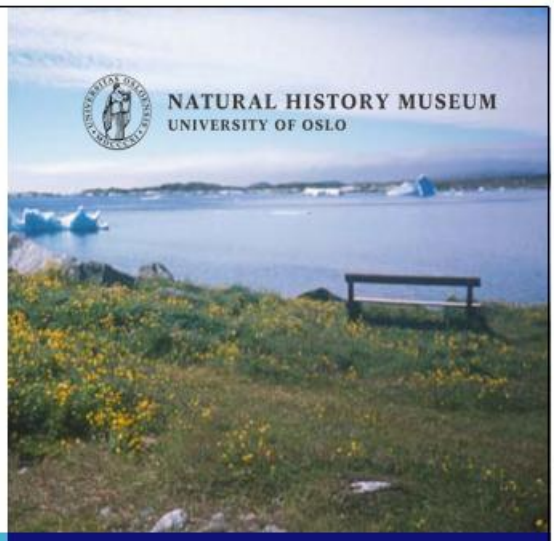

Immigration of the thermophilous, bird-dispersed *Empetrum nigrum* s. lat. to Svalbard

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Takk til:

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Tøyen, 28.11.05

Hilde

Abstract

The arctic archipelago of Svalbard was almost completely ice-covered during the last glaciation. If any, only very hardy species could have survived the glaciation *in situ*. *Empetrum nigrum* is one of the most thermophilous plants occurring on Svalbard today, and must have arrived postglacially by long-distance dispersal. In this study, AFLP fingerprinting and ploidal level determinations using flow cytometry were used to determine the source area(s) of the Svalbard populations of *E. nigrum*. A total of 435 individuals from 46 populations in the northern hemisphere, mostly from the North Atlantic area, were analysed.

PCO- and Bayesian clustering analyses separated the tetraploid *E. nigrum* in the North Atlantic area into a northern and a southern group. Svalbard belonged to the northern group. These two main groups of tetraploid *E. nigrum* probably reflect survival in different glacial refugia during the last and/or previous glaciations. A main refugium east of the Scandinavian ice sheet is suggested for the northern group, while a main refugium south of the Scandinavian ice sheet is suggested for the southern group. Allocation analyses strongly suggest that *E. nigrum* colonised Svalbard from East Greenland, which again was colonised from West Siberian/Ural Mountains source populations. The western North American and the East Siberian plants were separated into a third group that appeared to be most closely related to the northern Atlantic group. However, the present study comprises too few populations in the Beringian area to draw firm conclusions about this third group.

Two suture zones with high levels of genetic diversity were identified; the Ural Mountains and South West Greenland. The southern and northern group met in the Ural Mountains, while South West Greenland is probably influenced by both the southern and the northern group, and one or several Beringian lineages.

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Introduction

This master study is part of a larger ongoing five-year project entitled "Effects of climate change on ecosystems in Svalbard: past and future immigration of thermophilous key species". The main project comprises 18 species, nine occurring in Svalbard today and nine potential immigrants in case of a future temperature increase.

The arctic archipelago of Svalbard was completely ice-covered during the last glacial maximum, except for a few small mountain areas in the northwest (Landvik *et al.* 2003). The most thermophilous plants occurring there today can not have survived *in situ*, and must have arrived postglacially by long-distance dispersal. Paleobotanical records show that the thermophilous plants probably arrived during the postglacial warm period, the Hypsithermal, when the climate was 1-2°C warmer than today (Birks 1991). Climate models predict an arctic warming of about 4-7°C over the next 100 years, resulting in shorter and warmer winters (ACIA 2004). Many of the thermophilous species present in Svalbard today are dominant in more southern ecosystems (e.g. *Betula nana* L., *Empetrum nigrum* L.), but in the archipelago they are today restricted to particularly favourable places. A temperature increase is likely to result in range expansion of these species. In addition, even more thermophilous species may establish, given that they are able to cross the oceans. Several such species (e.g. *Betula pubescens* Ehrh., *Vaccinium myrtillus* L.) are dominant components of more southern vegetation types, and are likely to cause severe ecological cascade effects if they establish in Svalbard.

The geographic location of Svalbard makes northern Norway, East Greenland, and northwestern Russia the most likely source areas for immigration to Svalbard. One can expect the current thermophilous plant species in Greenland and northern Russia to migrate northwards on the continental landmasses following global warming (in Scandinavia, most of the thermophilous plant species in question are already present in the northernmost areas). Diaspores from these northern positions are most likely to reach Svalbard. Previous studies of more hardy, high arctic species in Svalbard indicate that species have immigrated once or several times from one or even several of these areas (Brochmann 1992; Abbott *et al.* 1995; Gabrielsen *et al.* 1997; Tollefsrud *et al.* 1998; Steen *et al.* 2000; Hagen *et al.* 2001).

The 18 species in the main project represent three different dispersal modes; wind-dispersal, bird-dispersal and without obvious mechanisms for long-distance dispersal. The objectives of the main project are to use AFLP markers (amplified fragment length

polymorphism; Vos *et al.* 1995), genotype assignment tests, and other statistic analyses to reveal the genetic pattern in each species, and then to:

1) *identify the source areas and frequency of previous immigrations to Svalbard of the established species*
2) *estimate the dispersal abilities of the potential immigrant species*
3) *compare the likelihood for immigration of species that are bird-dispersed, wind-dispersed, and without particular long-distance dispersal mechanisms*

In this Master student project I analyse one of the 18 species *Empetrum nigrum* (Fig.1), a relatively thermophilous, bird-dispersed species occurring in Svalbard. The genus *Empetrum* has a bipolar distribution. In the northern hemisphere it has a wide distribution throughout the circumarctic and boreal regions (Fig. 2; Hultén and Fries 1986). The taxonomy of the genus is rather unclear. Species delimitation has often been based on fruit colour, ploidal level and breeding system; i.e. red vs. black drupes, diploidy vs. tetraploidy, and dioecy vs. hermaphroditism. The number of species recognised in *Empetrum* varies from two to 18, depending on the author (Good 1927; Hagerup 1927; Löve 1960; Vassiliev 1961; Webb 1972). In a recent master study by Mirré (2004) based on AFLP data and ploidal level estimates, it was suggested that the genus has a complex history in the northern hemisphere, including glacial survival in different refugia, bottlenecking, rapid range expansion, and tetraploidisation. The morphological variation was, however, not unambiguously reflected in the genetic groups. She left the question unanswered whether *Empetrum* in the northern hemisphere should be recognised as a single species or a species complex.

In the northern Atlantic area, a single species with two subspecies is usually recognised, *E. nigrum* ssp. *nigrum* and ssp. *hermaphroditum* (Hagerup) Böcher (e.g., Webb 1972; Laurber and Wagner 1998; Lid and Lid 2005), although several authors give the two taxa species ranking (e.g., Hagerup 1927; Löve 1960). I choose to recognise them as subspecies. *Empetrum nigrum* is a dwarf shrub with wintergreen, strongly ericoid leaves and small, red flowers which are believed to be wind-pollinated (Good 1927). The fruit is a black, shiny drupe dispersed by birds. The species is long-lived and can propagate vegetatively through layering. Genets can spread over distances up to 40 m, and individuals as old as 80 years have been reported (Elvebakk and Spjelkavik 1995) (Szmidt *et al.* 2002). However, the maximum age is probably much higher.

Subspecies *nigrum* has been reported as diploid ($2n = 26$) and ssp. *hermaphroditum* as tetraploid ($2n = 52$; e.g., Hagerup 1927; Engelskjøn 1979; Teppner 1987). Usually, the diploid

Fig. 1. Empetrum nigrum *s. lat. (left; photo by Beate Adolfsen) and* E. nigrum *ssp.* hermaphroditum *drupe with remaining stamens (right; photo Arne Anderberg).*

plants are dioecious and most commonly occurring at low altitudes, whereas the tetraploid plants are hermaphroditic and dominate at high altitudes. Diploids and tetraploids may however form mixed populations (Suda 2002; Suda *et al.* 2004). Triploids, probably of hybrid origin, have been reported where diploids and tetraploids live in sympatry (Suda 2002).

The populations of *E. nigrum* occurring in Svalbard have been referred to as ssp. *hermaphroditum*, and two counts of 2*n* = 52 have been reported (Flovik 1940; Engelskjøn 1979). *Empetrum nigrum* ssp. *hermaphroditum* has an autodeposition efficiency of 0.9, meaning a large extent of self-pollination (Tikhmenev 1984). Whereas *Empetrum* is heath-forming in the southern parts of the Arctic, in Svalbard it only forms small patches, probably as relicts from the Hypsithermal. The current climatic conditions in Svalbard rarely allow *Empetrum* to produce ripe fruits (Elvebakk and Spjelkavik 1995).

This Master project is connected to the first goal of the main project, and aims to determine the source area(s) of the Svalbard populations of *E. nigrum* using ploidal level determinations (flow cytometry), and AFLP fingerprinting.. The project is based on broad sampling in the northern hemisphere but with emphasis on the North Atlantic area. The frequency of previous immigrations will not be considered here, as the procedure for calculating this frequency is still under development, but will be calculated and published later on (Alsos et al. in prep).

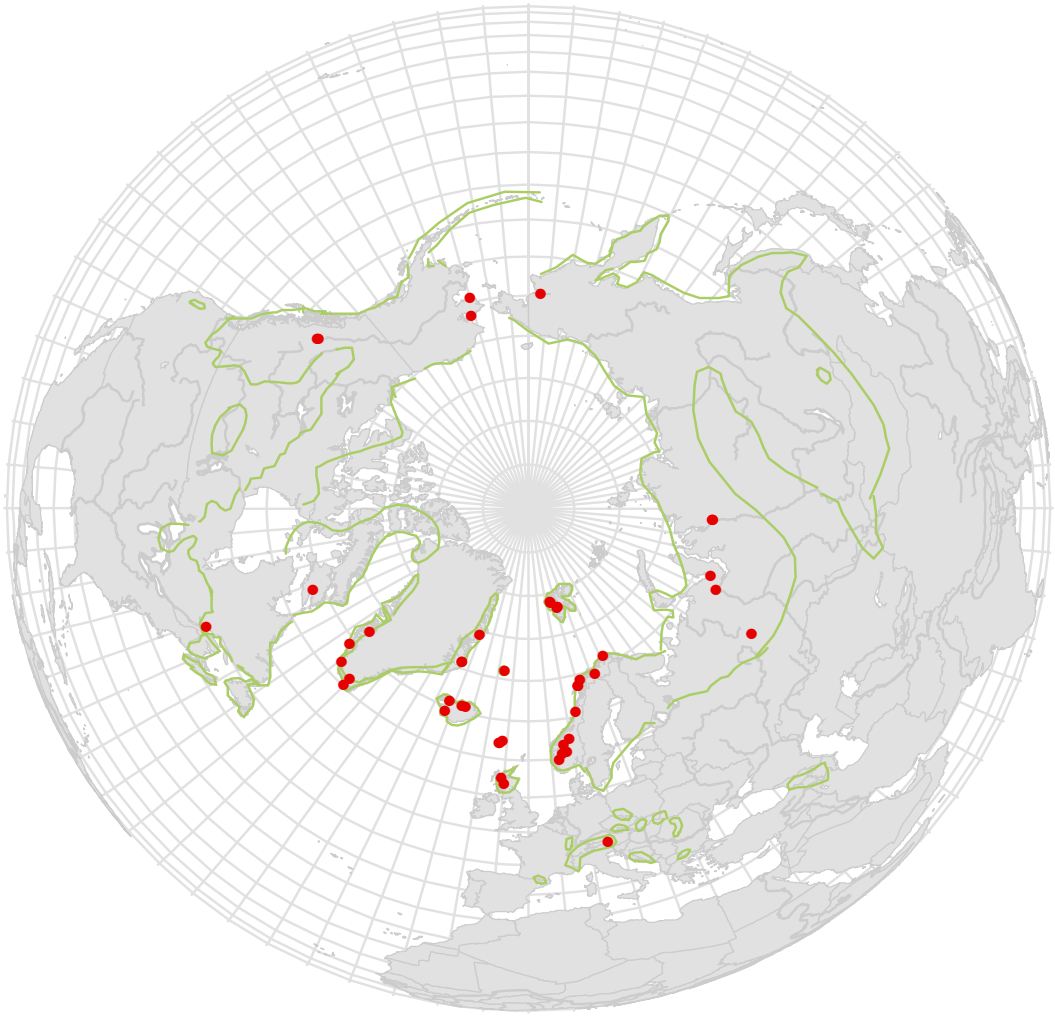


Fig. 2. *Geographic distribution of the monoecious* Empetrum nigrum *s. lat. redrawn from Hultén and Fries (1986), and locations of the 46 populations analysed in this study (red dots).*

Material and methods

Sampling

Material was collected with emphasis on the North Atlantic region. In addition some more distant populations were included as reference material (Fig. 2). The total geographic distribution of the species and the sampling in Svalbard are summarised in Fig. 3. Individuals growing within an area of 250 m x 250 m were defined as one population. From each population, leaves and young shoots from 11 plants were collected approximately 25 m apart along a straight line, and stored in silica gel. For practical reasons in the laboratory it was convenient to sample 11 individuals per population. Due to sampling errors and samples that failed in the laboratory not all populations consist of 11 specimens (see Table 1). For each population an additional sample X, which was a duplication of one of the 11 individuals, was included. The purpose of sampling the X was to make a reproducibility test for AFLPs and to discover possible mix-ups in the laboratory. One voucher was collected per population and stored at the Botanical Museum, University of Oslo (O). Living material from some of the populations were collected and cultivated in a phytotron at the Department of Biology, University of Oslo. A similar study of *Dryas octopetala* L.(Skrede 2004) suggested that including several populations from more distant regions would give a broader picture of the genetic variation throughout the entire distribution areas and make it easier to understand the phylogeography in the areas surrounding Svalbard in details. Thus, six populations of *Empetrum nigrum* s. lat. from areas outside the North Atlantic region were included. These were obtained from the study by Mirré (2004), and each consisted of five specimens. In total, 435 individuals from 46 populations were successfully analysed.

Table 1. *Collection data for* Empetrum nigrum *s.lat populations used in this study. AFLP - number of plants analysed by AFLPs, FC - number of plants of which the ploidal level is determined by flow cytometry, numbers in parenthesis are number of plants which ploidy level is estimated but not analysed by AFLPs.*

Main geographic area	Pop. name	Pop. ref.	Locality	Year	Latitude	Longitude	Altitude (m)	No. of plants AFLP	No. of plants FC	Ploidal level	Gene diversity*	Collectors**
Svalbard	Dyrevika	AK-102	Svalbard, Haakon VII Land, Dyrevika	2003	N 79˚00'07"	E 12˚20'48"		11			0.05	IGA, BES
	Hotellneset	AK-547	Svalbard, Nordenskiöldland, Hotellneset	2002	N 78˚14'20"	E 15˚31'12"	50	6			0.05	IGA, KW
	Colesdalen	AK-567	Svalbard, Nordenskiöldland, Colesdalen	2002	N 78˚05'82"	E 15˚12'35"	100	10	2	4x	0.07	IGA, KW
	Ossian Sars	AK-733	Svalbard, Haakon VII Land, Ossian Sars	2003	N 78˚57'07"	E 12˚26'42"	30-50	11	2	4x	0.06	IGA, BES
W Siberia/Ural	Dudinka 1	AK-1282	Russia, Krasnoyarskiy territory, Taymyr, Dudinka	2003	N 69˚25'34"	E 86˚14'11"		9	1 (2)	4x	0.16	DE
	North Ural	AK-145	Russia, North Ural, Denezhkin Kamen Rock	2002	N 60˚26'	E 59˚33'	1000-1200	10	2	4x	0.24	MK
	Polar Ural	AK-146	Russia, Polar Ural, Slanzevaya Mountain	2002	N 66˚55'	E 65˚46'	200-400	10	1 (1)	4x	0.16	MK
	South Yamal	AK-147	Russia, South Yamal, Ercuta-yaha River	2002	N 68˚12'	E 68˚54'		9	1 (1)	4x	0.08	MK
	Dudinka 2	AK-378	Russia, Krasnoyarskiy territory, Taymyr, Dudinka	2003	N 69˚24'36"	E 86˚15'00"		10	2	4x	0.15	DE
E Siberia	Chukotka	AK-600	Russia, Southern Chukotka, Anadyr Bay, Onemen Bay	2002	N 64˚47'	E 176˚58'		11	3	2x/3x	0.15	VR
W North America	Cape Nome	AK-584	USA, Alaska, Seward Peninsula, Cape Nome	2002	N 64˚26'37"	W 164˚58'23"	5-7	11	2	4x	0.12	RE, TMG, MHJ
	Cape Espenberg	SUP03-032	USA, Alaska , Seward Peninsula, Cape Espenberg	2003	N 66˚34'09"	W 164˚01'12"	2-8	5	4	4x	0.07	RE, HS
	Gnat Pass	SUP03-163	Canada, British Columbia, Cassiar Mountains, Gnat Pass	2003	N 58˚17'00"	W 129˚52'00"	1241	5	2	4x	0.11	RE, HS
	Dease Lake	SUP03-371	Canada, British Columbia, SW Cassiar Mountains, Dease Lake	2003	N 58˚27'00"	W 129˚59'00"	840-850	5	2	4x	0.13	RE, HS
E Canada	Baffin Island	SUP03-025	Canada, Baffin Island, Iqaluit	2003	N 63˚44'00"	W 68˚30'00"		5	(1)	4x	0.04	CM
	Forestville	SUP03-034	Canada, Quebec, Côte-Nord, Forestville	2003	N 48˚43'50"	W 69˚05'47"		5	1	4x	0.02	RE, AE
E Greenland	Zackenberg	AK-122	Greenland, Kong Christian X Land, Zackenberg	2002	N 74˚28'	W 20˚33'		9	1 (1)	4x	0.08	KA
	Primula River	AK-350	Greenland, Kong Christian IX Land, Constable Point, Primula River	2002	N 70˚44'52"	W 22˚41'52"	212	8	1	4x	0.13	IS, LL
	Hare River	AK-364	Greenland, Kong Christian IX Land, Constable Point, Hare River	2002	N 70˚42'42"	W 22˚40'51"	122	11	(1)	4x	0.14	IS, LL
SW Greenland	Kangerlussuaq	AK-203	Greenland, Kong Frederik IX Land, Kangerlussuaq	2002	N 67˚	W 51˚		4	(2)	4x	0.03	CP
	Paamiut	AK-245	Greenland, Kong Frederik IX Land, Paamiut	2002	N 62˚00'14"	W 49˚37'09"	78	10	2 (1)	4x	0.19	PBE, GHJ
	Nuuk	AK-252	Greenland, Kong Frederik IX Land, Nuuk	2002	N 64˚11'38"	W 51˚41'55"	36	9	1 (3)	4x	0.18	PBE, GHJ
	Narsarsuaq	AK-258	Greenland, Kong Frederik IX Land, Narsarsuaq	2002	N 61˚12'29"	W 45˚18'34"	258	11	1	4x	0.16	PBE, GHJ
	Nanortalik	AK-287	Greenland, Kong Frederik IX Land, Nanortalik	2002	N 60˚08'45"	W 45˚13'37"	14	11	(1)	4x	0.17	PBE, GHJ

Table 1. *Continued*

Main geographic area	Pop. name	Pop. ref.	Locality	Year	Latitude	Longitude	Altitude (m)	No. of plants AFLP	No. of plants FC	Ploidal level	Gene diversity*	Collectors**
N Norway	Brønnøy	AK-481	Norway, Nordland, Brønnøy, Gåsheia	2002	N 65˚29'92"	E 12˚23'00"	100	10	2	2x/4x	0.22	KW
	Nordkapp	AK-705	Norway, Finnmark, Nordkapp, Duken	2002	N 71˚03'91"	E 25˚47'66"	120	11	2	4x	0.14	KW
	Kåfjordfjellet	AK-743	Norway, Troms, Kåfjord, Kåfjordfjellet	2002	N 69˚23'63"	E 21˚02'10"	636	10			0.12	KW
	Andøy	AK-747	Norway, Nordland, Andøy, Røyken	2002	N 69˚06'48"	E 16˚00'26"	350	11	7	2x/4x	0.19	KW
	Tromsø	AK-752	Norway, Troms, Tromsø, Storsteinen	2002	N 68˚33'14"	E 14˚55'77"	545	10	2	4x	0.10	IGA, KW
	Hadsel	AK-762	Norway, Nordland, Hadsel, Storheia	2002	N 68˚33'14"	E 14˚55'77"		11	2	4x	0.09	IGA, BES, MLAS
S Norway	Ål	AK-430	Norway, Buskerud, Ål, N of Helsingset	2002	N 60˚44'17"	E 08˚37'2"	950-1000	11	(3)	4x	0.13	GHJ, VM
	Lom	AK-435	Norway, Oppland, Lom, Bøverdalen	2002	N 61˚40'54"	E 08˚08'54"	761	11	(2)	4x	0.08	PBE, IS, GHJ
	Oppdal	AK-466	Norway, Sør-Trøndlag, Oppdal, Vårstigen	2002	N 62˚20'34"	E 09˚37'26"	993	11	(2)	4x	0.16	PBE, IS, GHJ
	Røldal	AK-498	Norway, Hordaland, Odda, Røldal	2002	N 59˚50'04"	E 06˚44'04"	1000-1050	10	(1)	4x	0.02	PBE
	Finse	AK-513	Norway, Hordaland, Finse	2002	N 60˚35'14"	E 07˚29'57"	1260	10	(1)	4x	0.14	MHJ, IS, GHJ
Iceland	Akureyri	AK-815	Iceland, Akureyri	2002	N 65˚41'24"	W 18˚02'03"	200	11	6 (2)	2x/3x/4x	0.16	IS, SK, LL
	Hvalfjordur	AK-838	Iceland, Hvalfjördur S	2002	N 64˚21'01"	W 21˚26'75"	86	11	4 (2)	2x/3x/4x	0.13	IS, SK, LL
	Drangsnes	AK-845	Iceland, West Fjords, Drangsnes/ Hólmavík	2002	N 65˚45'56"	W 21˚38'10"	191	11	4	2x/4x	0.13	IS, SK, LL
	Myvatn	AK-876	Iceland, Mývatnssveit, Myvatn	2002	N 65˚36'37"	W 16˚55'0"	290	10	8 (1)	2x/4x	0.14	IS, SK, LL
Jan Mayen	Jan Mayen	AK-110	Norway, Jan Mayen, Kreklinghaugen	2002	N 71˚00'00"	E 08˚20'00"	10-30	11	2	4x	0.03	AW
Faroe Islands	Eystorøy	AK-826	Faroe Islands, Faeroe, near Sutrugøta town	2003	N 62˚	W 07˚00'		10	1	4x	0.11	C
	Fugløy	AK-1154	Faroe Islands, Fugløy, Vansdal	2003	N 62˚19'	W 06˚15'		11	2	4x	0.08	GB
	Torshavn	AK-1175	Faroe Islands, Torshavn, Høyvik	2003	N 62˚	W 06˚45'		11	2	4x	0.10	GB
Scotland	Glencoe	AK-1313	United Kingdom, Scotland, Glencoe, Coire nan lochan Valley	2003	N 56˚39'14"	W 05˚00'43"	644-782	11	2	4x	0.09	IS, PBE
	Torridon	AK-1321	United Kingdom, Scotland, Torridon	2003	N 57˚34'35"	W 05˚34'57"	738	11	4	2x/4x	0.14	IS, PBE
The Alps	Austria	SUP02-724	Austria, Salzburg	2002	N 47˚10'00"	E 12˚50'46"	2240	5	2	4x	0.13	PS

**Diploids and triploids were excluded*

Collectors:* *KA – Kristian Albert, IGA – Inger Greve Alsos, GB – Guri Bugge, C – Caroline, DE – Dorothee Ehrich, PBE – Pernille Bronken Eidesen, AE – Anne Elven, RE – Reidar Elven, TMG – Tove M Gabrielsen, GHJ – Gro Hilde Jacobsen, MHJ – Marte H Jørgensen, MK – Maxim Kapralov, SK – Siri Kjølner, LL – Leidulf Lund, CM – Carolyn Mallory, VM – Virginia Mirré, CP – Christian Pedersen, VR - Volodya Razzhivin, MLAS - Mai Lene Alsos Sandbakk, BES – Bjørn Erik Sandbakk, PS – Peter Schönswetter, IS – Inger Skrede, HS – Heidi Solstad, KW – Kristine Westergaard, AW – Anders Wollan

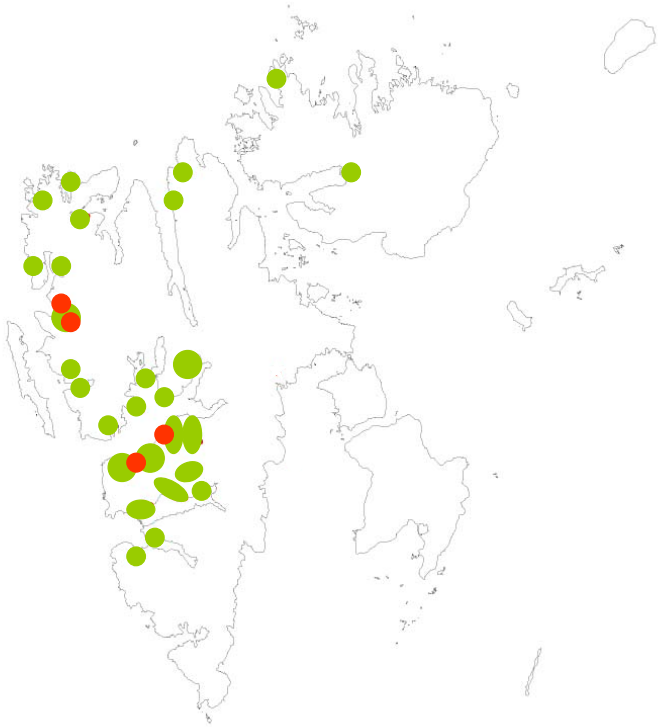


Fig. 3. *Geographic distribution of Empetrum* nigrum *s. lat. in Svalbard (in green), and locations of the four populations analysed in this study (red dots).*

Ploidal level determination

Ploidal levels were estimated for 111 specimens from 43 populations distributed throughout the whole sampling area. Usually one or two specimens per population were examined. When different DNA ratios within one population were detected, more samples were analysed. Living-, herbarium- or silica dried material was used for the analysis (Table 1; Appendix 2). All analyses were conducted by Jan Suda (http://botany.natur.cuni.cz, Department of Botany, Charles University, Prague). Ploidal levels were estimated with a Partec PA II flow cytometer (Partec GmbH, Germany) with a HBO-100 mercury arc lamp as excitation source.

A modified two-step procedure of nuclei isolation and staining, as originally described by Otto (1990) was used. Approximately 20 mg of leaves from each specimen with or without an internal standard were chopped with a razor blade in 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20). The solution was filtered (42 µm nylon mesh), stained with 1 ml Otto II buffer (0.4 M Na_2HPO_4 . 12 H_2O) with DAPI (4 µg/ml) and ß-mercaptoethanol (2 µg/ml). After incubation for 1–2 min at room temperature, the samples were measured on the

flow cytometer. The cytometer was adjusted so that the fluorescence of G_0/G_1 nuclei of a fresh diploid (2n = 26, Czech Republic, Šumava Mts.;Suda *et al.* 2004) was localised on channel 100. The flow rate was 30-50 fluorescent events per sec and the fluorescence of 5000 nuclei was usually recorded. The coefficient of variance (CV) of analysed plants did not exceed 3% in fresh material, and varied between 2.57% and 8.37% in herbarium and silica-dried vouchers, depending primarily on the age of the material and the ploidal level. Each plant was analysed at least twice and the same ploidal level was estimated with both of the following modifications:

1) Without internal standard to determine the peak quality and obtain preliminary information about the ploidal level. The above-mentioned chromosome-counted diploid material was used as an external standard, and the position of the diploid peak was always checked before the unknown sample analysis.
2) With fresh *Zea mays* L CE-777 (2C = 5.43 pg; Lysak and Dolezel 1998) as an internal standard. This was the same internal standard as used in ploidal level estimation of *Empetrum* material by Suda et al. (2004).

DNA extraction

Silica-dried branches with leaves, ~1 cm^2 per sample, were ground in a mixer mill (MM301, Retsch GmbH & Co., Haan) for two min at 20 Hz with two tungsten carbide beads in 2.0 ml tubes. DNA extractions were performed using DNeasy™ Plant Mini Kit (Qiagen 2001) according to the manufacturer's protocol; except that the samples were frozen at -80°C after adding buffer AP1 and the incubation step was prolonged to 20 min. To increase DNA concentration, the DNA was eluted using 50 µl AE buffer twice.

AFLP analysis

AFLP analysis consists of four steps. The total genome is digested with two restriction enzymes, one frequent cutter, *Mse*I, and one rare cutter, *Eco*RI. Adaptors are ligated onto the fragment ends and generate the primer binding sites. Two PCR-amplifications are run, one preselective amplification where the primers are extended with one additional nucleotide, and one selective amplification with primers extended with three nucleotides compared to the

adaptors. These steps reduce the number of fragments. The last step separates the fragments using electrophoresis.

AFLP analysis was performed according to the AFLP™ Plant Mapping protocol (AppliedBiosystems 1996) except using half of the recommended volume in the PCR reactions (GeneAmp PCR system 9700, Applied Biosystems, Foster City). Distilled, autoclaved water were used instead of Tris-EDTA-buffer ($TE_{0.1}$). This should only effect long term storage of the products, which were frozen instead of stored at 2-6˚C. Separation of the fragments was done using an ABI 3100 sequencer (Applied Biosystems, Foster City). 11.5 µl HiDi (formamide) and 0.5 µl GeneScan-500 ROX was loaded with 2.0 µl PCR product for dye set NED and 1.8 µl for dye set 5-FAM and run with 40 sec injection time.

A total of 45 primer combinations for the selective amplification were tested for four individuals from three different geographic regions (Norway, Iceland, and Greenland; Table 2). Four primer combinations that had well separated peaks and an appropriate amount of polymorphism were chosen (EcoRI-ACT/MseI-CAA, EcoRI-ACC/ MseI-CTG, EcoRI-ACC/ MseI-CTA, and EcoRI-AAC/ MseI-CTA).

Table 2. *AFLP primer combinations tested for* Empetrum nigrum *s. lat. in this study.* **-** *denotes primer combination tested,* **!** *denotes primer combinations used. 5-FAM-, NED-, and JOE primers are colored blue, yellow, and green respectively.*

***Eco*RI Primers**	***Mse*I Primers**							
	-CAA	**-CAC**	**-CAG**	**-CAT**	**-CTA**	**-CTC**	**-CTG**	**-CTT**
-AAC	-	-	-		!		-	
-AAG	-	-	-	-	-	-	-	
-ACA		-		-	-	-	-	
-ACC	-		-		!		!	-
-ACG	-	-		-	-	-	-	
-ACT	!	-	-		-		-	-
-AGC		-	-		-	-		
-AGG	-	-	-	-	-	-	-	

Prior to scoring, the profiles were aligned to the size standard and checked in GeneScan, version 3.7 (Applied Biosystems; Foster City). The AFLP bands were scored as present (1) or absent (0) in the range from 50 bp to 500 bp using Genographer, version 1.6.0 (http://hordeum.oscs.montana.edu/genographer). Due to very variable intensity of the profiles it was impossible to have the same threshold level for all profiles.

A Neighbour joining tree was calculated for the markers to check for linkage between them. Linked markers were removed from the dataset.

Data analysis

The AFLP data set was analysed by distant-based and model-based methods. Principle coordinate analysis (PCO) was performed with simple matching (SM), a similarity measure taking both absence and presence of markers into account, and Dice's similarity coefficient based on shared bands only, using NTSYSpc version 2.0 (Rohlf 1990).

A midpoint-rooted Neighbour joining tree was calculated using PAUP 4.0 (Swofford 2002) based on Nei and Li distance measure and with 1000 jackknife replicates.

Structure version 2 (Pritchard *et al.* 2000), a Bayesian MCMC model-based clustering method for inferring population structure and assigning individuals to populations probabilistically without specifying populations *a priori*, was used to group the specimens. The program was originally designed for co-dominant markers, but can be used for dominant markers under a no admixture model if no linkage between the loci is assumed. The number of groups (K) was used as a prior value, and set to 1-10. Ten replicates were run for each K with a burn-in of 100 000 and 1000 000 iterations for the whole data set. Different subsets were run with 10 replicates for each K, using a burn-in of 100 000 and 200 000 iterations.

Multilocus assignment tests were performed using AFLPOP (Duchesne and Bernatchez 2002). Individuals are assigned to predefined source population on the basis of differences in frequencies among polymorphic loci, based on the AFLP phenotypes. The likelihood for an individual to belong in a population is calculated for each population. The individual is assigned to the population with highest likelihood. The stringency level for an individual to assign is defined as a minimal likelihood difference between the two populations (A and B) with the highest likelihood given by: $\left(\left|\log L_A - \log L_B\right|\right) > \varepsilon$. In this study ε was set to 1.

Analysis of molecular variance (AMOVA) was calculated using the program Arlequin version 2.000 (Schneider *et al.* 1997). AMOVA is designed for co-dominant markers but can be used for AFLP markers as haplotypic data if the same mating pattern is assumed in all populations (Schneider *et al.* 1997).

Diversity indexes were calculated for each population using Nei's gene diversity with the formula $D = [n/ (n\text{-}1)] * [1 – (\text{freq } (1)^2 + \text{freq } (0)^2)]$, where n is the sample size, for each marker and then making the average across markers (Nei and Li 1979).

Results

Ploidal levels

Three ploidal levels were estimated among the 111 samples examined: 26 specimens were diploid, 4 triploid and 81 tetraploid (Table 1; Appendix 2). The 1/DNA ratio varied from 0.340-0.370 in the diploids, 0.668-0.730 in the tetraploids, and the triploids were in between with a 1/DNA ratio of 0.523-0.526 (Fig. 4; Appendix 2).

The geographic distribution of all known ploidal levels in *Empetrum* are illustrated in Fig. 5, based on the master study by Mirré (2004), chromosome counts from literature (summarised by Mirré), and results from this study. The tetraploids had a circumpolar distribution. The diploids appeared more disjunct and were found in Pacific North America, Chukotka, Iceland, mainland Europe, Altai, and Japan; but were absent from Svalbard, Greenland, East Canada, East Siberia, the Ural Mountains, and the northernmost part of Norway. Triploids were found in five areas where diploids and tetraploids were living in sympatry; southern Norway, Faroe Islands, Iceland, Czech Republic, and Alaska. Triploids were also found in Chukotka.

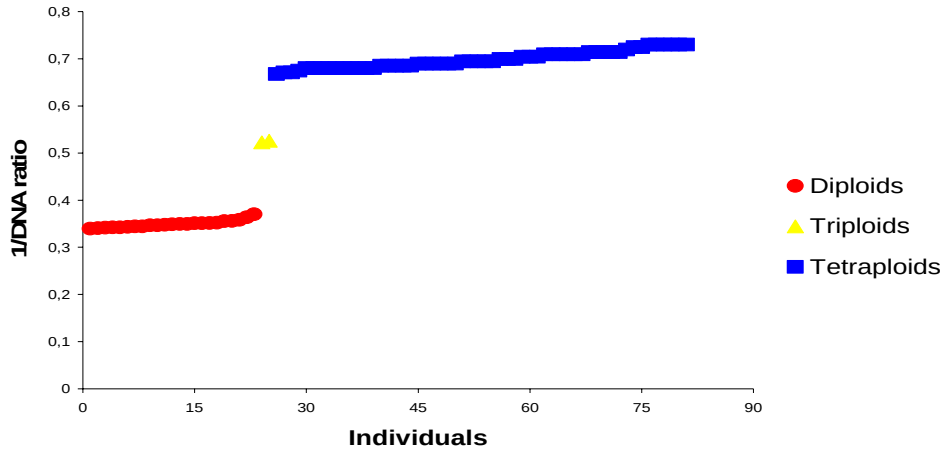


Fig. 4. *1/DNA ratio for 81* Empetrum nigrum *s. lat. plants determined by flow cytometry using DAPI staining and* Zea mays *as internal standard.*

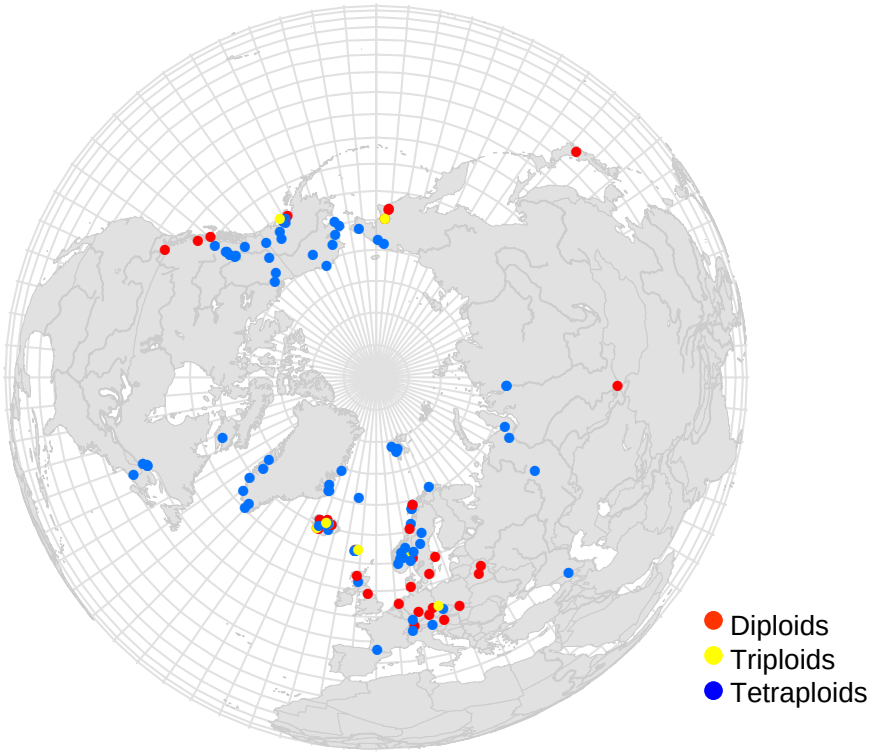


Fig. 5. *Geographic distribution of* Empetrum nigrum *s. lat. ploidal levels in the northern hemisphere, including chromosome counts available in the literature (summarised in Mirré 2004), results from the master study by Mirré (2004), and results obtained in this study (Table 1 and Appendix 2).*

Only tetraploid *E. nigrum* was found to be present in Svalbard. In this study, eight populations with mixed ploidal levels were found, located in Iceland, Scotland, northern Norway and Chukotka. The diploids and triploids found in these areas are apparently of little interest for this study of immigration to Svalbard, and were therefore excluded from several analyses.

AFLP analysis

The final AFLP data matrix consisted of 435 individuals and 78 polymorphic markers (Appendix 1 on CD). The reproducibility test based on 30 replicates (X-samples) gave an error rate of 2.3%. Four markers were removed from the original data set due to linkage, and five markers were removed due to a frequency lower than the error rate.

Structure analyses

Structure analysis was run for the whole AFLP data set to analyse which groups that were present in the material. The probability of the data given a number of groups (K) increased with number of groups inferred. Above K=3, the assignment to groups differed considerably between each replicated run. Two groups gave the same result for each run and divided the material into a northern circumpolar group, including Svalbard, and a southern North Atlantic group. The third group that was introduced when increasing K, was unstable and contained representatives from both the northern and southern group. The East Siberian, western North American, and some of the South West Greenland and South Norwegian plants were always assigned to the third group that was inferred, while the plants from Svalbard always belonged to the northern circumpolar group.

Neither the East Siberian and the West North American plants nor the diploids and triploids were apparently important when considering possible immigration routes to Svalbard. Therefore, Structure was also run without these plants. The probability of the data given a number of groups increased with number of groups (Fig. 6), but only K=2 gave a stable result. The same north-south pattern as for the whole data set was recognised (Fig. 7).

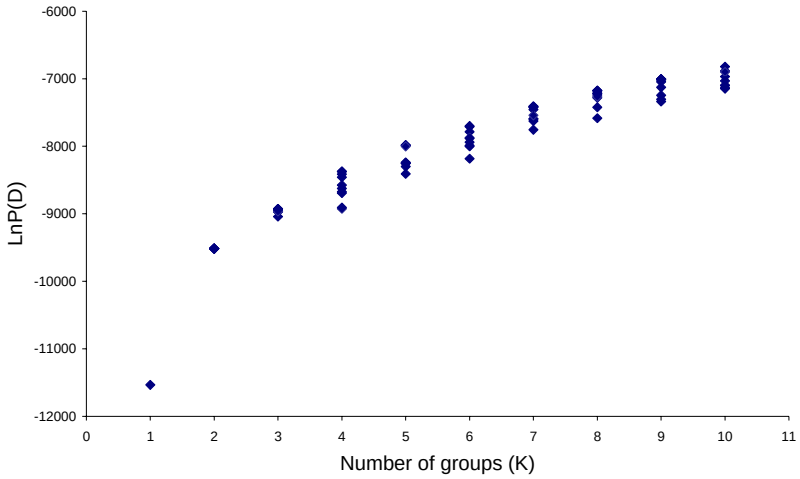


Fig. 6. *Logarithmic probability of data given a number of groups (K) resulting from Structure analysis of 78 AFLP markers on 376* Empetrum nigrum *s. lat. plants. Ten replicates are shown for each number of groups.*

The northern group consisted of plants from Svalbard, East Canada, East Greenland, Kangerlussuaq (South West Greenland), and West Siberia/Ural Mountains, except for four plants from the Ural Mountains, which were placed in the southern group. Plants from Europe and South West Greenland belonged to the southern group, except for single plants from southern Norway, South West Greenland, and the Alps, which belonged to the northern group.

The northern group was also analysed separately in Structure. Two groups were stable across different runs, and divided the northern group into a Svalbard group and a Russian/East Canadian group. Plants from East Greenland were present in both groups (Fig. 8).

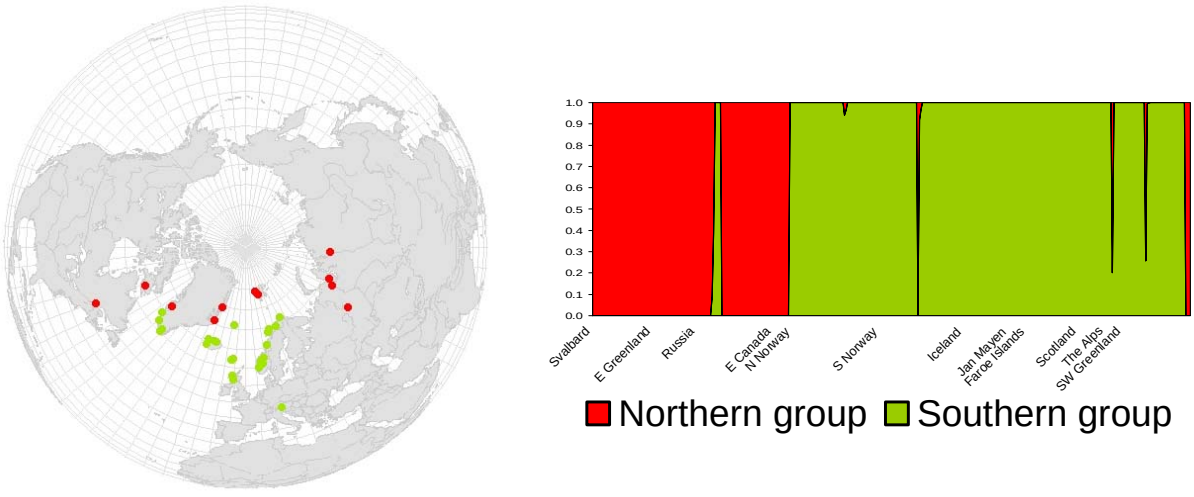


Fig. 7. *376* Empetrum nigrum *s. lat. plants from a broad amphi-Atlantic subsample of the dataset, excluding the diploids and tetraploids, sorted in a northern and a southern group by Structure.*

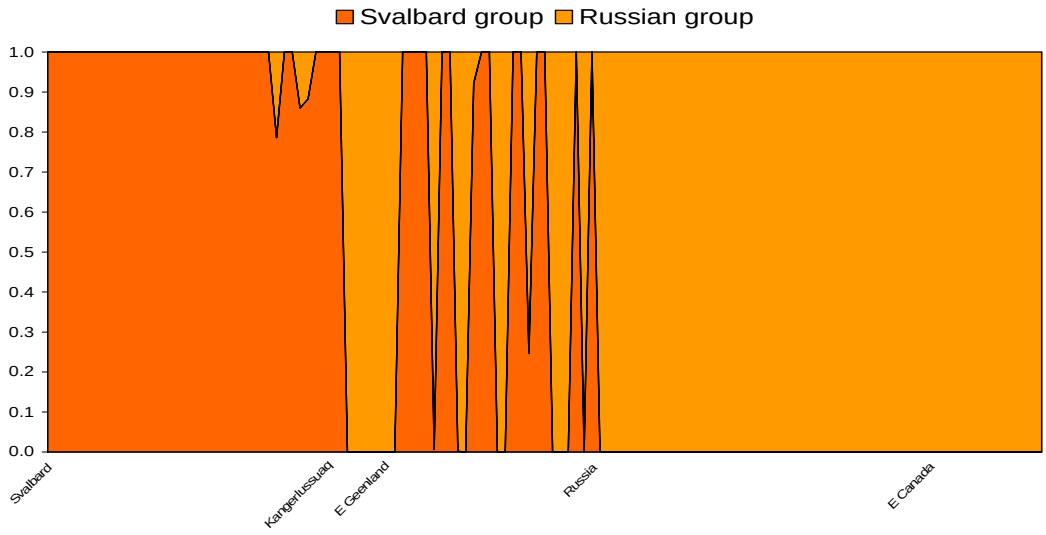
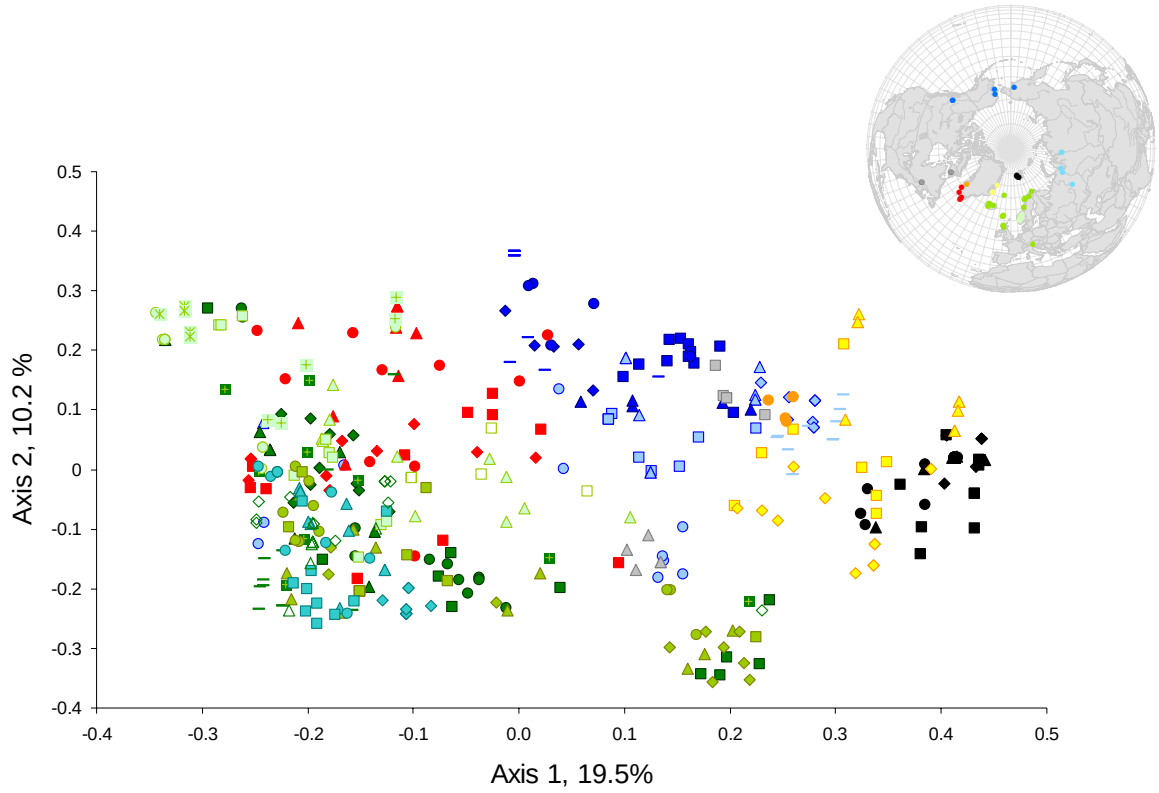


Fig. 8. *The northern group, 127* Empetrum nigrum *s. lat. plants, inferred from the Structure analyses of the broad amphi -Atlantic subsample, sorted in two groups by Structure. The Svalbard group consisted of the plants from Svalbard and half of the plants from East Greenland. The Russian group consisted of the plants from West Siberia/Ural, East Canada, Kangerlussuaq (South West Greenland), West Greenland and the other half of the plants from East Greenland.*

PCO analyses

A PCO analysis of all the 435 plants revealed a geographic structure in the AFLP dataset (Fig. 9; Fig. 10). The first axis spanned 19.5% of the variation, and reflected a north-south pattern, with the northern circumpolar populations on one side, and the southern North Atlantic populations on the other side. The only exception from this pattern was the European diploids, which are treated below. The second axis (10.2%) separated the East Siberian and West North American plants somewhat from the rest of the circumpolar group.

A PCO plot with the ploidal levels superimposed (Fig. 11) showed that the European plants estimated to be diploids and triploids formed a group separated from the tetraploid plants, even those from the same locality. The European diploids were placed in the northern circumpolar group along the first axis but were clearly separated from them along the second axis. The triploid from Iceland was placed between the diploids and tetraploids from the same population along the first axis. The diploids and the triploid from Chukotka grouped together and were placed among tetraploids from the same area.



◆ Svalbard, Hotellneset
■ Svalbard, Colesdalen
▲ Svalbard, Dyrevika
● Svalbard, Ossian Sars
□ W Siberia/Ural, Dudinka 1
○ W Siberia/Ural, North Ural
△ W Siberia/Ural, Polar Ural
◇ W Siberia/Ural, South Yamal
– W Siberia/Ural, Dudinka 2
■ E Siberia, Chukotka
▲ W North America, Cape Espenberg
– W North America, Cape Nome
◆ W North America, Gnat Pass
● W North America, Dease Lake
■ E Canada, Baffin Island
△ E Canada, Forrestville
△ E Greenland, Zackenberg
□ E Greenland, Primula River
◇ E Greenland, Hare River
● SW Greenland, Kangerlussuaq
● SW Greenland,Paamiut
▲ SW Greenland, Nuuk
■ SW Greenland, Narsarsuaq
◆ SW Greenland, Nanortalik
■ N Norway, Andøy
◆ N Norway, Tromsø
● N Norway, Nordkapp
▲ N Norway, Kåfjordfjellet
– N Norway, Hadsel
+ N Norway, Brønnøy
○ S Norway, Ål
+ S Norway, Lom
△ S Norway, Oppdal
✳ S Norway, Røldal
□ S Norway, Finse
● Iceland, Hvalfjordur
■ Iceland, Drangsnes
▲ Iceland, Akureyri
◆ Iceland, Myvatn
◇ Jan Mayen
△ Faroe Islands, Fugløy
■ Faroe Islands, Eystorøy
● Faroe Islands, Torshavn
△ Scotland, Glencoe
◇ Scotland, Torridon
□ The Alps, Austria

Fig. 9. *PCO analysis (axis 1 and 2) based on simple matching similarity between 435 AFLP phenotypes of* Empetrum nigrum *s. lat. plants based on 78 polymorphic markers. Colors indicate different geographical regions as indicated on the map. Populations are represented by symbols.*

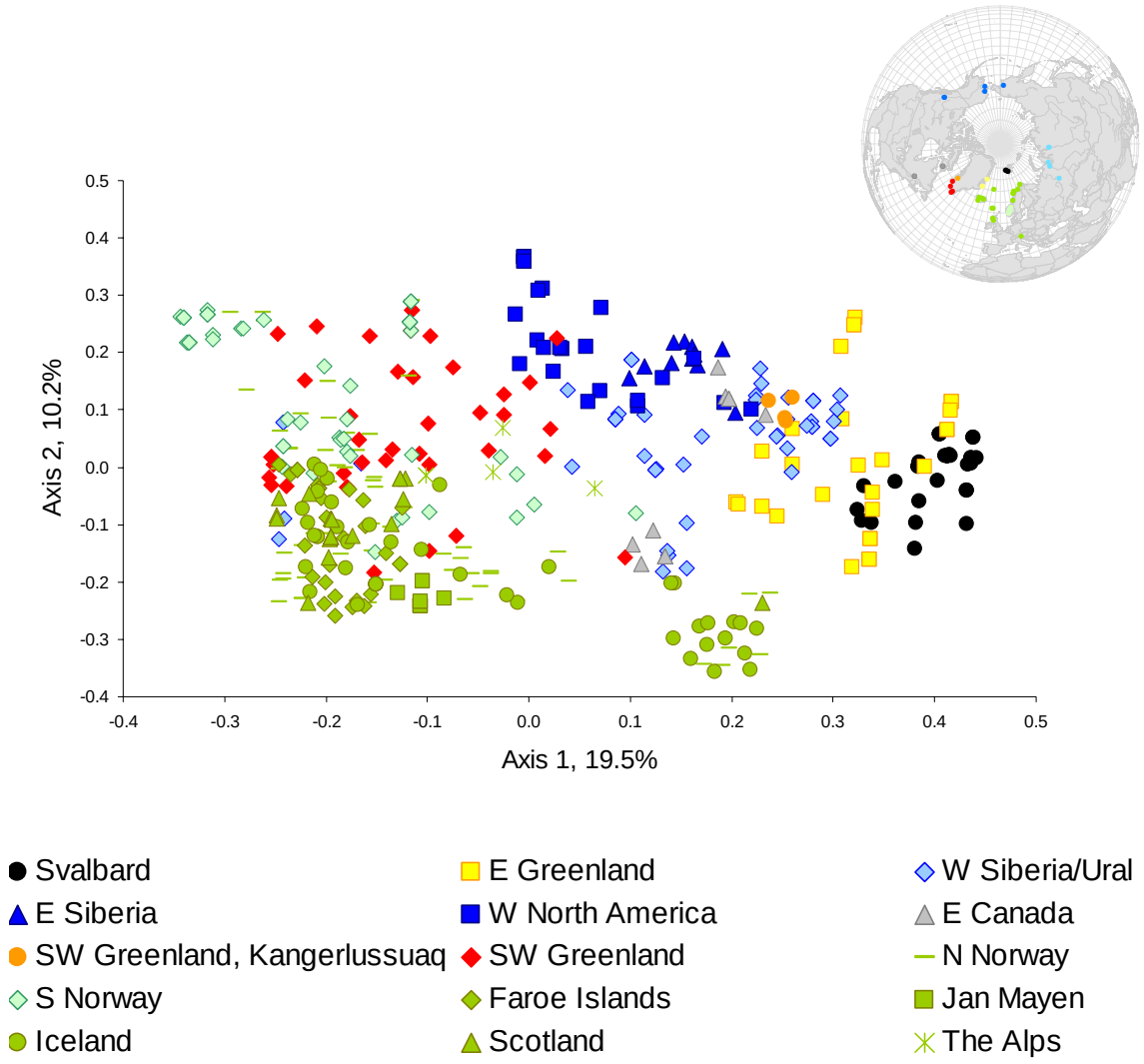


Fig. 10. *Simplified presentation of figure 9 (PCO analysis based on simple matching similarity between 435 AFLP phenotypes of* Empetrum nigrum *s. lat. plants based on 78 polymorphic markers). Colors indicate different geographical regions as denoted on the map. Symbols represent smaller geographic entities within each region.*

A PCO analysis was thus performed on a subset of the data, where the diploids, the triploids, and the plants from West North America and East Siberia were excluded (Fig. 12). The same north-south pattern as for the whole data set was recognised. The first axis spanned 22.1% of the variation and separated the plants from the northern areas (Svalbard, West Siberia/Ural Mountains, East Greenland, and East Canada) and the plants from the southern North Atlantic areas (Europe and South West Greenland). Exceptions from this pattern were four plants from West Siberia/Ural Mountains (North Ural and Polar Ural populations) which

were placed in the southern group and one population from West Greenland (Kangerlussuaq) which was placed in the northern group. There was some phylogeographic structure within the northern group, where the plants from Svalbard were grouped on the one extreme along axis 1. The East Greenland plants were situated in an intermediate position between the Svalbard plants, and the rest of the northern group. There was no apparent structure within the southern group along the first axis.

The second axis in this PCO analysis spanned 8.6% of the variation, but yielded little extra phylogeographic information for the northern group, except that the East Canadian populations were split into two groups. In the southern group, the South West Greenlandic and South Norwegian plants were separated from the rest of the Europeans, whereas the North Norwegian plants were scattered all over the group. The third axis spanned 6.4% of the variation, but gave little new information (not shown).

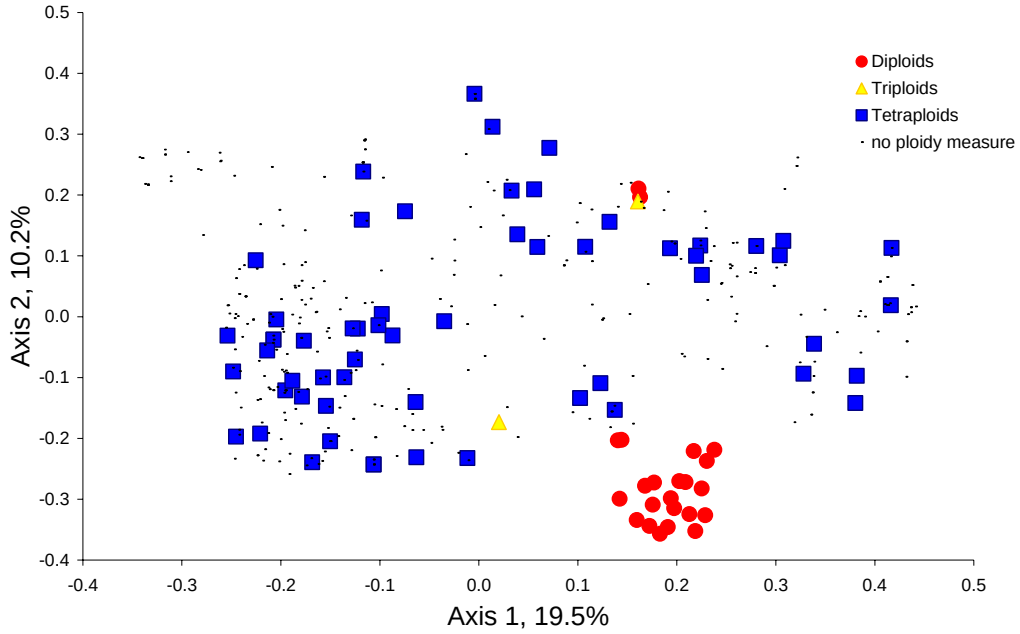


Fig. 11. *PCO analysis based on simple matching similarity between 435 AFLP phenotypes of* Empetrum nigrum *s. lat plants based on 78 polymorphic markers, where the specimens with ploidal estimates are highlighted (see also Fig. 9; Fig. 10).*

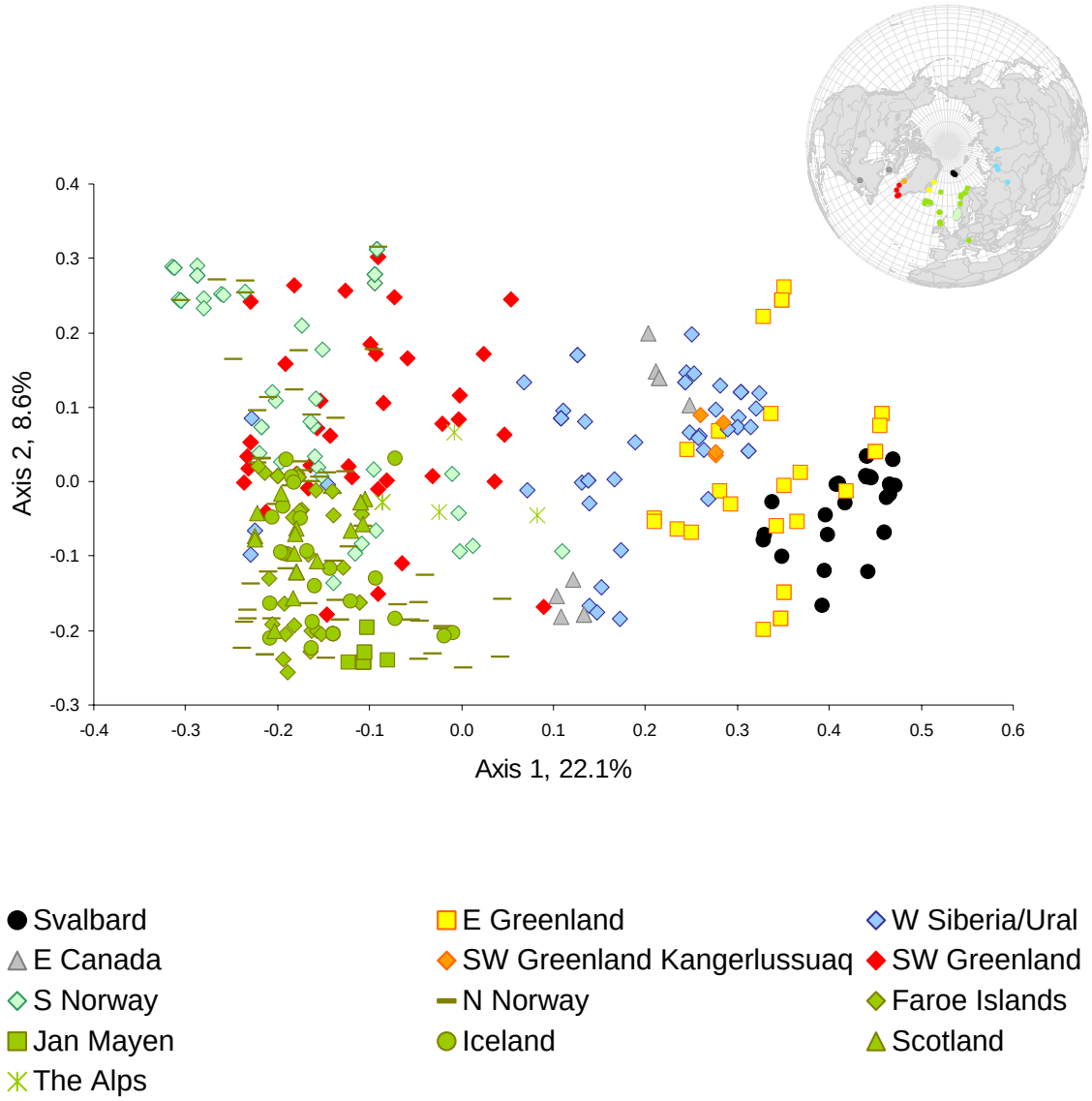


Fig. 12. *PCO analysis (axis 1 and 2) of a broad amphi-Atlantic subsample of the data excluding the diploids and triploids, based on simple matching similarity between 376 AFLP phenotypes of* Empetrum nigrum *s. lat. plants based on 78 polymorphic markers. Colors indicate different geographical regions as indicated on the map. Symbols represent smaller geographic entities within each region.*

Neighbour joining analysis

The neighbour joining tree divided the plants into two main groups, one northern and one southern, as in the former analyses (result not shown). The support was however very low with no jackknife values above 50%.

AFLPOP analyses

Reallocation

In a reallocation analysis of the southern and northern group inferred from the Structure analysis of the broad amphi-Atlantic subset with the diploids and triploids excluded, all plants allocated to the group of origin, except that three plants did not allocate.

Reallocation analyses were performed both to populations (Table 3) and to geographic areas, as defined in Table 1, except that the Kangerlussuaq population was defined as a separate area (Table 4). The diploids and tetraploids were excluded from the data set. In reallocation to populations most of the plants which allocated, assigned to their own population. Some plants assigned to another population within the same region as they originated, and a few plants assigned to other geographical regions. The proportion of assignment to the correct geographical area of origin was higher in the northern circumpolar area than in the southern North Atlantic region. When assigning to regions, the fraction of assignment decreased in most areas compared to population assignment.

Allocation

Allocation analyses were performed for plants from Svalbard, East Greenland, and West Siberia/Ural Mountains. The plants were assigned both to population (Table 5a) and to geographic areas as defined in Table 1, with exception of the Kangerlussuaq population which were defined as a separate area (Table 5b). The diploids and tetraploids were excluded from the data set. In this analysis, samples were not given the opportunity to assign to their own area. Of the Svalbard plants, 63% were assigned to East Greenland when they were assigned to populations, and the remaining 37% failed to assign. When assigning the Svalbard plants to other geographic areas, all plants assigned to East Greenland. The plants from East Greenland were mostly assigned to Svalbard (57%) and West Siberia/Ural Mountains (29%) when allocating to populations. When assigning to geographic areas, they assigned to Svalbard and West Siberia/Ural Mountains in equal amounts (46%). The plants from West Siberia/Ural Mountains were assigned to East- and South West Greenland in equal proportions (35%) when assigning to populations while 23% failed to assign. When these plants were assigned to geographic areas, 50% assigned to East Greenland and only 2% assigned to South West Greenland, while 31% failed to assign.

Table 3. *Proportion of assignment of* Empetrum nigrum *s. lat. plants to populations within geographic areas after reallocation of the 46 populations, based on AFLP data using AFLPOP. Diploids and triploids were excluded from the analyses.*

Assigned from \ Assigned to	Svalbard	W Siberia/Ural	E Greenland	Kangerlussuaq	E Canada	E Siberia	W North America	N Norway	S Norway	Jan Mayen	Faroe Islands	SW Greenland	Iceland	Scotland	The Alps	No assignment	No. of plants	No. of populations within areas
Svalbard	**0.63**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.37**	38	4
W Siberia/Ural	-	**0.90**	-	-	-	-	-	0.06	-	-	-	-	-	-	-	0.04	48	5
E Greenland	0.07	-	**0.86**	-	-	-	-	-	-	-	-	-	-	-	-	0.07	28	3
Kangerlussuaq	-	-	-	**1.00**	-	-	-	-	-	-	-	-	-	-	-	-	4	1
E Canada	-	-	-	-	**1.00**	-	-	-	-	-	-	-	-	-	-	-	10	2
E Siberia	-	-	-	-	-	**1.00**	-	-	-	-	-	-	-	-	-	-	8	1
W North America	-	-	-	-	-	0.04	**0.96**	-	-	-	-	-	-	-	-	-	26	4
N Norway	-	0.04	-	-	-	-	-	**0.68**	0.12	-	-	0.04	-	0.02	-	0.11	57	6
S Norway	-	-	-	-	-	-	-	0.04	**0.92**	-	-	-	-	-	-	0.04	53	5
Jan Mayen	-	-	-	-	-	-	-	-	-	**0.91**	-	-	0.09	-	-	-	11	1
Faroe Islands	-	-	-	-	-	-	-	-	-	-	**0.66**	-	-	0.03	-	**0.28**	32	3
SW Greenland	-	-	-	-	-	-	-	0.07	0.07	-	-	**0.66**	-	-	-	**0.20**	41	4
Iceland	-	-	-	-	-	-	-	-	-	0.07	-	0.04	**0.75**	0.04	-	0.11	28	4
Scotland	-	-	-	-	-	-	-	0.05	-	-	0.05	-	0.10	**0.62**	-	0.19	21	2
The Alps	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.80**	**0.20**	5	1

Table 4. *Proportion of assignment of* Empetrum nigrum *s. lat plants to geographic areas after reallocation of 15 geographic areas, based on AFLP data using AFLPOP. Diploids and triploids were excluded from the analyses.*

Assigned from \ Assigned to	Svalbard	W Siberia/Ural	E Greenland	Kangerlussuaq	E Canada	E Siberia	W North America	N Norway	S Norway	Jan Mayen	Faroe Islands	SW Greenland	Iceland	Scotland	The Alps	No assignment	No of. plants
Svalbard	**0.97**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	38
W Siberia/Ural	-	**0.92**	-	-	-	-	-	0.04	0.02	-	-	-	-	-	-	0.02	48
E Greenland	-	-	**0.86**	-	-	-	-	-	-	-	-	0.04	-	-	-	0.11	28
Kangerlussuaq	-	-	-	**1.00**	-	-	-	-	-	-	-	-	-	-	-	-	4
E Canada	-	-	-	-	**1.00**	-	-	-	-	-	-	-	-	-	-	-	10
E Siberia	-	-	-	-	-	**0.75**	-	-	-	-	-	-	-	-	-	**0.25**	8
W North America	-	0.04	-	-	-	-	**0.92**	-	-	-	-	-	-	-	-	0.04	26
N Norway	-	-	-	-	-	-	-	**0.33**	0.16	-	0.02	0.02	-	0.02	-	**0.46**	57
S Norway	-	0.02	-	-	-	-	-	0.11	**0.72**	-	-	-	-	-	-	0.15	53
Jan Mayen	-	-	-	-	-	-	-	-	-	**0.91**	-	-	0.09	-	-	-	11
Faroe Islands	-	-	-	-	-	-	-	-	-	-	**0.50**	-	0.06	-	-	**0.44**	32
SW Greenland	-	-	-	-	-	-	-	-	0.10	-	-	**0.78**	-	-	-	0.12	41
Iceland	-	0.04	-	-	-	-	-	-	-	-	-	0.11	**0.54**	-	-	**0.32**	28
Scotland	-	-	-	-	-	-	-	-	-	-	0.05	-	0.05	**0.52**	-	**0.38**	21
The Alps	-	0.20	-	-	-	-	-	-	-	-	-	-	-	-	**0.60**	0.20	5

Table 5. *Proportion of assignment of* Empetrum nigrum *s. lat plants to populations, and to geographic areas, in allocation of the Svalbard, E Greenland, and W Siberia/Ural plants, based on AFLP data. Diploids and triploids were excluded from the analyses. The plants were not given the opportunity to assign to their area of origin. a) Allocation to populations. b) Allocation to geographic areas.*

a)

Assigned from \ Assigned to	Svalbard	E Greenland	W Siberia/Ural	SW Greenland	N Norway	S Norway	Jan Mayen	Faroe Islands	Kangerlussuaq	E Canada	E Siberia	W North America	Iceland	Scotland	The Alps	No assignment	No. of plants
Svalbard	-	**0.63**	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.37**	38
Greenland	**0.57**	-	**0.29**	-	-	0.07	-	-	-	-	0.04	-	-	-	-	0.04	28
W Siberia/Ural	0.01	**0.35**	-	**0.35**	0.10	-	-	-	-	-	-	-	-	-	0.01	**0.23**	48

b)

Assigned from \ Assigned to	Svalbard	E Greenland	W Siberia/Ural	SW Greenland	N Norway	S Norway	Jan Mayen	Faroe Islands	Kangerlussuaq	E Canada	E Siberia	W North America	Iceland	Scotland	The Alps	No assignment	No. of plants
Svalbard	-	**1.00**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38
E Greenland	**0.46**	-	**0.46**	0.04	-	-	-	-	-	-	-	-	-	-	-	0.04	28
W Siberia/Ural	-	**0.50**	-	0.02	0.06	0.02	-	-	-	-	-	0.08	-	-	-	**0.31**	48

Diversity

In two of the Svalbard populations (Dyrevika and Hotelneset), four plants in each population had identical AFLP phenotypes. The plants were sampled within a small area in both of these populations, and possibly belonged to single clones. In the diversity analyses, these possible clones were treated as individuals (clone-corrected). The diploids and triploids were excluded from the analyses. The genetic diversity in each population varied from 0.02 in Røldal, South Norway, to 0.24 in North Ural (Table 1; Fig. 13). The diversity in the Svalbard populations varied from 0.05 to 0.07.

The average genetic diversity for the populations within each geographic area is summarised in Fig. 14. The genetic diversity was highest in South West Greenland, East Siberia, and West Siberia/Ural Mountains. The genetic diversity was higher in West Siberia/Ural Mountains than in East Greenland, and higher in East Greenland than in Svalbard.

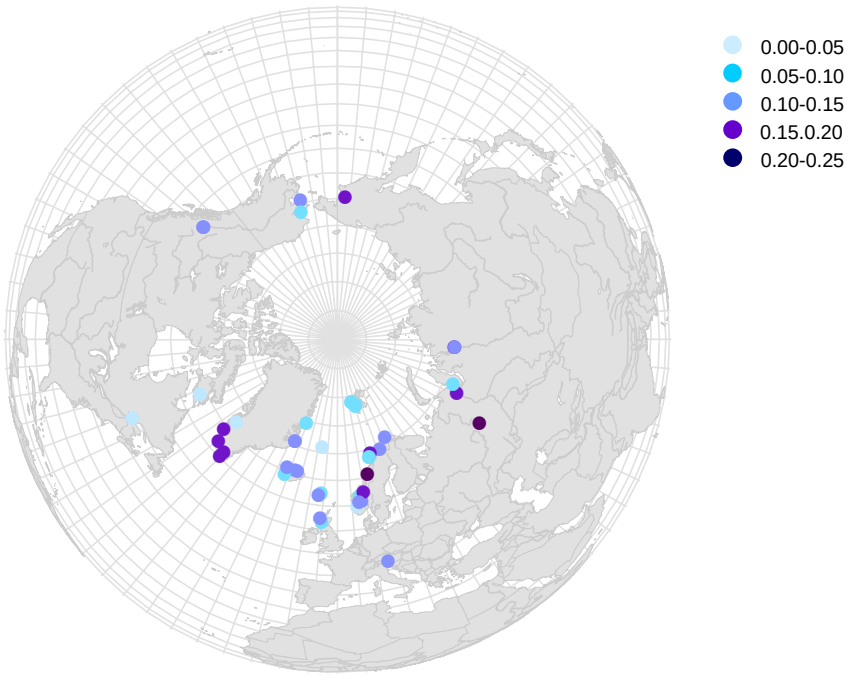


Fig. 13. *Genetic diversity within the 46 population of* Empetrum nigrum *s. lat., based on average number of pairwise differences. The diploid and tetraploid plants were excluded.*

Analyses of molecular variance (AMOVA)

An AMOVA analysis of the whole AFLP dataset (clone-corrected) excluding the diploids and triploids, resulted in 55.7% of the variation among the populations. Separate analyses were also performed for each geographic region (Fig. 14). The among-population variation varied from 19% in South West Greenland to 89% in East Canada. Svalbard had an among-population variation of 25.5%.

AMOVA analyses were also performed on a subset of the tetraploid data set, excluding the plants from West North America and East Siberia. In a hierarchical AMOVA, 30.6 % of the variation was explained between the northern- and the southern group indicated by the Structure and PCO analyses, and 32.3 % among the populations within the groups. Separate AMOVA analyses were performed for the two main groups. For the northern group, 57.5 % of the variation was explained among the populations, while for the southern group 41.8 % was explained among the populations

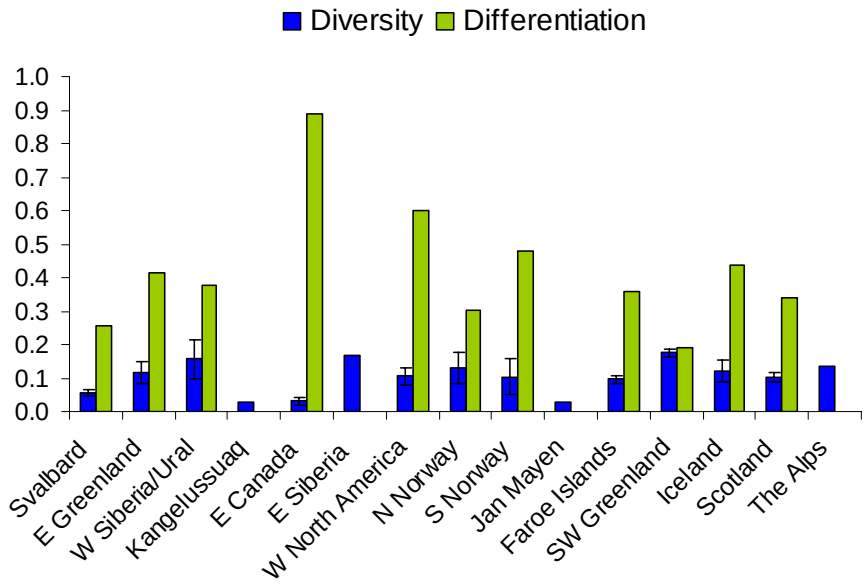


Fig. 14. *Interpopulation variation of* Empetrum nigrum *s. lat. for each geographic area based on AMOVA analyses, and average genetic diversity for the populations within the same areas, based on average number of pairwise differences. The diploid and tetraploid plants were excluded.*

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Discussion

Immigration of Empetrum nigrum *to Svalbard*

The AFLP data strongly suggest that the Svalbard populations of *Empetrum nigrum* originated from direct immigration from East Greenland. This result is consistent with the ploidal measurements, as only tetraploid *E. nigrum* was found in Svalbard as well as in the whole area of the northern group. The European diploids separated clearly from the tetraploids in the PCO analysis (cf. Fig. 11) and therefore seem to have a different history than the tetraploids, thus the exclusion of the diploids and triploids in many of the analyses is justified.

All methods are consistent in demonstrating that the Svalbard populations of *E. nigrum* originated from direct immigration from East Greenland, which again was colonised from West Siberian/Ural Mountains source populations. This hypothesised migration route is especially well demonstrated by the allocation analyses to geographic areas, where all Svalbard plants allocated to East Greenland, and the East Greenlandic plants allocated to Svalbard and West Siberia/Ural Mountains in equal amounts (cf. Table 5b). The analysis of ploidal levels is also consistent with this pattern, as there were only found tetraploids in these three areas. The direction of migration is further supported by the patterns of the genetic diversity. The leading edge model postulates that the diversity will decrease from the source population along the migration route due to founder effects (Hewitt 1996). In accordance with the leading edge model, the genetic diversity decreased along the migration route hypothesised above for *E. nigrum*. The genetic diversity was highest in West Siberia/Ural Mountains, intermediate in East Greenland, and lowest in Svalbard (cf. Fig. 14). The hypothesised direction of migration of *E. nigrum* from East Greenland to Svalbard is also consistent with the suggested time of colonisation of these areas. Thermophilous plants present in Svalbard today, like *E. nigrum*, probably arrived during the Hypsithermal, approximately 9500-4000 years BP (Birks 1991). Macrofossil findings have shown that *E. nigrum* has been present in East Greenland since 10 400 years BP, and it was one of the first woody plants to arrive (Bennike 1999; Bennike *et al.* 1999). Thus, *E. nigrum* colonised East Greenland prior to the suggested time of immigration of thermophilous plants to Svalbard.

There are few previous studies that suggest Greenland as main source area for immigration to Svalbard. Philipp and Siegismund (2003) suggested immigration from Greenland to Svalbard for *Dryas octopetala*. However, their results have later been refuted by Skrede *et al.* (in press), who showed that *D. octopetala* mainly colonised Svalbard from western Russia. Direct migration from Russia to Svalbard, or from northern Scandinavia to Svalbard, have been demonstrated for several plant species, i.e. *Saxifraga oppositifolia* L., *Saxifraga cespitosa* L., *Dryas octopetala*, and *Vaccinium uliginosum* L. (Gabrielsen *et al.* 1997; Tollefsrud *et al.* 1998; Skrede 2004; Eidesen, unpublished data). Nevertheless, the results from our main project indicate that East Greenland has been a main source area for immigration to Svalbard also for other species, e.g. for *Cassiope tetragona* (L.) D.Don (Eidesen unpublished data).

Empetrum nigrum is adapted to animal dispersal, and it is safe to assume that long-distance dispersal of the species has occurred mainly with birds. Little is known about past bird migration routes, but there are today no frequently used migration routes for seed-eating birds between Russia and Greenland, or between Greenland and Svalbard (Johansen and Hytteborn 2001, ref. therein). The only bird that is likely to disperse *E. nigrum* in these areas today is the snow bunting (*Plectrophenax nivalis* L.). The breeding range of the snow bunting is circumpolar, and the birds breeding in North East Greenland have their wintering areas on the Russian steppes (Alerstam 1990). However, long-distance dispersal is often just a matter of chance, and even if *E. nigrum* is adapted to bird-dispersal, other dispersal vectors might have been involved. It is suggested that drift ice and driftwood are important vectors for long-distance chance dispersal (Johansen and Hytteborn 2001). *Draba sibirica* (Pall.) Thell. and *Potentilla stipularis* L. are considered to be likely examples of direct long-distance dispersal with drift ice or driftwood from Siberia to Greenland (Johansen and Hytteborn 2001, ref. there in). After Younger Dryas, but before the onset of the Hypsithermal (approx. 10,500-9000 years BP), several geophysical conditions for successful transport of diaspores from Siberia to areas in the North Atlantic by drift ice or driftwood were present (Johansen and Hytteborn 2001, ref. therein). This “time window” corresponds to the earliest fossil findings of *E. nigrum* in East Greenland (Bennike 1999). Thus, whether birds have been migrating between these areas in the past or not, other plausible dispersal vectors for *E. nigrum* between these areas were present, such as wind, driftwood or drift ice.

Our sampling did not, however, cover the entire distribution area of *E. nigrum* in Svalbard, so possible additional source areas might have been overlooked.

The main genetic structure of Empetrum nigrum *in the North Atlantic area*

In the North Atlantic area, tetraploid *Empetrum nigrum* is divided into two main groups; one northern group including the plants from West Siberia/Ural Mountains, Svalbard, East Greenland, parts of West Greenland (Kangerlussuaq), and East Canada, and one southern group including the plants from Europe and South West Greenland (south of Kangerlussuaq). This was suggested by Structure analysis (cf. Fig. 7), and indicated in the PCO analysis (cf. Fig 12). The Structure groups were supported by the reallocation analyses of the two groups, where all plants allocated to the group of origin except for three plants that did not allocate. The Structure groups were also supported in the hierarchical AMOVA analysis. A similar pattern have also been observed in another plant species *Cerastium arcticum* Lange s. lat. (Hagen *et al.* 2001). These two groups of tetraploid *E. nigrum* probably reflect different genetic lineages with separate histories of survival in different glacial refugia during the last and/or previous glaciations.

The tundra south of the Scandinavian ice sheet could have served as a large main refugium for the southern group of tetraploids, as well as for the diploids. Based on fossil evidence and other genetic studies this area is known as refugium for other plant species during the Weichselian (Brochmann *et al.* 2003, ref. therein). When the ice retreated, *E. nigrum* probably migrated northwards to the British Isles, Iceland, Faroe Islands, Jan Mayen, westwards to the South West coast of Greenland and on the continental landmasses, all the way up to the northernmost part of Norway. Today, there are several bird migration routes between continental Europe, Iceland and southern parts of Greenland (Bakken *et al.* 2003). The southern group also seems to have migrated towards the northeast, to the Ural Mountains. In the PCO analysis, four of the individuals from the Ural Mountains were placed among the plants from the southern group. The same individuals were placed in the southern group in the Structure analysis as well.

Large areas in Taymyr and Siberia were ice-free during the last glaciation (Svendsen *et al.* 2004). Thus, refugia for the northern group of tetraploid *E. nigrum* could be located east of the Scandinavian ice sheet. This has previously been suggested for both *Saxifraga cernua* and *D. octopetala* (Bronken 2001; Skrede 2004). An eastern refugium followed by a westwards migration seems plausible for *E. nigrum* on the basis of the allocation analysis as discussed above concerning the immigration to Svalbard.

The two East Canadian populations were very divergent (cf. Fig. 14). Both populations were also analysed by Mirré (2004). She suggested that they belonged to different lineages. In her study, the Forrestville population belonged to a northern amphi-Atlantic group, which is in accordance with the results from this study. In contrast, Mirré ascribed the Baffin Island population to a genetic group which spanned from East Canada, via Beringia, to West Siberia. This lineage was not detected in this current study. However, this is probably a result of insufficient sampling outside the North Atlantic area. The strong differentiation between the two Canadian populations is most likely explained by different colonisation history. The Forrestville area was probably colonised from the suggested eastern refugium via East Greenland, while the Baffin Island population belongs to the lineage described by Mirré. A similar widespread genetic lineage has been described for another bird-dispersed species *Vaccinium uliginosum* (Alsos *et al.* 2005). The close relationship of the Baffin Island population to the northern group can probably be explained by a common history before the last glaciation. In the study of *Cerastium arcticum*, which showed a similar north-south split as *E. nigrum*, a refugium in East Canada was suggested for the northern group during the last glaciation (Hagen *et al.* 2001). In *E. nigrum*, East Canada might have served as a refugium for the lineage to which the Baffin Island population belongs.

There was more genetic structure within the northern group than within the southern group. The AMOVA analyses showed lower differentiation among the southern (41.8%) than among the northern populations (57.5%). This pattern was also indicated by the PCO analysis and the reallocation tests, where more individuals allocated to the region of origin in the northern group than in the southern group. The northern group probably comprises several genetic lineages, as previously discussed, which may cause a higher level of structure. Still the PCO analyses and the allocation to population indicate that there is more structure within the northern group than can be ascribed to presence of several lineages. Thus, the data indicate higher gene flow within the southern group. *Empetrum nigrum* seldom set ripe fruits in Svalbard (Elvebakk and Spjelkavik 1995), and the level of viable seed set in the whole arctic region is probably low. Higher level of viable seed set in the boreal region than in the arctic region has previously been demonstrated for thermophilous plants like *Betula nana*, *Vaccinium uliginosum*, and *Campanula rotundifolia* L. (Alsos 2003). This might be an explanation for the higher gene flow within the southern group. Another explanatory factor might be that the northern group inhabits a larger area than the southern group. Diploids and triploids were only found in the area of the southern group of tetraploid *Empetrum*. Thus, the lack of structure within this group, as well as the higher genetic diversity within some

populations with mixed ploidal levels (cf. Table 1), could be due to gene flow between the different ploidal levels.

Comments on the Beringian populations

The West North American and the East Siberian plants appear to be most closely related to the northern Atlantic group, and form a weak group (from now called the Beringian group). This was indicated by the structure analysis of the whole data set, and to some degree reflected in the PCO analysis. The variation in *Empetrum* in the entire northern hemisphere was more fully treated by Mirré (2004). Her study comprised a more extensive sampling in West North America and East Siberia. She suggested that the plants from these areas were separated into three different lineages. The present study comprises too few populations to recognise different lineages within the area.

Beringia was ice-free during the last glaciation (Frenzel *et al.* 1992). Thus, the Beringian group probably survived in that particular area. It is difficult to make interpretations of migration from a Beringian refugium, due to the lack of samples from large areas in North America and Siberia. However, the Structure and PCO analyses in the present thesis indicated an eastwards migration from a Beringian refugium to South West Greenland. Many of the South West Greenlandic plants were placed close to the West North American plants in the PCO analysis (cf. Fig. 10). In the Structure analysis of the whole data set, some plants from South West Greenland showed high affinity for belonging to the third unstable group, mainly consisting of the West North American plants. Survival in a Beringian refugium, followed by expansion both westwards and eastwards after the glaciation has previously been postulated for other circumpolar species, e.g., *Saxifraga cernua* (Bronken 2001) and *S. oppositifolia* (Abbott *et al.* 2000).

High genetic diversity: refugia or suture zones?

Three regions with particularly high genetic diversity were revealed in tetraploid *Empetrum nigrum*; East Siberia, West Siberia/Ural Mountains, and South West Greenland (cf. Fig. 13; Table 1). Several factors influence the level of genetic diversity in populations. The most important factors to cause high genetic diversity are perhaps the migration history of the

population, i.e. whether the population had a long *in situ* evolutionary history, and/or whether the population represents a mixture from various migration routes, so-called suture zones (Petit *et al.* 2003).

As previously discussed, areas east of the Scandinavian ice sheet were possible refugial areas for *E. nigrum*. Thus, the high level of genetic diversity within West Siberia and the Ural Mountains can be attributed to long evolutionary history. In addition, some of the diversity in the Ural Mountains can probably be ascribed to a suture zone between the southern and the northern group in this area (cf. migration of the southern group discussed above). Whether the presence of the southern group in the Ural Mountains results from occasional long-distance dispersal, or whether the area is a major suture zone cannot be addressed without more samples from the area between Central Europe and the Ural Mountains. However, a similar pattern has been observed for *Vaccinium uliginosum*, and for this species, the Ural Mountains clearly represent a suture zone (Alsos and Eidesen, unpublished).

In South West Greenland large ice-free uplands probably existed around 67°N and 72°N throughout the last glaciation (Funder and Hansen 1996). The conditions in this area were, however, so harsh that only hardy plants could have survived there, if any (Brochmann *et al.* 2003). Thus, the high genetic diversity in South West Greenland is probably not due to a long evolutionary history. As previously stated, the South West Greenland populations are probably descendents from immigrants from Europe. Nevertheless, colonisation from the west cannot be excluded as the plants from South West Greenland belonged to both the southern, and the Beringian group according to the Structure analysis. In the Structure analysis only including the North Atlantic tetraploids, some plants from South West Greenland also assigned to the northern group, and a population belonging to the northern group (Kangerlussuaq) was found on West Greenland. A reasonable explanation for the high genetic diversity in South West Greenland seems to be that the area is a suture zone for the southern- and the northern North Atlantic group and one or several Beringian lineages. All three Beringian lineages identified by Mirré were present in the Eastern Canada. Thus, migration from the west seems plausible.

Conclusions and future prospects

Empetrum nigrum is one of the most thermophilous plants occurring on Svalbard today, and must have arrived postglacially by long-distance dispersal. This study strongly suggests that *E. nigrum* immigrated directly from East Greenland, which again was colonised from source populations in West Siberia and the Ural Mountains. In the North Atlantic area tetraploid *E. nigrum* was divided into a northern and a southern group, where Svalbard belonged to the northern group. A main refugium east of the Scandinavian ice sheet is suggested for the northern group, while a main refugium south of the Scandinavian ice sheet is suggested for the southern group. The western North American and the East Siberian plants appear to be most closely related to the northern Atlantic group. However, the present study comprises too few populations to draw conclusions about the western North American and East Siberian areas.

There was less genetic structure within the southern than the northern group. The lack of structure within the southern group might be explained by higher level of viable seed set in the boreal region or by gene flow between the different ploidal levels.

Two suture zones with high levels of genetic diversity were identified; the Ural Mountains and South West Greenland. The southern and northern group met in the Ural Mountains, while South West Greenland is probably influenced by the southern- and the northern North Atlantic group and one or several Beringian lineages.

This study involves an extensive sampling in the North Atlantic area, while as the study by Mirré comprised more samples from Beringia. Combining these data sets and including more material from Canada, Siberia and North East Europe could bring us closer to the true phylogeograpy of *E. nigrum* s. lat in the northern hemisphere.

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Appendices

Appendix 1. *Data matrix of 78 AFLP markers and 435* Empetrum nigrum *s. lat. individuals (on CD).*

Appendix 2. *Flow cytometry results for 111* Empetrum nigrum *s. lat. plants.*

Pop. ref.	Pop. name	DNA ratio relative to internal standard Zea mays	Estimated ploidal level	plants not included in the AFLP analyses
AK-110-1	Jan Mayen	1.47	4x	
AK-110-5	Jan Mayen	*	4x	
AK-1154-5	Fugløy	1.41	4x	
AK-1154-6	Fugløy	1.44	4x	
AK-1175-2	Torshavn	1.40	4x	
AK-122-4	Zackenberg	1.47	4x	
AK-122-12	Zackenberg	*	4x	x
AK-1282-1	Dudinka 1	1.45	4x	x
AK-1282-2	Dudinka 1	1.47	4x	x
AK-1282-7	Dudinka 1	1.41	4x	
AK-1313-2	Glencoe	1.37	4x	
AK-1313-10	Glencoe	1.44	4x	
AK-1321-2	Torridon	1.50	4x	
AK-1321-3	Torridon	1.47	4x	
AK-1321-6	Torridon	2.84	2x	
AK-1321-7	Torridon	1.47	4x	
AK-145-10	North Ural	1.40	4x	
AK-145-2	North Ural	1.41	4x	
AK-146-12	Polar Ural	*	4x	x
AK-146-1	Polar Ural	1.44	4x	
AK-147-12	South Yamal	*	4x	x
AK-147-6	South Yamal	1.41	4x	
AK-203-12	Kangerlussuaq	*	4x	x
AK-203-6	Kangerlussuaq	1.45	4x	x
AK-245-13	Paamiut	*	4x	x
AK-245-2	Paamiut	1.43	4x	
AK-245-5	Paamiut	1.49	4x	
AK-252-12	Nuuk	*	4x	x
AK-252-13	Nuuk	*	4x	x
AK-252-14	Nuuk	*	4x	x
AK-252-2	Nuuk	1.47	4x	
AK-258-6	Narsarsuaq	*	4x	
AK-287-12	Nanortalik	*	4x	x
AK-350-1	Primula River	*	4x	
AK-364-12	Hare River	*	4x	x
AK-378-4	Dudinka 2	1.37	4x	
AK-378-5	Dudinka 2	1.38	4x	

Appendix 2. *Continued.*

Pop. ref.	Pop. name	DNA ratio relative to internal standard Zea mays	Estimated ploidal level	plants not included in the AFLP analyses
AK-430-12	Ål	*	4x	x
AK-430-13	Ål	*	4x	x
AK-430-14	Ål	*	4x	x
AK435-12	Lom	*	4x	x
AK435-13	Lom	*	4x	x
AK466-12	Oppdal	*	4x	x
AK466-13	Oppdal	*	4x	x
AK-481-2	Brønnøy	*	4x	
AK-481-6	Brønnøy	2.87	2x	
AK-498-13	Røldal	*	4x	x
AK-513-12	Finse	*	4x	x
AK-567-11	Colesdalen	1.37	4x	
AK-567-3	Colesdalen	1.46	4x	
AK-584-1	Cape Nome	*	4x	
AK-584-10	Cape Nome	1.37	4x	
AK-600-1	Chukotka	2.81	2x	
AK-600-5	Chukotka	2.86	2x	
AK-600-9	Chukotka	1.90	3x	
AK-705-3	Nordkapp	1.47	4x	
AK-705-8	Nordkapp	1.46	4x	
AK-733-11	Ossian Sars	1.42	4x	
AK-733-7	Ossian Sars	1.40	4x	
AK-747-10	Andøy	2.92	2x	
AK-747-11	Andøy	2.89	2x	
AK-747-2	Andøy	1.42	4x	
AK-747-4	Andøy	2.92	2x	
AK-747-5	Andøy	2.90	2x	
AK-747-6	Andøy	2.88	2x	
AK-747-8	Andøy	1.45	4x	
AK-752-2	Tromsø	1.42	4x	
AK-752-5	Tromsø	1.37	4x	
AK-762-3	Hadsel	1.44	4x	
AK-762-9	Hadsel	1.46	4x	
AK-815-1	Akureyri	1.91	3x	
AK-815-12	Akureyri	*	3x	x
AK-815-13	Akureyri	*	2x	x
AK-815-2	Akureyri	2.81	2x	

Appendix 2. *Continued.*

Pop. ref.	Pop. name	DNA ratio relative to internal standard Zea mays	Estimated ploidal level	plants not included in the AFLP analyses
AK-815-4	Akureyri	1.41	4x	
AK-815-6	Akureyri	1.48	4x	
AK-815-7	Akureyri	2.75	2x	
AK-815-8	Akureyri	2.79	2x	x
AK-826-1	Eystorøy	1.43	4x	
AK-838-1	Hvalfjordur	2.85	2x	
AK-838-12	Hvalfjordur	*	2x	x
AK-838-13	Hvalfjordur	*	3x	x
AK-838-4	Hvalfjordur	1.43	4x	
AK-838-6	Hvalfjordur	2.84	2x	
AK-838-7	Hvalfjordur	2.90	2x	
AK-845-1	Drangsnes	*	4x	
AK-845-11	Drangsnes	1.47	4x	
AK-845-5	Drangsnes	2.85	2x	
AK-845-6	Drangsnes	1.45	4x	
AK-876-1	Myvatn	*	2x	
AK-876-11	Myvatn	2.92	2x	
AK-876-2	Myvatn	2.91	2x	
AK-876-3	Myvatn	2.86	2x	x
AK-876-4	Myvatn	2.88	2x	
AK-876-6	Myvatn	2.95	2x	
AK-876-7	Myvatn	2.70	2x	
AK-876-8	Myvatn	2.93	2x	
AK-876-9	Myvatn	1.37	4x	
S02-724-3	Austria	1.47	4x	
S02-724-4	Austria	1.46	4x	
S03-163-2	Gnat Pass	1.45	4x	
S03-163-5	Gnat Pass	1.40	4x	
S03-32-1	Cape Espenberg	1.39	4x	
S03-32-2	Cape Espenberg	1.49	4x	
S03-32-4	Cape Espenberg	1.40	4x	
S03-32-5	Cape Espenberg	1.45	4x	
S03-34-1	Forestville	1.46	4x	
S03-34-5	Forestville	1.47	4x	
S03-371-3	Dease Lake	1.38	4x	
S03-371-5	Dease Lake	1.44	4x	
S03-25	Baffin Island	1.41	4x	

* DNA ratio not available.

Agent *Empetrum* melder til hovedkvarteret:
The Mystery of Krekling er løst.
Slipper snart ut fra hagens gjerder, og er klar for nye oppdrag.