Mori et al. 2008). The retreating Arklio Glacier has resulted in a series of moraines of different ages (Table 1), estimated as (A) 35,000–25,000, (B) 15,000–8,000, (C) 3,300–2,400, and (D) 400–250 years old (Okitsu et al. 2004). We also sampled two more southern populations, Resolute Bay on Cornwallis Island (Res) and Cambridge Bay (Cam) to compare their genetic diversity to Ellesmere Island populations. Leaves were collected from plants >5 m apart.

Ecological niche modeling to estimate current distribution

Ecological niche modeling was performed to visualize a current potential distribution and population locations in this study within the species' current range. We obtained 933 geo-referenced records from the Global Biodiversity Information Facility (GBIF) database (http://www.gbif.org/). Bioclimatic variables from current climate models were obtained from Global Climate Data (http://www.worldclim.org; Hijmans et al. 2005). Four bioclimatic variables, which had pairwise correlation coefficients less than 0.7, were used to model the species' range using the maximum entropy machine learning algorithm as implemented in MAXENT (Phillips et al. 2006). Binomial tests of omission were conducted by randomly selecting 30 % of the occurrence localities from the dataset as test data. We evaluated grid cells with a cumulative probability >10, as suggested by Waltari et al. (2007) and Peterson et al. (2007).

Chloroplast DNA sequence variation

DNA was extracted from air-dried leaf tissue using the modified CTAB method (Doyle and Doyle 1990). Eight chloroplast DNA regions (*trnV–ndhC, psbD–trnT, rps16–trnK, petL–psbE, psbJ–petA, atpI–atpH, trnK2–trnQr* (DumolinLapegue et al. 1997; Shaw et al. 2007) were tested. Four intergenic regions (*psbJ–petA, atpI–atpH, trnK2–trnQr,* and *trnL–trnF*) that successfully amplified were used in this study. The PCR-amplified products were sequenced using an ABI Prism BigDye Terminator v3.1 kit on an ABI3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Only high quality traces were considered. DNA sequences were aligned using ClustalW (Thompson et al. 1994; Larkin et al. 2007). All observed indels were excluded from the analysis, while an inversion was included as 5th state.

The observed number of haplotypes (H(n)), haplotype diversity (Hd), and nucleotide diversity (π) and theta (θ) from segregating sites were calculated for each population. Tajima's D (Tajima 1989) and Fu and Li's D* test (Fu and

Table 1 Population information and genetic diversity

Region	Population	Latitude	Longitude	Estimated age of moraines	Vascular plant cover (%)	Density of S. arctica (individual/m²)	n	H(n)	Hd	π	θ	Tajima' D	Fu & Li's D*
Ellesmere	Elles(A)	80° 50–52	82° 49–51	35,000–25,000 ya	66.0	10.1	22	5	0.762	0.00096	0.00081	0.587	0.650
	Elles(B)	80° 50–52	82° 49–51	15,000–8,000 ya	41.3	3.0	23	4	0.605	0.00040	0.00068	−1.260	−2.401*
	Elles(C)	80° 50–52	82° 49–51	3,300–2,400 ya	37.0	3.8	21	3	0.338	0.00015	0.00023	−0.843	−0.612
	Elles(D)	80° 50–52	82° 49–51	400–250 ya	0.8	0.2	22	3	0.394	0.00036	0.00035	0.121	0.992
Resolute	Res	74° 43	94° 59	n.a.	n.a.	n.a.	20	6	0.742	0.00068	0.00061	0.323	−0.413
Cambridge	Cam	69° 06	105° 07	n.a.	n.a.	n.a.	19	5	0.673	0.00134	0.00145	−0.292	−0.151
	Total						127	14	0.633	0.00073	0.00146	−1.384	−1.334

Estimated ages of the moraines on Ellesmere Island are from Okitsu et al. (2004). Vascular plant coverage (%) and plant density of S. arctica (number of individuals per m²) on the moraines were from Mori et al. (2008). n number of sampled individuals; H(n) number of haplotypes; Hd haplotype diversity; π nucleotide diversity; θ diversity based on combined 2,450 bp chloroplast regions; Hd haplotype diversity; π nucleotide diversity; θ diversity based on number of segregating sites. Asterisk in Tajima's D and Fu & Li's D* indicates statistical significance (p < 0.05)

Fig. 1 Haplotype variation.
a Observed haplotypes with a predicted distribution map. Four sample populations (*A–D*) were collected from moraines on Ellesmere Island. *Filled circles* are sampling locations, and *open squares* are geo-reference records obtained from the GBIF database. *Darker gray* indicates the potential range of the species predicted by niche modeling with the geo-referenced occurrence data (at least 10 % of cumulative probability). The *blue line* indicates the northern limit of the last glaciation. **b** Haplotype network of observed haplotypes. Each haplotype is represented by a *color-filled circle*. Branch length indicates the number of mutation events. (Color figure online)
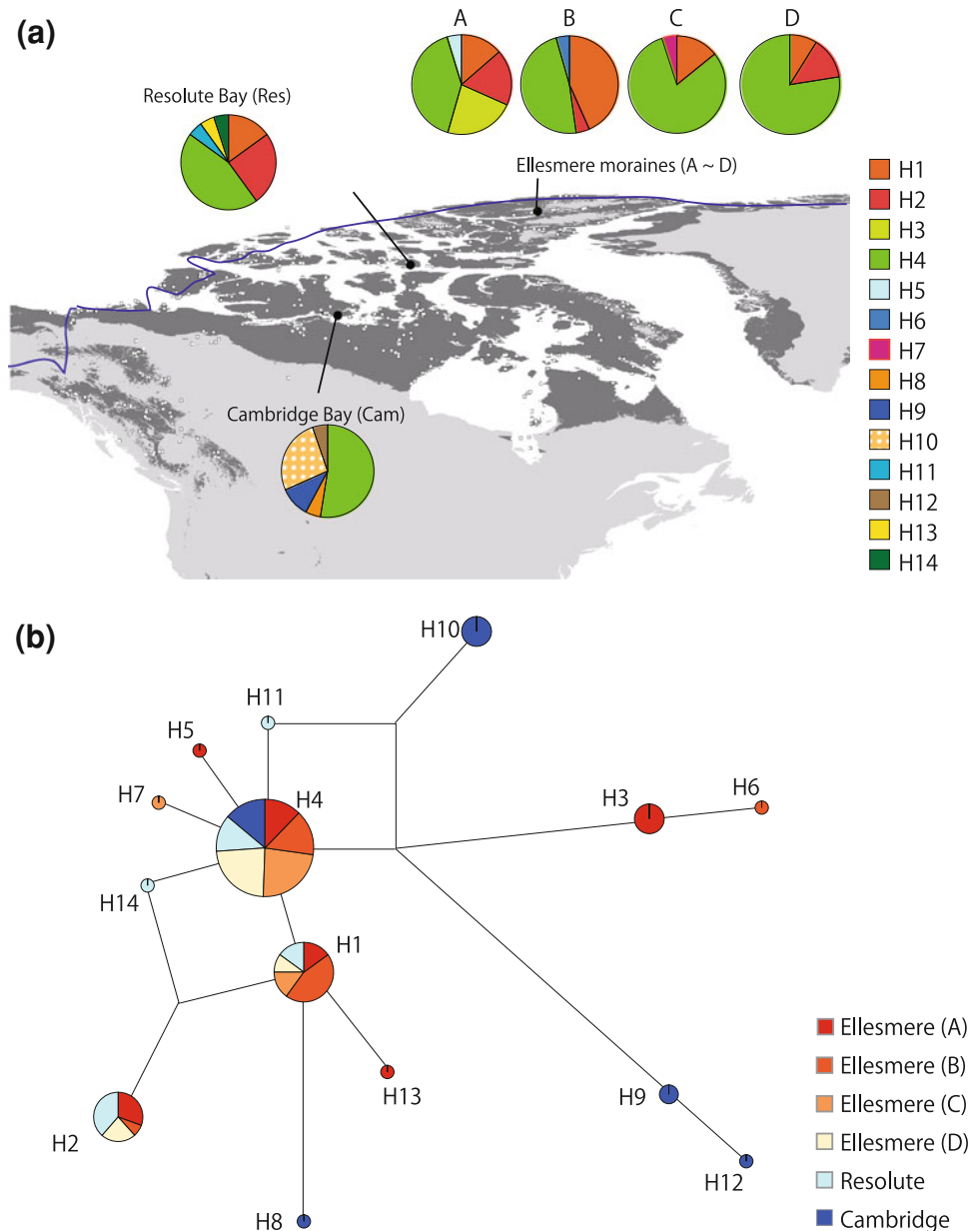
Li 1993) for departure from neutrality were calculated using DnaSP v5 (Librado and Rozas 2009). A haplotype network was constructed using NETWORK v4.6 (Fluxus Technology, http://www.fluxus-engineering.com/network_terms.htm) with the reduced median method (Brandelt et al. 1995). Pairwise population differentiations and population differentiation (N_{ST} in addition to G_{ST}) were estimated from haplotype frequency and distances between haplotypes (Pons and Petit 1996). The significance of differentiation between G_{ST} and N_{ST} was tested with 1,000 non-parametric permutation tests. Demographic inference was explored by mismatch distribution analysis of observed haplotype pairwise differences, following a population expansion model (Rogers and Harpending 1992) and a spatial expansion model (Excoffier 2004). We used

the sum of squared deviations between the observed and expected mismatch and the Raggedness Index (Harpending 1994) to test deviation from the model.

Results

Current distribution

Ecological niche modeling for *S. arctica* under current climate conditions determined the species distribution (Fig. 1a) with a high Area Under the ROC Curve (AUC) for the receiver operating characteristic (ROC) (AUC = 0.944 with test data), where a score of 0.5 indicates a random

model. If AUC equaled 1.0, this would indicate a perfect discrimination between an area where the species is present and where it is absent. Thus, the model performed well in projecting the current distribution of the species. The predicted range shows a continuous distribution throughout the Canadian Arctic expect absence in a few northern regions such as on Ellesmere Island and the surrounding islands. It also indicates a disjunct distribution in the alpine areas of North America. Our sampled populations from the moraines on the Ellesmere Islands were located at the northern edge of the species range relative to other populations. The other two populations were located at center and south of the large continuous distribution of the species in the High Arctic.

Genetic diversity in *S. arctica* in the High Arctic

A total of 2,450 bp of chloroplast sequence data were combined from aligned sequences of *psbJ–perA* (920 bp), *atpI–atpH* (1,004 bp), and *trnK2–trnQr* (526 bp). The combined dataset had a total of 14 haplotypes (Table 1). Among the moraine populations, genetic diversity was highest in the oldest moraine A (Hd: 0.762; π: 0.00096) and lowest in the younger moraines (Hd: 0.338; π: 0.00015 for moraine C and Hd: 0.394; π: 0.00036 for moraine D). A neutrality test (Fu and Li's D*) was significant only in the moraine B, the 2nd oldest moraine. There is a common shared haplotype (Hap4) in all studied populations, while all except two younger moraine populations had private haplotypes (Fig. 1a, b).

Population differentiations were significant among three regions, Ellesmere A–D, Resolute Bay (Res) and Cambridge Bay (Cam) ($N_{ST} = 0.139$, $p < 0.01$; Table S1). The differences between G_{ST} and N_{ST} were also significant, which indicates genetically more similar genotypes exist within regions. Population differentiation among the moraine populations on Ellesmere Islands was not significant ($N_{ST} = 0.085$).

Mismatch distribution analysis

For the youngest moraine population (D), the demographic expansion model was rejected. However, this result should be taken with caution because the number of segregated sites is very low in the younger moraine populations. Among the Ellesmere Island moraine populations, the mismatch distribution parameter τ, which is the time in mutational steps since the modeled expansion event, was constantly generally high in the oldest moraine in both population and spatial expansion models, compared to other younger moraines (Table 2). The parameter τ was the highest in the southern population (Cam). The Raggedness Index, which becomes larger in a stable population, was also highest in the Cam population, and a smooth distribution, which is typical for expanding populations, was rejected in this population.

Discussion

We detected generally high within-population haplotype variation in the High Arctic populations of *S. arctica*. The moraine populations we studied on Ellesmere Island have well-developed vegetation, as is occasionally observed for limited locations within polar deserts (Muc et al. 1994). Each Ellesmere Island moraine developed at a different time, and thus each has a different soil structure (Okitsu et al. 2004; Mori et al. 2008). According to

Table 2 Mismatch distribution analysis under two expansion models: population expansion and spatial expansion

	Elles(A)	Elles(B)	Elles(C)	Elles(D)	Res	Cam
Population expansion model						
τ	3.600	0.800	3.000	0.000	2.400	6.800
SSD	0.061	0.022	0.227	**0.226**	0.020	0.140
p Value (SSD)	0.145	0.104	0.136	0.000	0.328	0.062
Raggedness Index	0.183	0.163	0.210	0.312	0.073	**0.376**
p Value (Raggedness)	0.115	0.185	0.243	0.959	0.588	0.018
Spatial expansion model						
τ	2.843	0.848	0.428	2.825	1.294	4.726
SSD	0.053	0.022	0.004	0.024	0.013	0.104
p Value (SSD)	0.219	0.050	0.221	0.338	0.371	0.198
Raggedness Index	0.183	0.163	0.210	0.312	0.120	0.395
p Value (Raggedness)	0.298	0.165	0.448	0.674	0.447	0.242

Sum of squared deviations (SSD) and Raggedness Index are indicated as test statistics for the models. The demographic parameter τ indicates the time in mutational steps since the modeled expansion event. Bold text indicates where $p < 0.05$

Mori et al. ([2008](#)), the rocky environment of the youngest moraine has resulted in low species richness and lower occurrence of *S. arctica*. In their study, the occurrence of *S. arctica* was estimated as 100 % on the oldest moraine (moraine A in this study) and 20 % on the youngest moraines (moraine D) based on 20 observed plots per moraine. Larger overall population size with relatively longer habitat establishment may have contributed to sustaining relatively larger levels of genetic diversity within moraines. The oldest moraine population had a common private haplotype and equivalent genetic variation when compared with two other island populations sampled from the central and southern portion of the species' range, while two younger moraine populations on more recently developed sites (oldest estimated age of 3,300 years) had relatively lower haplotype and nucleotide diversity and had no private haplotype. Frequent sexual reproduction (Steltzer et al. [2008](#)) may boost genetic diversity in the small but a relatively old *S. arctica* moraine population located in the High Arctic. Although there is no significant population differentiation among moraine populations, limited dispersal of a common private allele in the oldest moraine suggests most dispersal may be limited within local regions, even in a species with a strong ability to disperse. This suggests such vegetation-rich patches would provide crucial sources of genetic variation and population expansion, and loss of these small patches may have significant effects on total genetic diversity and evolutionary potential of the species in High Arctic ecosystems.

The Canadian High Arctic was inferred to have been one of multiple refugia in North America during the last glaciation for arctic plants such as *Dryas integrifolia* (Tremblay and Schoen [1999](#)) and *Saxifraga oppositifolia* (Abbott et al. [2000](#)) and the arctic rodent *Dicrostonyx groenlandicus* (Fedorov and Stenseth [2002](#)). Recent genetic analysis of fossils revealed the presence of a tree species in Scandinavia where trees are believed to have been absent during the last glaciation (Parducci et al. [2012](#)), indicating survivability of woody species adapted to cold environments in small and isolated conditions. Since the oldest moraine is estimated to have developed before the Last Glacial Maximum (Okitsu et al. [2004](#)), Mori et al. ([2008](#)) and Okitsu et al. ([2004](#)) have posited that moraines and nearby districts may have been covered by vegetation during the full glaciation period. In our study, the mismatch distribution analysis suggested that the population size of the Cambridge Bay site has been more stable over time, compared to other populations. If the species generally retreated to the south during the glacial period, higher diversity and more stable population size compared to the other northern population would be expected as seen in this study. However, significant population divergence among three regions (moraines, Res and Cam populations) and the presence of regional private haplotypes support the hypothesis that there have been at least multiple sources contributing to post-glacial colonization of *S. arctica* in Canadian Arctic regions. Further investigation is required to clarify this hypothesis in *S. arctica*. Nonetheless, with such strong population structure created by historical events and/or relatively limited gene dispersal, loss of local populations can directly result in loss of unique genetic resources.

Conclusion

Salix arctica is a foundation species in the High Arctic as a low trophic-level species, which acutely and chronically modulates fundamental ecosystem properties and processes (Ellison et al. [2005](#)). Wind dispersal with fluffy seeds, which is a typical feature of *Salix* species, may promote high levels of seed dispersal and colonization as a dominant species. High gene flow enhances homogenization of genetic variation among populations (Hartl and Clark [2007](#)). However, our findings in *S. arctica* suggest gene flow may not be so high that it rapidly homogenizes genetic variation among populations and that recovery of genetic variation within populations may take a few thousands of years even if source populations are close by. This implies rapid environmental changes may influence population recovery, thus ultimately slowing the recovery of High Arctic vegetation. Also, the implication that there may be multiple refugia has recently received increased attention as an alternative explanation for the rapid range expansion of woody species in response to past climate changes (e.g. Petit et al. [2008](#)). Arctic and alpine species will experience expanding growth, increased competition and loss of habitat with the climate changes occurring globally (Birks [2008](#)). Revisiting local processes of population colonization and recovery from small populations in the High Arctic are essential in projecting the response of the High Arctic to environmental changes.

References

Abbott RJ, Smith LC, Milne RI, Crawford RMM, Wolff K, Balfour J (2000) Molecular analysis of plant migration and refugia in the Arctic. Science 289:1343–1346

Alsos IG, Alm T, Normand S, Brochmann C (2009) Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modelling. Global Ecol Biogeogr 18:223–239

Birks HH (2008) The late-quaternary history of Arctic and alpine plants. Plant Ecol Divers 1:135–146

Bliss LC, Gold WG (1994) The patterning of plant communities and edaphic factors along a high arctic coastline: implications for succession. Can J Bot 72:1095–1107

Brandelt HJ, Forster P, Sykes BC, Richards MB (1995) Mitochondrial portraits of human populations using median networks. Genetics 141:743–753

Chapin FS III, Walker LR, Fastie CL (1994) Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. Ecol Bull 38:69–76

Davis MB, Shaw RG (2001) Range shift and adaptive responses to quaternary climate changes. Science 292:673–679

Doyle JJ, Doyle JL (1990) A rapid total DNA preparation procedure for fresh plant tissue. Focus 12:13–15

DumolinLapegue S, Pemonge MH, Petit RJ (1997) An enlarged set of consensus primers for the study of organelle DNA in plants. Mol Ecol 6:393–397

Dyke AS, Andrews JT, Clark PU, England JH, Miller GH, Shaw J et al (2002) The Laurentide and Innuitian ice sheets during the Last Glacial Maximum. Quat Sci Rev 21:9–31

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Front Ecol Environ 3:479–486

Excoffier L (2004) Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. Mol Ecol 13:853–864

Fedorov VB, Stenseth NC (2002) Multiple glacial refugia in the North American Arctic: inference from phylogeography of the collared lemming (*Dicrostonyx groenlandicus*). Proc Royal Soc B-Biol Sci 269:2071–2077

Freedman B, Svoboda J, Henry GHR (1994) Alexandra Fiord—an ecological oasis in the polar desert. In: Svoboda J, Freedman B (eds) Ecology of a polar oasis: Alexandra Fiord, Ellesmere, Island, Canada. Captus University Publications, Toronto

Fu Y-X, Li W-H (1993) Statistical tests of neutrality of mutations. Genetics 133:693–709

Harpending HC (1994) Signature of ancient population-growth in a low-resolution mitochondrial-DNA mismatch distribution. Hum Biol 66:591–600

Hartl DL, Clark AG (2007) Principles of population genetics, 4th edn. Sinauer Associates, Sunderland

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978

Horn HS, Nathan R, Kaplan SR (2001) Long-distance dispersal of tree seeds by wind. Ecol Res 16:877–885

King L (1981) Studies in glacial history of the area between Oobloyah Bay and Esayoo Bay, northern Ellesmere Island, N.W.T., Canada. In: Barsch D, King L (eds) Results of the Heidelberg Ellesmere Island expedition. Geographischen Instituts der Universtät Heidelberg, Heidelberg

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and clustal X version 2.0. Bioinformatics 23:2947–2948

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Mori AS, Osono T, Uchida M, Kanda H (2008) Changes in the structure and heterogeneity of vegetation and microsite environments with the chronosequence of primary succession on a glacier foreland in Ellesmere Island, High Arctic Canada. Ecol Res 23:363–370

Muc M, Freedman B, Svoboda J (1994) Vascular plant communities of a polar oasis at Alexandra Fiord, Ellesmere Island. In: Svoboda J, Freedman B (eds) Ecology of a polar oasis: Alexandra Fiord, Ellesmere Island, Canada. Captus University Publications, Toronto

Okitsu S, Sawaguchi S-I, Hasegawa H, Kanda H (2004) Vegetation development on the glacier moraines in Oobloyah Valley, Ellesmere Island, High Arctic Canada. Polar Biosciences 17:83–94

Parducci L, Jørgensen T, Tollefsrud MM, Elverland E, Alm T, Fontana SL, Bennett KD, Haile J, Matetovici I, Suyama Y, Edwards ME, Andersen K, Rasmussen M, Boessenkool S, Coissac E, Brochmann C, Taberlet P, Houmark-Nielsen M, Larsen NK, Orlando L, Gilbert MTP, Kjær KH, Alsos IG, Willerslev E (2012) Glacial survival of boreal trees in Northern Scandinavia. Science 335:1083–1086

Petit RJ, Hu FS, Dick CW (2008) Forests of the past: a window to future changes. Science 320:1450–1452

Peterson AT et al (2007) Transferability and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. Ecography 30:550–560

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecol Model 190:231–259

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144:1237–1245

Rogers AR, Harpending H (1992) Population-growth makes waves in the distribution of pairwise genetic-differences. Mol Biol Evol 9:552–569

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94:275–288

Steltzer H, Hufbauer RA, Welker JM, Casalis M, Sullivan PF Chimner R (2008) Frequent sexual reproduction and high intraspecific variation in *Salix arctica*: implications for a terrestrial feedback to climate change in the High Arctic. J Geophys Res 113:G03S10

Tajima F (1989) The effect of change in population-size on DNA polymorphism. Genetics 123:597–601

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tremblay NO, Schoen DJ (1999) Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. Mol Ecol 8:1187–1198

Waltari E, Hijmans RJ, Peterson AT, Nyári ÁS, Perkins SL, Guralnick RP (2007) Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. PLoS ONE, 2, e563