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High genetic variation in the boreal forest moss *Hylocomium splendens* through its distribution range

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Abstract

Many mosses produce diaspores which are suitable for long-distance dispersal by wind and long-time storage in diaspore banks. Spores are commonly found high in the atmosphere and far from their origin, supporting the hypothesis of frequent long-distance dispersal. *Hylocomium splendens* is a haploid unisexual moss that is widely distributed and common in most of the northern hemisphere. It is found in widely different environmental conditions, from temperate and boreal forest to arctic and alpine heath. The studied populations of *H. splendens* showed no sign of isolation by distance across great distances, but high genetic variation (mean H_E = 0.701) compared to other bryophytes. This can be explained by high gene flow through efficient spore dispersal by wind over the whole Northern Hemisphere.

Introduction

The changes in ice cover following the glacial cycles in the Northern Hemisphere have constantly changed the environment. When the habitat has changed, the organisms have either moved to more suitable habitats, gone locally extinct or adapted to the new habitat. Stress tolerant organisms might have survived the cold periods in northern refugia within the glaciated areas of the Northern Hemisphere (Dahl 1955; Dahl 1987) and bryophytes are stress tolerant and common in boreal and arctic areas. But Abbott and Brochmann (2003) argue that molecular evidence fails to support the hypothesis of Arctic vascular plant species surviving in glaciated areas. They suggest that the North Atlantic has not been a great barrier to dispersal in vascular plants because of genetically very similar populations east and west of the Atlantic Ocean. However, recent studies have found refugia both within and East and South of the glaciated areas in a number of different organisms (Ehrich et al. 2008; Westergaard et al. 2011; Sonsthagen et al. 2011).

The bryophytes are a paraphyletic group consisting of the hornworts (Anthocerophyta), liverworts (Marchatiophyta) and mosses (Bryophyta) and are basal in the evolution of land plants (Qiu et al. 2006). Bryophytes can disperse by means of gametes (spermatozoids) or diaspores. The male spermatozoids depend on water for movement and usually disperse a few centimetres (Wyatt 1994). The dispersal capacity of diaspores varies with size (Söderström 1994), and small spores have the potential of long-distance dispersal (Van Zanten & Pocs 1978). Spores are produced by sexual reproduction and are the haploid products of meiosis in the diploid sporophytes. Sporophytes are not produced every year in all bryophytes and the number of spores produced by a sporophyte varies between species,

individuals and years (Miles & Longton 1990). Long-distance dispersal of bryophytes depends on successful production, transport and establishment of haploid spores. The combined probability of success in transport and establishment in bryophytes is perceived to be low (Crum 1972). So a successful spore producing year would be necessary to produce enough spores to ensure successful establishment and reproduction following long-distance dispersal (Rydgren & Økland 2002). But even though the probability of one spore resulting in a gametophyte is low, a clonal organism can produce many gametophytes and each gametophyte can produce spore capsules containing many hundred thousand spores (Hassel & Söderström 1999). Taking this into account the lifetime reproduction success of a female clone can be high.

Wind is believed to be the most important dispersal vector of bryophytes (Goffinet & Shaw 2009) and transports spores far away from the gametophyte (eg. Stenøien et al. 2011). Bryophyte spores are commonly found in the atmosphere (Gregory 1973; Despres 2007). Sundberg et al. (2006) found that species frequency of 19 *Sphagnum* species on land uplift islands in the Baltic Sea mainly depended on the spore output of the mainland population and of habitat preference. This indicates that mainland populations are important sources for colonization in *Sphagnum*. Söderström (1994) reviewed the scope and significance of studies on reproductive biology in bryophytes, and found that most experimental studies on spore transport are of ranges less than 15 m. The studies indicate that a fraction of the spores (varying from 10% to 80%) are transported further than 15 m from the gametophyte, a so called "fat tail" of an inverse exponential probability distribution. The spores of the fat tail are the ones that possibly contribute to long-distance dispersal.

The physical condition of the spores during and after wind mediated transport is important for successful long-distance dispersal. Van Zanten (1978) tested the effect of desiccation on spores for four different time periods, and found a general trend of germination capacity to drop gradually with increasing length of desiccation. There was also a strong positive correlation between the spores' germination rates and the geographical distribution areas of the species involved. Miles and Longton (1990) did experimental plantings using four common bryophytes and found spore germination leading to shoot production to vary consistently between species with different life-history strategies. Sundberg and Rydin (2000) buried spore capsules of four *Sphagnum* species at different depths in a peat bog. They found no correlation between spore size and longevity across all species, but within species small spores seemed to have longer longevity than larger spores. In *Sphagnum*, small spores seem

to be better suited for the conditions encountered during long distance dispersal than large spores (Sundberg & Rydin 2000).

It is not clear what physical conditions are important for spore establishment in *H. splendens*, but studies have found some factors that were important for spore establishment in other bryophytes. Lloret (1994) found no significant effect of physical conditions (temperature, annual rainfall, aspect, slope) on establishment, but Löbel et al. (2006) found that small spores can be more dependent on the environmental conditions in the establishment phase in epiphytes. Germination from diaspore banks is important in some bryophytes (Jonsson 1993; Hassel 2000) and *H. splendens* has been found to germinate in previously unoccupied substrates, which might indicate that diaspore banks are important in disturbed sites. Most studies follow the plants for less than five years. When considering the possibly very long generation times of many perennial bryophytes, five years might be too short to understand the importance of establishment of spores in bryophytes.

Genetic methods have not previously been used to study long-distance dispersal mechanisms in *H. splendens*, but Cronberg et al. (1997) and Cronberg (2002) have studied the dispersal of *H. splendens* in Scandinavia using izosyme markers, and found relatively high genetic variation and little genetic structure. Microsatellites are suitable for inferring evolutionary processes happening over short timescales, and may therefore be ideal for within-species population genetic studies (Powell et al. 1996; Jarne & Lagoda 1996). It is of interest to know the dispersal capacity of bryophytes to understand their distribution ranges. In this study I use neutral molecular markers to infer the dispersal capacity of *H. splendens* (Hedw.) B. S. G., sampling most of its range, comprising the whole Northern Hemisphere. Økland (1995) states that *H. splendens* is a highly successful bryophyte through its range and that this might be because of rapid growth in optimal habitats, by size build-up and survival or by rapid colonization of open patches. A thorough sample of the species' range should make it possible to disentangle the species' recent history. The main aim is to understand the processes shaping the Northern Hemisphere distribution and the importance of dispersal in *H. splendens*; and more specifically: 1) Is there high genetic variation in this species also on the continental scale? 2) Is there isolation by distance between populations? and 3) How are genetic clusters distributed across the species range? The clustering of populations might also give an insight to the taxonomical status of the subspecies *H. splendens* var. *alaskanum* (Lesq. & James) and *H. splendens* ssp. *giganteum* Vitt.

Materials and methods

Hylocomium splendens is a widely distributed, haploid, unisexual moss. It is medium to large in size, perennial, clonal, pleurocarpous and has modular growth due to annual periodicity in the emergence of new modules. It produces spores with a median size of 14.1 µm and a range of 12.8 µm-17.5 µm (Boros et al. 1993). It has a circum arctic and circum boreal distribution on the Northern Hemisphere (Fig. 1). It is also known from the Azores, Canaries and further south it is known from alpine areas in North Africa and Asia (Nyholm 1981), but is only known from New Zealand and Mt. Kilimanjaro in the Southern Hemisphere (Smith 1978). *H. splendens* shows a remarkable capacity to grow under widely different environmental conditions, from temperate and boreal forest to arctic and alpine heath and has high morphological variation. *H. splendens* var. *alaskanum* is found in arctic tundra and is more compact than the nominate form. It lacks the arrangement of annual segments and broadly pointed leaves (Crum & Anderson 1981; Persson & Viereck 1983). *H. splendens* ssp. g*iganteum* is a huge form from the Pacific Northwest which has stem leaves that end with a long, nearly hair-line acumen (Persson & Viereck 1983).

168 samples were included in the study (Fig. 1, Table S1) and most of the samples used were taken from herbarium specimens (Table S1). Microsatellites enabled us to use old and stored material collected by others in the study. Most samples are collected after 1990, the oldest sample is from 1943 and the youngest from 2010. Tissue samples of material originating from herbarium LE and MHA, together with vouchers from Steinnes' and Cronberg's private herbaria are kept at the herbarium (TRH) in Trondheim.

The samples were chosen to reflect the known distribution range of *H. splendens* (Fig. 1). The sampling is denser in Europe, with Norway as the most densely sampled location. We made an effort to sample Asia as evenly as possible. Sampling density is higher along the coast of North America than in the central regions of North America. One individual was sampled from each location. We did not succeed in acquiring samples from New Zealand and have only one sample from China. The samples obtained from Tanzania (Table S1) had too high levels of missing data to be included in the study.

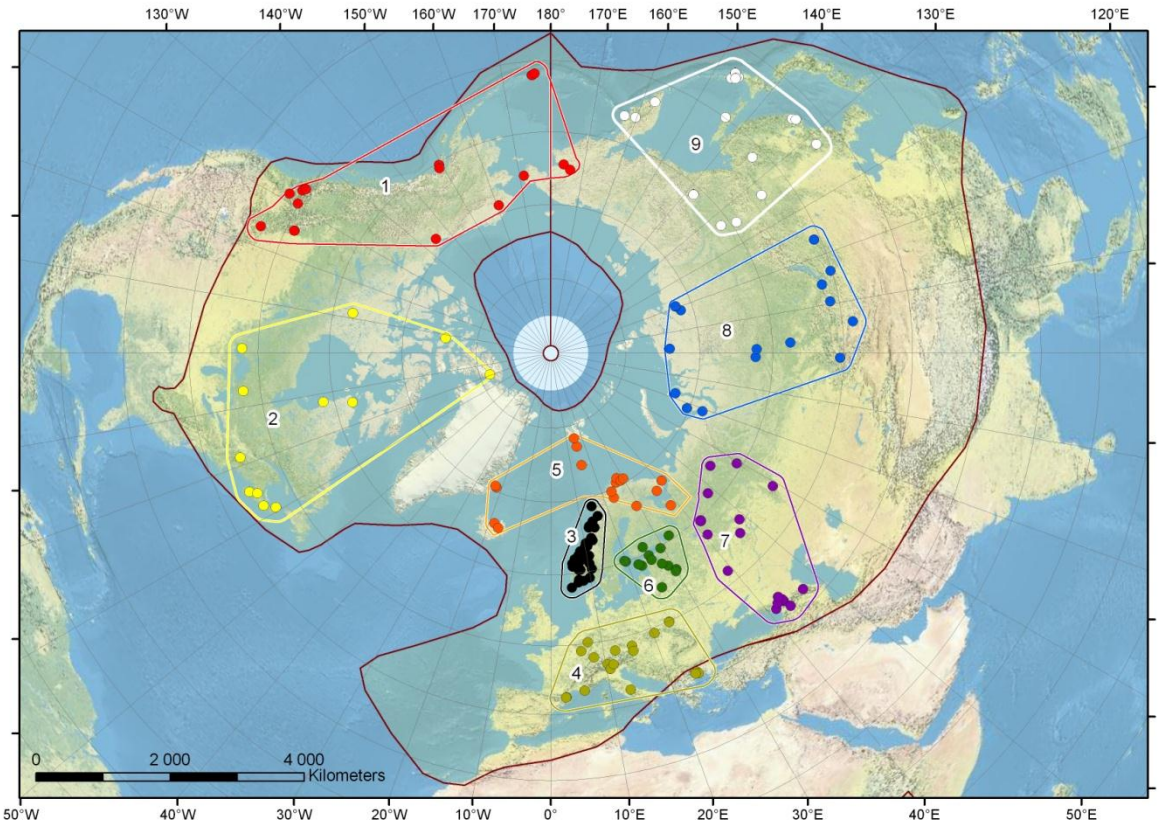

Fig. 1: Total distribution range of *H. splendens* (after Schofield (1985)) in the Northern Hemisphere indicated by a red line and all samples used in the present study on the global phylogeography of the species are indicated by dots. The colours of the dots indicate the nine populations used for the population genetic analyses, assigned as this: West North America (1), East North America (2), West North Europe (3), South Europe (4), Arctic Europe (5), Baltic (6), East Europe (7), North West Asia (8), East Asia (9).

All samples were fragmented into small pieces to enable crushing in a Tissue Lyser 2 (QIAGEN). The apex of one shoot (approximately 10 µg) was used in DNA extraction with the EZNA plant mini kit according to the manufacturers' protocol. The quantity of the DNA was controlled with agarose gel electrophoresis and the gel electrophoresis revealed very strong bands.

Initially 14 microsatellite markers (Korpelainen et al. 2007; Vik et al. in press) were tested and grouped into four multiplex primer mixes (QIAGEN) (Table 1). Microsatellites were amplified using the Multiplex PCR Handbook, but with less DNA per run (5 µL Multiplex mix, 1 µL primermix, 1 µL DNA, 3 µL H_2O) than suggested in the manual. Two protocols differing in annealing temperature were used due to lower annealing temperature of one marker. The first protocol was run with 95°C for 15 min, 94°C for 30 sec, (56°C for 45 sec, 72°C for 45 sec) which was repeated 30 times, and then then 60°C for 30sec. The other protocol was run with 95°C for 15 min, (54°C for 45 sec, 72°C for 45 sec) which was

repeated 30 times, ending with 60°C for 30 min. Three samples (IE8, IE19, IE29) were diluted 1:10 because of very strong agarose gel electrophoresis bands. Microsatellite analysis was done by the lab at the Department of Biology, Norwegian University of Science and Technology (NTNU) with 1 µL PCR product prepared with 8.85 µL Hi-Di ™ Formamide (Applied Biosystems, Foster City, CA) and 0.15 µL GSLizz500 in optical plates on an ABI 3730 sequencer. PCR was done twice for more than 10% of the samples to test reproducibility. The preparations for the microsatellite analysis were done at the molecular lab at the Museum of Natural History and Archaeology (NTNU).

The results were sized and genotyped in GENEMAPPER® (Applied Biosystems). All ladders were adjusted manually and peaks were evaluated based on smoothness, how pointy they were, if they had a smaller peak before the main one and if the height of the peak was distinctly larger than the height of the noise. A conservative approach was used to evaluate bin patterns of the markers. Bin sets were assigned to all markers and all peaks were either assigned a bin or rejected. All identical runs that gave different results were deleted to ensure high reproducibility. Because of a relatively high number of alleles per marker, two measures were followed to decrease genetic variation to avoid unnecessary noise in the data set; 1) very uncommon alleles were deleted if an allele 1 bp longer or shorter was more than four times as common 2) alleles were treated as one if they were separated by 1 bp. This means that in a string of alleles separated by 1 bp, pairs of alleles would be renamed and if there were three alleles at the end of the string, all three would be treated as one allele. For further analysis loci were excluded if they had low reproducibility (<90%) or high levels of missing data (>5%) (Table 2). Samples were excluded when containing more than 40% missing data (41 samples).

Table 1: A list of the markers developed for *H. splendens* (Korpelainen et al. 2007; Vik et al. 2012). Primer ID, marker sequence forward (F) and reverse (R) in 5'-3', repeat type, size in base pairs, annealing (T), primer mix (mix), number of alleles (No. of alleles) per marker after joining adjacent alleles.

Primer ID	Primer sequence, F Primer sequence, R	Repeat type	Size	T (C°)	Mix	Developed by	No. of alleles
HySp01	GCTCCAACACCCGCGTGTATA TAGCCTTGGTATGACCGCATCA	(CA)6	60-80	56	3	Korpelainen	1
HySp03	GACGAGAGTGAGACAGCGGAA CCTTAACTAGCTCCCCTTTCTTTCC	(GGCT)4GG(GGCTT)5	80-120	52	4	Korpelainen	9
HySp04	GGCCTGCTCCCCCTTTTTTAAG CTGACAAATCTGTTGAGCAGCA	(CT)7	80-110	56	3	Korpelainen	9
HySp06	CCACACTGTACAGTACACTACATTG CCAGGACAACAGAGACAGACTC	(GA)21	140-200	56	3	Korpelainen	12
Hylspl05	TTAAGGACCCCACTCACTCG CATGGAGGAAGGGTGAACAG	(AAC)6(ACA)(AC)12	140-280	56	1	Ecogenics	19
Hylspl06	TTCATGCCTACGACACCAAC GCCTTGGCAAAGTGTTTGAG	(TG)15	180-240	56	2	Ecogenics	20
Hylspl07	TTGCAAACGGTATCCAAATG ATCTCACCTGAGGTCCAACG	(CAA)8	160-180	56	2	Ecogenics	6
Hylspl08	ACTGACCAGTCATGCACACG GCTACGACCGCATCTGAATC	(GTT)7	110-170	56	1	Ecogenics	11
Hylspl09	AAACCGCAACTGTGCAAATC CATCCAAAGTGGCAAAAACC	(AAC)7	110-130	56	2	Ecogenics	11
Hylspl14	TTTACTGCTGCCTGCCTACC CAGAGCAACCTCCCACTCTC	(TC)9…(TC)17	200-290	56	1	Ecogenics	18
Hylspl18	CATCTTGTCAGGGCTTGTAGG ATGCCGAAACAAAAGAATCG	(AG)24	160-220	56	3	Ecogenics	16
Hylspl19	CACCAAATGGTGTAAATGGTG ACGACAAACACCGCATGTAG	(GT)14	180-230	56	4	Ecogenics	17
Hylspl23	TTGCAAGCTCAAGGAATTTG CAGATGAATCATACAGCAATGG	(CTT)8	80-120	56	4	Ecogenics	10
Hylspl30	TCGATAGATGACCTAACTTATGAGC GAAGGAAGCATCCCCAAAAG	(TGT)6	70-95	56	2	Ecogenics	6

Initial analyses were done by dividing the data into a varying number of populations, all based on the geographic location of the samples. Nine populations were chosen based on the initial analyses to study variation between large-scale regions.

Expected heterozygosity (H_E) within and genetic differentiation (F_{ST}) between populations were calculated using Arlequin 3.5.1.3 (Excoffier & Lischer 2010). The software package GENALEX 6 (Peakall & Smouse 2006) was used to calculate isolation by distance and Analysis of Molecular Variance (AMOVA).

STRUCTURE 2.3.3 (Falush et al. , 2003; 2007; Hubisz et al., 2009; Pritchard et al., 2000) is a software for multi-locus genotype data to investigate population structure and can infer the presence of distinct populations and assign individuals to genetic clusters, it identifies groups of individuals corresponding to the uppermost hierarchical level, and is shown to perform well with both dominant and co-dominant markers (Evanno et al. 2005). The program uses Bayesian inference and MCMC (Markov Chain Monte Carlo) to discover the multi parameter space. The user sets the number of burn-in steps, the total number of MCMC steps in each analysis and the number of iterations for each value of K.

Evanno, Regnaut, and Goudet (2005) tested STRUCTUREs ability to identify the real number of genetic clusters (K) in datasets of AFLPs and microsatellites and concluded that the parameter delta K is a good predictor of the real number of clusters. Delta K is an ad hoc quantity related to the second order rate of change of the log probability of data with respect to the number of clusters that can be used instead of the direct likelihood.

STRUCTURE was run with 200 000 burn in, 1 million MCMC steps and 15 iterations per K-value. Both the no admixture model (individuals are discretely from one population or another) and the admixture model (each individual draws some fraction of its genome from each of the K populations) were tested with and without pre-set geographical settings (locprior) both assuming independent alleles. In the final STRUCTURE run genetic admixture was assumed and nine populations were assigned as locprior. The display of genetic clusters (Fig. 4) was calculated based on the best STRUCTURE run. The best run was chosen based on the highest likelihood of the 15 iterations run with the best K value evaluated by delta K.

Results

Of 209 specimens sampled, 168 gave reliable results. All samples are from different locations, 29 from North America, 102 from Europe (as far as 60° East) and 37 from Asia (North of the Himalayas). 13 of the 14 markers were found to be polymorphic and all samples carried unique haplotypes. The 14 markers were found to have approximately the same number of alleles as Vik et al. (in press). Marker information is presented in Table 1.

Table 2: Missing data and reproducibility measures for 14 microsatellite markers (Korpelainen et al. 2007; Vik et al. in press) in *H. splendens*. Presented are missing data values of 13 loci and 209 samples (M.D 13-209), missing data values of 13 loci and 168 samples (M.D 13-168), missing data values of four loci and 168 samples (M.D 4-168) and reproducibility of all 14 loci (R). Number of alleles (No. Alleles) are presented for all 14 microsatellite loci.

Primer ID	Developed by	M.D 13-209	M.D 13-168	M.D 4-168	R	No. Alleles
HySp01	Korpelainen	NA	NA	NA	NA	1
HySp03	Korpelainen	0.656	0.643	NA	0.333	9
HySp04	Korpelainen	0.751	0.708	NA	0.333	9
HySp06	Korpelainen	0.852	0.821	NA	0.667	12
Hylspl05	Ecogenics	0.804	0.804	NA	0.806	19
Hylspl06	Ecogenics	0.081	0.030	0.030	0.955	20
Hylspl07	Ecogenics	0.847	0.815	NA	0.933	6
Hylspl08	Ecogenics	0.158	0.125	0.107	0.931	11
Hylspl09	Ecogenics	0.139	0.065	0.065	1.000	11
Hylspl14	Ecogenics	0.823	0.833	NA	0.964	18
Hylspl18	Ecogenics	0.163	0.107	NA	NA	16
Hylspl19	Ecogenics	0.507	0.446	NA	0.500	17
Hylspl23	Ecogenics	0.584	0.577	NA	0.800	10
Hylspl30	Ecogenics	0.124	0.065	0.077	0.952	6

Most of the missing data points were caused by double peaks in GENEMAPPER where there should only have been single peaks. These data points were removed to avoid unreliability in the data set. As a consequence of this, nine of the microsatellite loci were too unreliable to use in the study caused by a combination of too much missing data (M.D) and too low reproducibility (R). This is shown in more detail in Table 2. Reproducibility was found to be lower than in Vik et al. (in press). HylSpl06, HylSpl08, HylSpl09 and HylSpl30 had more than 90% reproducibility and less than 5% missing data (Table 2). The number of alleles (Table 3) is quite high in all four loci and nine populations. The number of alleles for each marker and each population is presented in Table 3.

Table 3: The number of alleles counted for all nine populations of *H. splendens* and the four microsatellite loci, HylSpl06, HylSpl08, HylSpl09 and HylSpl30.

Locus#	W North America	E North America	N Europe	S Europe	Arctic Europe	Baltic	East Europe	NW Asia	E Asia	Mean ±S.D	Total
Hylspl06	7	4	14	12	9	11	6	9	7	8.778 ±3.15	20
Hylspl08	2	5	5	3	3	4	3	3	3	3.444 ±1.01	6
Hylspl09	6	7	8	5	4	6	6	6	4	5.778 ±1.30	14
Hylspl30	6	5	7	6	3	5	5	7	6	5.556 ±1.23	11
Mean ±S.D	*5.25 ±2.21*	*5.25 ±1.25*	*8.5 ±3.87*	*6.5 ±3.87*	*4.75 ±2.87*	*6.5 ±3.10*	*5 ±1.41*	*6.25 ±2.5*	*5 ±1.82*	*5.889 ±1.19*	*12.7 ±5*

There were no notable differences in expected heterozygosity and F_{ST}-values when comparing a hierarchical structure of "populations and regions" and a non-hierarchical structure of "only populations". The division of the samples into nine populations (West North America, East North America, North Europe, South Europe, Arctic Europe, Baltic, East Europe, North West Asia, East Asia) (Fig. 1) was chosen to reduce parameterization, but keep important geographical areas. Expected heterozygosity is shown in Table 4 and was very high for all loci and all populations (mean H_E = 0.701±0.07). The W North American population had the lowest mean H_E (0.592±0.263) while the E North American had the highest mean H_E (0.784±0.080).

Table 4: Gene diversity measured by expected heterozygosity calculated for all nine populations in *H. splendens* using four microsatellite loci.

Locus#	W North America	E North America	N Europe	S Europe	Arctic Europe	Baltic	E Europe	NW Asia	East Asia	Mean ±S.D.
Hylspl06	0.772	0.682	0.886	0.937	0.922	0.905	0.783	0.895	0.795	0.842 ±0.09
Hylspl08	0.221	0.788	0.676	0.416	0.451	0.619	0.658	0.503	0.602	0.548 ±0.17
Hylspl09	0.588	0.879	0.680	0.716	0.529	0.648	0.717	0.725	0.380	0.651 ±0.14
Hylspl30	0.787	0.788	0.746	0.768	0.523	0.790	0.758	0.837	0.848	0.761 ±0.10
Mean ±S.D	*0.592 ±0.26*	*0.784 ±0.08*	*0.747 ±0.09*	*0.709 ±0.21*	*0.606 ±0.21*	*0.740 ±0.13*	*0.729 ±0.05*	*0.740 ±0.17*	*0.656 ±0.21*	*0.701 ±0.07*

F_{ST} values (Table 5) were intermediate for all nine populations (F_{ST} < 0.2). The highest differentiation was found between NW Asia and W North America (F_{ST} = 0.152). W North America and E North America (F_{ST} = 0.146), E Europe and Arctic Europe (F_{ST} = 0.125), and NW Asia and Arctic Europe (F_{ST} = 0.117) were the next most differentiated populations. The

most similar populations were Arctic Europe and N Europe (F_{ST} = 0.027), NW Asia and N Europe (F_{ST} = 0.034) and E Asia and E Europe (F_{ST} = 0.042).

Table 5: F_{ST} values between nine populations of *H. splendens* in the Northern Hemisphere. Statistically significant values are indicated with *. The cut off value was $\alpha = 0.05$.

	West North America	East North America	North Europe	South Europe	Arctic Europe	Baltic	East Europe	North West Asia	East Asia
West North America	0.000								
East North America	0.146*	0.000							
North Europe	0.097*	0.025	0.000						
South Europe	0.052*	0.028	0.000	0.000					
Arctic Europe	0.075*	0.074*	0.027*	0.000	0.000				
Baltic	0.032	0.046*	0.017	0.006	0.052*	0.000			
East Europe	0.106*	0.055*	0.028	0.047*	0.125*	0.005	0.000		
North West Asia	0.153*	0.090*	0.035*	0.075*	0.118*	0.050*	0.023	0.000	
East Asia	0.054*	0.063*	0.013	0.023	0.057*	0.000	0.042*	0.062*	0.000

An analysis of molecular variance (AMOVA) was calculated in GENALEX 6 and showed most variation within populations (94%), some variation among populations (5%) and almost no variation among regions (1%) when divided into nine populations and three regions (North America, Europe and Asia) (Table 6).

Table 6: A summary table of the AMOVA for *H. splendens* with nine populations and three regions.

Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Among Regions	2	6.904	3.452	0.017	1 %
Among Pops	6	15.675	2.612	0.070	5 %
Within Pops	159	208.629	1.312	1.312	94 %
Total	167	231.208		1.399	100 %

A Mantel test was done to test for isolation by distance using GENALEX 6. The slope of the regression line was $y = 0.278x + 1.9013$ with $R^2 = 0.077$. The R^2 shows clearly that geographic distance does not explain the variation in genetic distance. Several different organisations and transformations of the data were tried (results not shown), but all gave qualitatively similar results; no effect of geographic distance on genetic similarity (Fig. 2). GENALEX 6 overestimated the geographical distance between populations, so a scatter plot of F_{ST} values versus geographic distance (Fig. 2) is presented to give a more accurate presentation of the lack of isolation by distance. A transformation of F_{ST} to $F_{ST}/(1-F_{ST})$ (Rousset 1997), did not differ considerably (not shown) from Fig. 2.

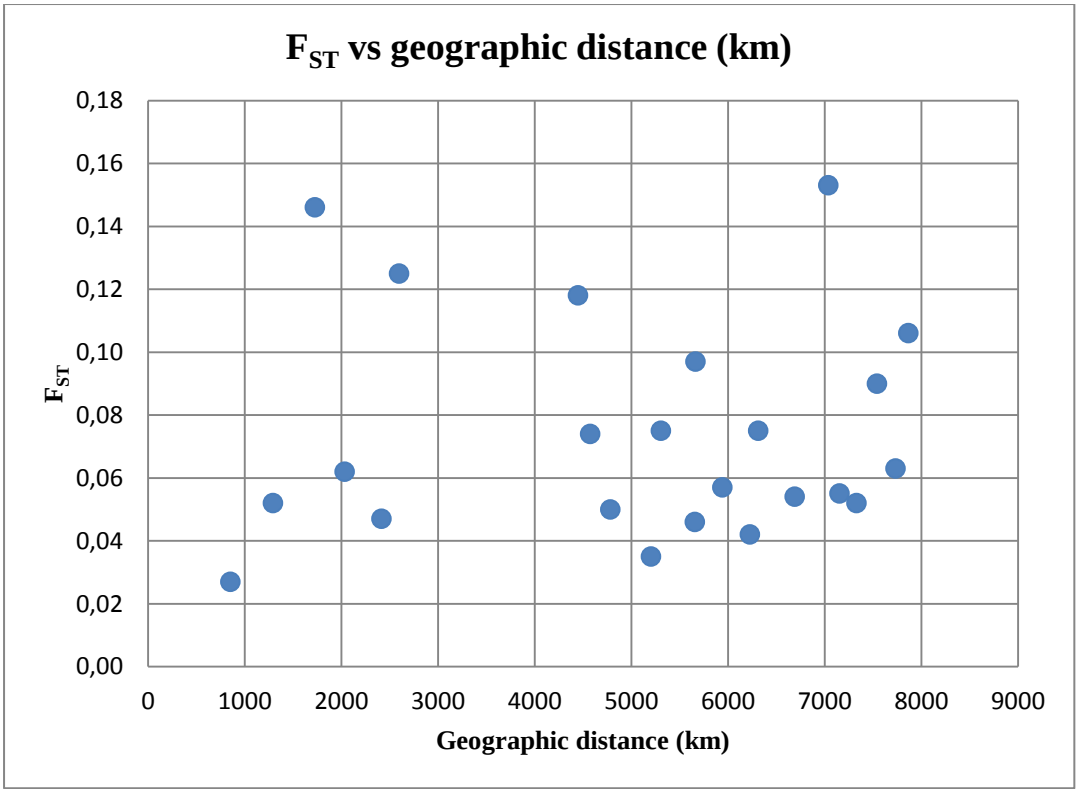

Fig. 2: A scatter plot of genetic isolation (F_{ST}) versus geographic distance for all nine populations.

K was calculated and plotted (Fig. 3) by the program package R (R Development Core Team 2011) using the R script Structure.deltaK (Ehrich 2006) and the output from STRUCTURE. The best result file of the 15 iterations with K = 7 was chosen to calculate the shares of the seven genetic clusters in all nine populations. The shares of the genetic variation for each of the seven clusters were averaged for each of the nine populations and were then plotted as pie a chart for each population. The seven clusters seem not to have differing geographical distributions (Fig. 4). One cluster (cluster 5) of the seven dominates all nine populations throughout the species' distribution range.

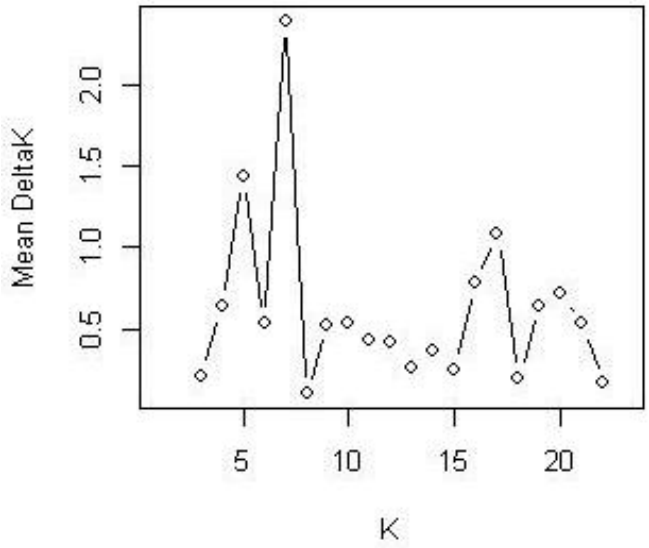

Fig. 3: The plot of a STRUCTURE run on *H. splendens* assuming admixture, independent allele frequencies, nine populations used as locprior and K-values of 1-30 with 15 iterations. Analysis was run with 200 000 burn in and 1 mill MCMC steps. K = 7 returned the highest delta K value.

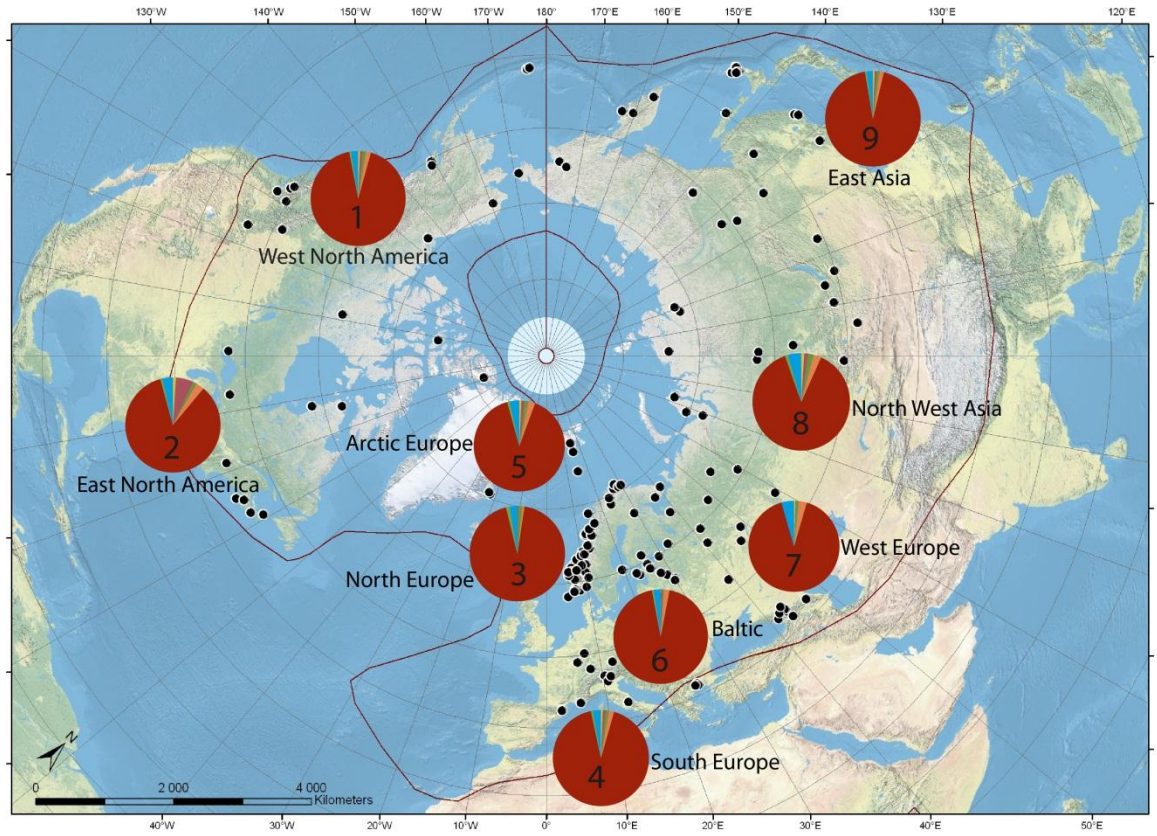

Fig. 4: Map showing the seven genetic clusters inferred from the STRUCTURE analysis of microsatellite loci in *Hylocomium splendens* in its distribution range, using delta K to determine the most likely number of clusters (K). The pie charts represent the relative contribution of the seven genetic clusters to the nine populations, West North America (1), East North America (2), West North Europe (3), South Europe (4), Arctic Europe (5), Baltic (6), East Europe (7), North West Asia (8) and East Asia (9). The black dots are all 168 samples used in the study.

In the analyses, we found no genetic foundation for the subspecies *H. splendens* var. *alaskanum* and *H. splendens* ssp. *giganteum*. A population comprising of the northernmost samples was run through analysis of genetic diversity and genetic differentiation, but the arctic population did not differ from the remaining populations (results not shown).

Discussion

Microsatellite analysis of *H. splendens* was performed to understand the history and genetic structure of the species. Most of the distribution range was sampled in order to try to understand species-level processes as well as inferring the species' response to its changing distribution range in the Northern Hemisphere over geological time. The analyses revealed high genetic variation (mean $H_E = 0.701$) across all samples and populations. No evidence of isolation by distance was found, but the samples seem to group into seven genetic clusters that are found throughout the distribution range.

Genetic variation was found to be high over the whole species range, with mean expected heterozygosity for each of the nine populations found between 0.59 and 0.78. It is worth noting that E North America has the highest expected heterozygosity (0.78±0.08), but W North America has the lowest (0.59±0.26), as these two populations share no evident physical barriers. As microsatellites can be expected to be effectively neutral (Jarne & Lagoda 1996), effective population size should be the most important factor explaining high, within-population genetic variation.

In Europe, the populations of this study lie along a latitudinal gradient; Arctic Europe (H_E = 0.606±0.213), N Europe (H_E = 0.747±0.098), Baltic (H_E = 0.740±0.133) and S Europe (H_E = 0.709±0.217). There is little difference in mean expected heterozygosity between the three southernmost populations. The lower mean expected heterozygosity in Arctic Europe compared to the three other populations might indicate a more recent introduction of *H. splendens* to arctic areas in Europe. But genetic differentiation in Europe in general does not seem to follow a latitudinal gradient. The genetic differentiation between Arctic Europe and N Europe or Baltic is significant but low, but there is no significant genetic differentiation between Arctic Europe and S Europe. The lack of differentiation along the latitudinal gradient in Europe might be related to the fact that S Europe only is significantly different from one of the European populations (E Europe). The lack of differentiation along a north to south gradient in Europe and the lack of significant genetic differentiation between S Europe and the majority of the populations in the study indicate that there might be other forces

influencing the genetic variation and differentiation of *H. splendens* in Europe than isolation by distance and a northward expansion pattern. The division of samples into populations makes it difficult to study processes along latitudinal gradients on other continents, but in the initial analyses a population of arctic samples from all continents was included, but it did not result in any significant clustering or genetic differentiation between the arctic population and the remaining populations.

Both E North America and NW Asia are in areas that were glaciated during the last glacial maximum. Regardless of whether *H. splendens* has occupied northern refugia within the glaciated area or not, the large area where there was ice must have been re-colonised during the last 10-15 000 years. Given the high levels of expected heterozygosity in *H. splendens* in these areas, effective population sizes must be large to avoid an edge effect (lower genetic diversity along the edges of the distribution) or dispersal must be very effective to rapidly "overwrite" the low genetic diversity resulting from the initial founder effect in the most distant areas of the distribution range. Areas known to have been ice free during the last glacial maximum (Siberia and Beringia) contain populations both with some of the lowest expected heterozygosities (W North America and E Asia) and with high expected heterozygosity (NW Asia). This inconsistency gives indications of random spore dispersal.

Disjunct and wide distributions can be caused by the rare events of long-distance dispersal. Rare events are difficult to model, but given a long time span a rare event can be assumed to have happened many times. And when diaspore production is high, as is the case in many bryophytes (Hassel & Söderström 1999; Söderström & Jonsson 1989; Hassel 2000), rare events involving spore dispersal can be important for regular gene flow between distant populations. Sporophyte production in *H. splendens* can be seen as a rare event as it is known to have a large variation in sporophyte production between years (Rydgren et al. 2006; Rydgren & Økland 2002). Sundberg (2005) did an in-depth study on spore dispersal in six *Sphagnum* species and the dependence of dispersal on spore and capsule size. He found deposition patterns to fit well to the inverse power law, but even when the curves were extended to infinity they failed to explain the dispersal of all the spores. Release height was found to be more important for short-distance dispersal than for long-distance dispersal (Sundberg 2005) an important factor for long-distance dispersal.

In closely related taxa, overall mutation rates can be expected to be similar, so the differences in genetic variation are primarily related to the histories of the species. More genetic variation

is expected in populations that have not experienced bottlenecks or founder events, while highly divergent genetic clusters are expected in populations with little gene flow. In *H. splendens* there is high genetic variation in all sampled populations, indicating a long time since the last bottleneck. Cronberg et al. (1997) similarly found little differentiation between populations on a much smaller scale than in this study, which could suggest fairly high levels of gene flow. High genetic variation is not uncommon in plants, Tian et al. (2009) found high total genetic diversity in the five-needled pine *Pinus kwangtungensis* in the isolated mountains of southern China and northern Vietnam, but unlike *H. splendens*, they found three distinct clades and a weak but significant correlation between genetic and geographic distance.

From the present study it is clear that there is much genetic variation in *H. splendens* (mean H_E = 0.701) compared to other bryophytes (Akiyama 1994; Stenøien & Såstad 1999; Kyrkjeeide et al. 2012; Derda & Wyatt 1990). Stenøien and Såstad (1999) found somewhat lower values of genetic diversity in *Sphagnum augustifolium* than in *H. splendens* and quite low values in the remaining *Sphagnum* species, Kyrkjeeide et al. (2012) found gene diversities almost one order of magnitude smaller in *Sphagnum wulfianum* than in *H. splendens* using similar markers and techniques, while Derda and Wyatt (1990) found the mean genotypic diversity of *Polytrichum commune* to be lower than for *H. splendens*. The use of different markers [Stenøien & Såstad (1999): RAPDs and isozymes, Kyrkjeeide et al. (2012): microsatellites and Derda & Wyatt (1990): allozymes] and population definitions make the comparisons only suggestive, but it is clear that the level of genetic diversity found in *H. splendens* is unusual for a bryophyte.

In the AMOVA, most of the genetic variation was found within populations and only 5 % among populations. This is in accordance with the high levels of genetic diversity through the species' distribution range. The high levels of genetic diversity indicate an absence of structure between populations and continents, i.e. effective dispersal across great geographic distances. The seven genetic clusters found by STRUCTURE are found in all nine populations, but one cluster dominates every population. There is some variation in the share of the other six clusters between the populations, but not enough for any of the populations to seem significantly different from the others. The dominance of one genetic cluster combined with high genetic variation indicates high levels of gene flow. The highest genetic differentiation was found between NW Asia and W North America. The samples of the two populations are separated by between 3500km and 10 000km, and this distance could be

expected to lead to some genetic differentiation. Populations with similar geographic distances are found in this study and the genetic differentiation does not seem to be correlated with distance (E North America/E Asia, W North America/E Europe, S Europe/E Asia). Specific geographic location seems to be more important than distance, which is exemplified by W and E North America. The two populations are the second most genetically differentiated, so rather than a general isolation by distance throughout the distribution range, there seems to be little genetic differentiation in total and a random long-distance dispersal of spores. *H. splendens* is highly variable in morphological traits and even though the general level of genetic diversity is also high, no support was found in the microsatellite data for *H. splendens* var. *alaskanum and H. splendens* ssp. *giganteum*.

It is quite extraordinary to find no isolation by distance in a study where the geographic distances between populations are commonly more than 2000 km. Because *H. splendens* has motile sperm with a limited dispersal distance (mean 5.0 cm, max 12 cm (Rydgren et al. 2006)), the most probable explanation for this pattern is efficient spore dispersal. Cronberg et al. (2006) found abundant sporophyte production and sexual reproduction to be common in *H. splendens*, a necessity for high spore production and dispersal. Akiyama (1994) found spore dispersal to be the most likely factor for the population structure in five species of *Leucodon.* Van Der Velde and Bijlsma (2003) investigated five species of the bryophyte genus *Polytrichum* along a north-south gradient in Europe using both allozyme and microsatellite markers. They did not find geographical structure of the genetic variation in three of the species (*P. commune, P. uliginosum* and *P. piliferum*) and proposed abundant gene flow caused by efficient spore dispersal to be a probable cause. Results comparable to those found for *H. splendens* are found in other spore producing organisms. Hovmøller et al. (2002) using AFLP variation in a biotrophic fungus found on wheat, showed that there was one single clonal population of the species inhabiting the UK, Germany, France and Denmark, up to 1700 km apart. This was most likely explained by long-distance wind dispersal of spores (Hovmøller et al. 2002). Travadon et al. (2011) found similar results between two regions of a fungal plant pathogen in France. There was high genetic variation and haplotypic richness, more than 97% of the variation was found within populations, there was low differentiation between populations and there was no isolation by distance. They concluded that high dispersal rates and/or large effective populations sizes to account for this pattern.

Earlier it was thought that species with large or disjunct distribution ranges were relicts from earlier glacial cycles (Abbott & Brochmann 2003). But long distance dispersal has been found to be of higher importance than previously thought in both vascular plants (Alsos et al. 2007; Brochmann et al. 1996; Alsos et al. 2005; Westergaard et al. 2010; Ehrich et al. 2008), bryophytes (Kyrkjeeide et al. 2012; Stenøien et al. 2011) and lichens (Geml et al. 2011; Geml et al. 2010). Heinrichs et al. (2009) reviewed the recent research on the molecular phylogeny and phylogenetic biogeography of bryophytes and concluded that intercontinental ranges of bryophytes often are caused by dispersal rather than geographical vicariance. Wind is the most probable vector for long-distance dispersal in bryophytes (Goffinet & Shaw 2009) and Van Zanten (1978) emphasised that wind-dispersal is the most plausible explanation for the similarities between populations of the same species in the Southern Hemisphere. In their review of distribution and dispersal of bryophytes, Van Zanten and Pocs (1981) state that aerial spore transport over moderately long and long-distance within a climatological belt is common for bryophytes with spores smaller than 25 µm. Wind dispersal of spores seems to be the only thing that can properly explain the current distribution of *H. splendens* together with the lack of structuring and isolation by distance. Wind dispersal has been found to be important in other bryophytes, Hassel and Söderström (2005) mapped the expansion of two alien mosses in Britain and found the two species to have different lengths of acclimatisation phase before being able to produce spores, but both had exponential expansion patterns.

More powerful computers make allow us to model complicated and computationally intensive scenarios that would be too costly to study in the field. Wilkinson et al. (2011) modelled the effect of size on the aerial dispersal of microorganisms and found that there was extensive within-hemisphere distribution of microbes of 9 µm and 20 µm in a simulation where microbes were released in Mexico and could spread over the whole globe averaged over a whole year. The spores of *H. splendens* are 12.8 µm-17.5 µm (Boros et al. 1993) and can be expected to behave in a similar fashion as the microbes. This high probability of spores reaching most of the Northern Hemisphere during a normal year (Wilkinson et al. 2012) makes the hypothesis of high rates of dispersal more probable.

Even if the dispersal of spores over long distances can be expected to be common, the establishment of spores is still necessary for gene flow. Rydgren and Økland (2002) report that establishment from spores never has been observed in *H. splendens*, but they still think that sexual spores may play a role in establishment of *H. splendens* on new substrates. Hassel and Söderström (1999) looked at the germination and establishment of spores of *Pogonatum*

dentatum in northern Sweden. The mean number of spores per capsule was 712 000 and in a sowing regime of one capsule in a 10×10 cm block, 11 shoots emerged after one year. In a review on seedling survival and seed size, the size of the seed was found to influence the seedling survival, but the advantages of large seeds in establishment under hazardous environments did not counterbalance the advantage of a great number of seeds (Moles & Westoby 2004) which also has been found for bryophyte spores (Sundberg & Rydin 2002). A large number of spores seem to be more important than spore establishment. Extreme longevity of genets due to extensive clonal growth can lead to an accumulation of genets in the population through time, which will lead to high genetic variation in the population. The maximum age of genets in *H. splendens* is not known, but it is not unlikely that they can be as old as the forest where they are found (Hassel, pers. com.), which could lead to a high number of genets in populations. It has been shown for *H. splendens* (Cronberg et al. 2006; Cronberg et al. 1997; Cronberg 2002) that populations of different patches are occupied by different sets of genets that are genetically different. They often persist for more than 50 years and with a steady production of sporophytes, the possibility for high spore production is present.

From the F_{ST} values and the Mantel test there seem to be random long-distance dispersal of spores in *H. splendens* and both large genetic differences over short geographic distances and small genetic differences over considerable geographic distances (Fig. 2). The Mantel test revealed high genetic variation across short geographical distances in our study, which is usually a sign of extreme genetic drift between closely situated populations. As the populations in this study comprise of single individuals sampled from distant locations, little is known of the local effective population size (N_e) in all sampled areas. But extreme genetic drift is not compatible with the local and small-scale genetic variation found by Cronberg et al. (1997) and Cronberg (2002).

Conclusion

The low genetic structure evident in this study is supported by earlier studies (eg Cronberg 2002), where no isolation by distance was found on islands in a Baltic uplift area. Combining the results of Cronberg et al. (1997) and Cronberg (2002) with the results from this study it seems as if *H. splendens* has no genetic structure either on local or large-scale geographical levels. Seven genetic clusters were found in the data, but one cluster dominated in all populations. This indicates high genetic similarity across the whole species. AMOVA showed that 94% of the variation is explained within populations confirming these results

together with high levels of heterozygosity and low F_{ST} values. Extreme genetic drift in neighbouring populations is not compatible with large effective population sizes that are proposed to be the cause of the general high genetic diversity across *H. splendens'* distribution range. The apparent randomness of genetic variation and structure indicate local random gene flow mediated by wind-dispersed spores. The results indicate a high ability for long-distance dispersal, high rates of establishment and high rates of sexual reproduction in *H. splendens*.

Many have looked into the histories and dynamics of arctic-alpine species and found vicariance and long-distance dispersal respectively to cause present day distributions. From the results of this study it is not possible to make any assumptions of glacial refugia in the whole of *H. splendens'* distribution range because all genetic signals are over-written by frequent long-distance dispersal. But there is no doubt that plants can disperse far across or around the North Pole, the time frame depending on the dispersal abilities of the species. The more common a plant is and the more propagules it can produce, the more probable it is that it can disperse successfully. Given the high genetic variation in *H. splendens*, the weak genetic structure, the wide distribution range and the high probability of spore dispersal high gene flow between all populations is probable.

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Supplementary material

Table S1: Table of all samples of *H. splendens* used in the analysis. Continent, country and state/region, collector, year sample was collected, herbarium where the sample is from (LE: St. Petersburg, MHA: Moscow, TRH: Trondheim, DUKE: North Carolina), ID number used in this study and assigned population. The herbaria in St. Petersburg and Moscow do not used herbarium numbers. Tissue samples of material from LE, MHA and DUKE are kept at TRH, except where indicated with *.

Continent	Country	State/region	Collector	Year	Herbarium	ID#	Pop #
Asia	Russia	Chukotka	O. M. Afonina	1978	LE	IE120	1
Asia	Russia	Chukotka	O. M. Afonina	1981	LE	IE139	1
Asia	Russia	Far East	O. M. Afonina	1983	LE	IE119	1
North America	Alaska	Brooks range	G. L. Smith, W. C. Steere	1966	LE	IE082	1
North America	Canada	Alberta	H. A. Crum	1955	DUKE-179531	IE197	1
North America	Canada	Alberta	W. B. Schofield, H. A. Crum	1955	DUKE-179529	IE196	1
North America	Canada	British Columbia	W. B. Schofield	1979	LE	IE075	1
North America	Canada	British Columbia	W. B. Schofield, S. Ellis	2005	DUKE-177596	IE185	1
North America	Canada	British Columbia	W. B. Schofield, S. Ellis	2005	DUKE-177666	IE186	1
North America	Canada	Northwest Territories	R. Kuramoto, K. Wade, V. J. Kraiina	1963	DUKE-179524	IE194	1
North America	USA	Alaska	W. B. Schofield, S. S. Talbot	2006	DUKE-176582	IE182	1
North America	USA	Alaska	W. B. Schofield, S. S. Talbot	2006	DUKE-176683	IE178	1
North America	USA	Alaska	W. B. Schofield, S. S. Talbot	2006	DUKE-176684	IE179	1
North America	USA	Alaska	W. B. Schofield, S. S. Talbot	2006	DUKE-176685	IE180	1
North America	USA	Alaska	W. B. Schofield, S. S. Talbot	2006	DUKE-177148	IE184	1
North America	USA	Montana	W. B. Schofield, R. Helliwell, K. L. Gray	2003	DUKE-176942	IE181	1
North America	USA	Washington	W. B. Schofield, J. A. Harpel	2002	DUKE-177069	IE183	1
North America	Canada	British Columbia	R. R. Ireland	1989	LE	IE077	2
North America	Canada	Northwest Territories	B. H. Allen	1997	DUKE-179521	IE191	2
North America	Canada	Northwest Territories	K. G. Foote	1963	DUKE-179523	IE193	2
North America	Canada	Northwest Territories	R. R. Ireland, Jr., I. M. Brodo	1973	DUKE-179522	IE192	2
North America	Canada	Nova Scotia	R. M. Garvey, R. M. King	2005	DUKE-177908	IE187	2
North America	Canada	Nova Scotia	W. B. Schofield, M. I. Schofield	1996	DUKE-179519	IE190	2

Continent	Country	State/region	Collector	Year	Herbarium	ID#	Pop #
North America	Canada	Nova Scotia	W. B. Schofield, R. J. Belland	1991	DUKE-179518	IE189	2
North America	Canada	Nova Scotia	W. B. Schofield, R. J. Belland	1992	DUKE-179517	IE188	2
North America	Canada	Quebec	B. Flatberg, K. I. Flatberg	2007	TRH-694614	IE220	2
North America	Canada	Quebec	B. Flatberg, K. I. Flatberg	2007	TRH-694615	IE221	2
North America	USA	Minnesota	F. D. Bowers	1995	DUKE-179533	IE199	2
North America	USA	New Hampshire	B. Shaw	2008	DUKE-68735	IE177	2
Europe	Faroe Islands	Nolsøy	N. Cronberg	2000	TRH-694605	IE123	3
Europe	Faroe Islands	Suderøy	N. Cronberg	2000	TRH-694606	IE124	3
Europe	Norway	Aust-Agder	K. I. Flatberg	1987	TRH-157682	IE007	3
Europe	Norway	Buskerud	E. Steinnes	2010	TRH-694616	IE200	3
Europe	Norway	Buskerud	E. Steinnes	2010	TRH-694617	IE202	3
Europe	Norway	Hedmark	K. Hassel, K. I. Flatberg	2007	TRH-690039	IE005	3
Europe	Norway	Hordaland	E. Steinnes	2010	TRH-694624	IE209	3
Europe	Norway	Hordaland	E. Steinnes	2010	TRH-694625	IE210	3
Europe	Norway	Møre og Romsdal	A. Stølen	1983	TRH-20035	IE009	3
Europe	Norway	Møre og Romsdal	B. Wilmann	2002	TRH-671341	IE011	3
Europe	Norway	Nordland	E. Steinnes	2010	TRH-694626	IE211	3
Europe	Norway	Nordland	E. Steinnes	2010	TRH-694627	IE212	3
Europe	Norway	Nordland	E. Steinnes	2010	TRH-694628	IE213	3
Europe	Norway	Nordland	E. Steinnes	2010	TRH-694629	IE214	3
Europe	Norway	Nordland	T. Prestø	2001	TRH-672669	IE021	3
Europe	Norway	Nordland	T. Prestø	2002	TRH-670812	IE020	3
Europe	Norway	Nord-Trøndelag	B. Wilmann	2002	TRH-671371	IE017	3
Europe	Norway	Nord-Trøndelag	K. Hassel	2008	TRH-690502	IE016	3
Europe	Norway	Nord-Trøndelag	T. Prestø	1998	TRH-1641	IE018	3
Europe	Norway	Oppland	M. O. Kyrkjeeide	2010	TRH-692973	IE022	3
Europe	Norway	Oppland	M. O. Kyrkjeeide, E. Sjetne	2010	TRH-692970	IE023	3
Europe	Norway	Rogaland	E. Steinnes	2010	TRH-694630	IE215	3
Europe	Norway	Sogn og Fjordane	E. Steinnes	2010	TRH-694631	IE217	3

Continent	Country	State/region	Collector	Year	Herbarium	ID#	Pop #
Europe	Norway	Sogn og Fjordane	E. Steinnes	2010	TRH-694632	IE218	3
Europe	Norway	Sogn og Fjordane	K. Hassel	2005	TRH-6575	IE008	3
Europe	Norway	Sør-Trøndelag	A. A. Frisvoll	1982	TRH-20069	IE013	3
Europe	Norway	Sør-Trøndelag	K. Hassel	2008	TRH-672908	IE014	3
Europe	Norway	Sør-Trøndelag	K. Hassel, K. I. Flatberg	2007	TRH-690042	IE015	3
Europe	Norway	Telemark	E. Steinnes	2010	TRH-694633	IE219	3
Europe	Norway	Vest-Agder	M. O. Kyrkjeeide	2010	TRH-692968	IE026	3
Europe	Norway	Vest-Agder	M. O. Kyrkjeeide, E. Sjetne	2010	TRH-692967	IE024	3
Europe	Norway	Vest-Agder	M. O. Kyrkjeeide, I. Engh	2010	TRH-692969	IE025	3
Europe	Scotland		D. Quandt	2001	TRH-694613	IE136	3
Europe	Austria	Lohnbach fall	D. Quandt	2000	TRH-694587	IE047	4
Europe	Austria	Neuberg	D. Quandt	2000	TRH-694594	IE055	4
Europe	Bulgaria	Smolyan	N. Cronberg	1997	TRH-694596	IE058	4
Europe	France	Alpes-Maritimes	J. Kortselius, H. Siebel	2001	TRH-694604	IE069	4
Europe	France	Pyrineene	D. Quandt	2001	TRH-694600	IE065	4
Europe	France	Pyrineene	D. Quandt	2001	TRH-694602	IE067	4
Europe	France	Vallon de Botton	D. Quandt	2001	TRH-694593	IE054	4
Europe	Germany	Black forest	D. Quandt	2002	TRH-694589	IE050	4
Europe	Germany	Rheinland pfalz	D. Quandt	2002	TRH-694590	IE051	4
Europe	Germany	Velden	J. C. Vogel	2002	TRH-694591	IE052	4
Europe	Greece	Demario	N. Cronberg	1997	TRH-694597	IE059	4
Europe	Greece	Rodopezna	N. Cronberg	1995	TRH-694595	IE057	4
Europe	Greece	Rodopi	N. Cronberg	1995	TRH-694598	IE060	4
Europe	Italy	Abruzzo	D. Quandt	2001	TRH-694599	IE064	4
Europe	Italy	Bozen	D. Quandt	2000	TRH-694603	IE068	4
Europe	Italy	Ledro	D. Quandt	2000	TRH-694601	IE066	4
Europe	Slovakia	Karpatene	N. Cronberg	1995	TRH-694588	IE048	4

Continent	Country	State/region	Collector	Year	Herbarium	ID#	Pop #
Europe	Switzerland	Val Scharl	I. Bisang	1996	TRH-694592	IE053	4
Europe	Ukraine	Ivano-Frankivsk Oblast	I. Danylkiv	2000	TRH-694612	IE135	4
Europe	Ukraine	Lozyna	I. Danylkiv	2000	TRH-694611	IE134	4
Europe	Finland	Lapland	E. Steinnes	2010	TRH-694618	IE203	5
Europe	Finland	Oulanka	L. Appelgren	1997	TRH-694583	IE043	5
Europe	Greenland	East Greenland	K. Hassel, B. Pedersen	2007	TRH-690019	IE037	5
Europe	Greenland	East Greenland	K. Hassel, B. Pedersen	2007	TRH-690020	IE036	5
Europe	Iceland	Eyjafjardarsysla	K. Westergaard, T. Dahl	2007	TRH-690030	IE029	5
Europe	Island	Eyjafjardarsysla	K. Hassel, B. Pedersen	2007	TRH-690014	IE027	5
Europe	Norway	Finnmark	E. Steinnes	2010	TRH-694619	IE204	5
Europe	Norway	Finnmark	E. Steinnes	2010	TRH-694620	IE205	5
Europe	Norway	Finnmark	E. Steinnes	2010	TRH-694621	IE206	5
Europe	Norway	Finnmark	E. Steinnes	2010	TRH-694622	IE207	5
Europe	Norway	Finnmark	E. Steinnes	2010	TRH-694623	IE208	5
Europe	Russia	Arkhangelsk prov.	A. V. Bykov	2005	MHA	IE155	5
Europe	Russia	Kola peninsula	N. Cronberg	1994	TRH-694609	IE131	5
Europe	Russia	Murmansky Region, Terskiy district	E. Seman	1971	LE	IE109	5
Europe	Russia	Vologda republic	M. Noskova	2002	TRH-694607	IE127	5
Europe	Svalbard	Bjørnøya	S. Dunfjeld, T. Engelskjøn	1983	TRH-67534	IE030	5
Europe	Svalbard	Svalbard	A. A. Frisvoll	1977	TRH-162621	IE034	5
Europe	Svalbard	Svalbard	B. Godzik, K. Grodzinska	1985	TRH-67157	IE032	5
Europe	Estonia	Käsmu	N. Cronberg	2003	TRH-694581	IE041	6
Europe	Estonia	Norra	N. Cronberg	2003	TRH-694585	IE045	6
Europe	Estonia	West-Estonian archipelago	L. Kannukene	1997	LE	IE110	6
Europe	Estonia	Üüc-v cliff	N. Cronberg	2003	TRH-694582	IE042	6
Europe	Finland	Lammi	L. Appelgren	1997	TRH-694580	IE040	6
Europe	Lithuania	Vilnius district	N. Kalinawskaité	2000	TRH-694578	IE038	6
Europe	Lithuania	Vilnius district	N. Kalinawskaité	2000	TRH-694579	IE039	6
Europe	Russia	Leningradsky region	G. Vjunova	1974	LE	IE111	6

Continent	Country	State/region	Collector	Year	Herbarium	ID#	Pop #
Europe	Russia	Pskov Prov	V. I. Zolotov, T. M. Zolotova	1998	MHA	IE146	6
Europe	Russia	Pskov Prov	V. I. Zolotov, T. M. Zolotova	2001	MHA	IE150	6
Europe	Russia	Pskov Province	M. S. Ignatov, V. I. Zolotov	1997	MHA	IE149	6
Europe	Russia	Pushkinskyje Gory	O. M. Afonina	2005	LE	IE107	6
Europe	Russia	River Zubets	M. Noskova	2002	TRH-694608	IE128	6
Europe	Finland	Åland	N. Cronberg	1997	TRH-694584	IE044	6
Europe	Finland	Åland	N. Cronberg	1997	TRH-694586	IE046	6
Europe	Georgia	Khashuri	T. Shulkina	1997	MHA	IE159	7
Europe	Russia	Abhazia	I. V. Radzimovskaja	1984	LE	IE113	7
Europe	Russia	Archangelsk	M. Noskova	2002	TRH-694610	IE132	7
Europe	Russia	Chelyabinsk Oblast	S. V. Balandin, P. V. Kulikov	1997	MHA	IE153	7
Europe	Russia	Chuvash Republic	S. A. Moshkovskij	1999	MHA	IE137	7
Europe	Russia	Dagestan	M. E. Ignatov, G. Urbanskaia	2009	LE	IE114	7
Europe	Russia	Kabardino-Balkaria	M. Ignatov, E. Ignatova, Z. Kharzinov	2004	MHA	IE157	7
Europe	Russia	Kabardino-Balkaria	M. Ignatov, E. Ignatova, Z. Kharzinov	2005	MHA	IE158	7
Europe	Russia	Komi Republic	I. B. Kuttsjerov, S. A. Kutenkov	2007	MHA	IE154	7
Europe	Russia	Kostroma region	D. Donskov	2008	MHA	IE152	7
Europe	Russia	Permskaya Sverdlovskaya oblast	E. N. Smirnova	1955	LE	IE115	7
Europe	Russia	Stavropo'skij Krai	E. A. Ignatova	1986	MHA	IE160	7
Europe	Russia	Stavropo'skij Krai	G. Urbanskaia	1993	LE	IE112	7
Europe	Russia	Stavropo'skij Krai	T. Konovalova	2007	MHA	IE161	7
Europe	Russia	Tatarstan Republic	M. Ignatov, E. Ignatova	2003	MHA	IE151	7
Europe	Russia	Voronezh reservation	N. N. Popova	1982	LE	IE108	7
Asia	Mongolia	Arhanskiy aymak	D. Bangzrach, A. I. Bannikova, N. Manibazar, Bumtsent, Munkhbajar	1972	LE	IE105	8
Asia	Mongolia	Iro Somon	A.A. Junatov	1943	LE	IE104	8
Asia	Mongolia	Khovsgol Lake	E. I. Ignatova	2007	MHA	IE143	8

Continent	Country	State/region	Collector	Year	Herbarium	ID#	Pop #
Asia	Russia	Altai mountains	M. Ignatov	1989	MHA	IE165	8
Asia	Russia	Altai mountains	M. Ignatov	1989	MHA	IE164	8
Asia	Russia	Irkutsk	N. Sjevireva, T. Konovalova	1993	MHA	IE167	8
Asia	Russia	Krasnoyarsk Krai	A. Rodenkov	1997	MHA	IE163	8
Asia	Russia	Krasnoyarsk Krai	I. N. Pospelov	2003	MHA	IE168	8
Asia	Russia	Krasnoyarsk Krai	S. S. Shcherbina	1994	MHA	IE166	8
Asia	Russia	Krasnoyarsk Krai	V. E. Fedosov	2008	MHA	IE169	8
Asia	Russia	Krasnoyarsk Krai	V. E. Fedosov	2008	MHA	IE170	8
Asia	Russia	Krasnoyarsk Krai	V. Kuvaer, D. Sjakhin	1994	MHA	IE162	8
Asia	Russia	Southern Siberia	O. M. Afonina	2006	LE	IE142	8
Asia	Russia	Southern Siberia	O. M. Afonina	2007	LE	IE079	8
Asia	Russia	West-Siberia	I. V. Czernyadjeva	1994	LE	IE078	8
Asia	Russia	West-Siberia	Matvejeva	1965	LE	IE122	8
Asia	Russia	West-Siberia	O. V. Rebristaia	1981	LE	IE116	8
Asia	Russia	West-Siberia	O. V. Rebristaia	1991	LE	IE121	8
Asia	China	Jilin province	E. Enkhzhargal	1983	MHA	IE145	9
Asia	Russia	Amurskaya territory	D. A. Petelin	1979	MHA	IE101	9
Asia	Russia	Sakha/Yakutia Republic	M. Ignatov	2000	MHA	IE172	9
Asia	Russia	Far East	I. V. Czernyadjeva	2002	LE	IE118	9
Asia	Russia	Far East	I. V. Czernyadjeva	2005	LE	IE080	9
Asia	Russia	Far East	T. G. Polozova	1956	LE	IE138	9
Asia	Russia	Kamchatka	I. V. Czernyadjeva	2005	LE	IE140	9
Asia	Russia	Kuril islands	V. A. Bakalin	2007	MHA	IE095	9
Asia	Russia	Kuril islands	V. A. Bakalin	2007	MHA	IE096	9
Asia	Russia	Kuril islands	V. A. Bakalin	2007	MHA	IE173	9
Asia	Russia	Russian Far East	M. Ignatov	1997	MHA	IE100	9
Asia	Russia	Russian Far East	M. Ignatov	2006	MHA	IE097	9
Asia	Russia	Russian Far East	M. Ignatov	2006	MHA	IE174	9
Asia	Russia	Russian Far East	M. Ignatov	2006	MHA	IE175	9

Continent	Country	State/region	Collector	Year	Herbarium	ID#	Pop #
Asia	Russia	Russian Far East	M. Ignatov	2007	MHA	IE099	9
Asia	Russia	Russian Far East	M. Ignatov, E. Ignatova, V. Cherdantseva	2006	MHA	IE176	9
Asia	Russia	Russian Far East	M. Ignatov, V. Teleganova	2006	MHA	IE098	9
Asia	Russia	Sakha/Yakutia Republic	E. I. Ivanova	2000	LE	IE141	9
Asia	Russia	Sakha/Yakutia Republic	M. Ignatov	2000	MHA	IE147	9

Table S2: The results from the analysis of *H. splendens* using four microsatellite markers. ID number used in the study, continent, country and allele for each marker shown for all samples.

ID#	Continent	Country	hyl6	30	9	8
IE005	Europe	Norway	192	87	115	146
IE007	Europe	Norway	205	87	121	146
IE008	Europe	Norway	192	87	118	146
IE009	Europe	Norway	225	87	115	146
IE011	Europe	Norway	190	83	115	143
IE013	Europe	Norway	212	83	115	146
IE014	Europe	Norway	205	87	115	146
IE015	Europe	Norway	194	87	0	146
IE016	Europe	Norway	240	87	115	146
IE017	Europe	Norway	192	87	0	146
IE018	Europe	Norway	194	0	115	146
IE020	Europe	Norway	199	87	115	149
IE021	Europe	Norway	192	87	115	146
IE022	Europe	Norway	203	83	121	143
IE023	Europe	Norway	205	87	0	146
IE024	Europe	Norway	183	87	115	116
IE025	Europe	Norway	194	87	115	146
IE026	Europe	Norway	208	87	115	146
IE027	Europe	Island	192	83	115	146
IE029	Europe	Iceland	190	83	121	146
IE030	Europe	Svalbard	190	87	0	116
IE032	Europe	Svalbard	203	0	115	146
IE034	Europe	Svalbard	214	83	115	116
IE036	Europe	Greenland	240	87	115	146
IE037	Europe	Greenland	203	87	115	146
IE038	Europe	Lithuania	183	87	115	143
IE039	Europe	Lithuania	223	87	115	143
IE040	Europe	Finland	205	87	118	116
IE041	Europe	Estonia	190	0	127	162
IE042	Europe	Estonia	192	87	108	116
IE043	Europe	Finland	203	87	0	116
IE044	Europe	Sweden	192	0	115	116
IE045	Europe	Estonia	196	87	115	116
IE046	Europe	Sweden	212	83	129	0

ID#	Continent	Country	hyl6	30	9	8
IE047	Europe	Austria	196	87	115	146
IE048	Europe	Slovakia	203	87	121	143
IE050	Europe	Germany	192	87	118	146
IE051	Europe	Germany	192	87	121	146
IE052	Europe	Germany	203	87	115	146
IE053	Europe	Switzerland	240	87	115	146
IE054	Europe	France	194	87	115	146
IE055	Europe	Austria	205	87	0	139
IE057	Europe	Greece	194	87	115	146
IE058	Europe	Bulgaria	190	87	121	146
IE059	Europe	Greece	210	83	115	162
IE060	Europe	Greece	196	87	115	143
IE064	Europe	Italy	192	87	115	162
IE065	Europe	France	203	87	0	0
IE066	Europe	Italy	192	87	108	162
IE067	Europe	France	208	83	118	0
IE068	Europe	Italy	190	0	115	116
IE069	Europe	France	214	83	115	116
IE075	North America	Canada	205	87	103	146
IE077	North America	Canada	203	83	115	156
IE078	Asia	Russia	192	83	115	156
IE079	Asia	Russia	192	83	115	162
IE080	Asia	Russia	0	87	115	143
IE082	North America	USA	201	87	115	146
IE095	Asia	Russia	190	87	115	149
IE096	Asia	Russia	192	87	103	146
IE097	Asia	Russia	190	0	115	146
IE098	Asia	Russia	190	83	115	116
IE099	Asia	Russia	208	87	115	146
IE100	Asia	Russia	0	83	118	146
IE101	Asia	Russia	0	83	115	146
IE104	Asia	Mongolia	205	83	115	162
IE105	Asia	Mongolia	183	83	112	116
IE107	Europe	Russia	192	80	115	162
IE108	Europe	Russia	192	83	115	0
IE109	Europe	Russia	192	87	0	146

ID#	Continent	Country	hyl6	30	9	8
IE110	Europe	Estonia	201	83	115	162
IE111	Europe	Russia	192	87	115	116
IE112	Europe	Russia	199	87	115	0
IE113	Europe	Russia	199	87	115	116
IE114	Europe	Russia	192	87	115	0
IE115	Europe	Russia	192	87	118	116
IE116	Asia	Russia	188	83	115	162
IE118	Asia	Russia	192	87	115	116
IE119	Asia	Russia	190	83	115	0
IE120	Asia	Russia	190	87	103	116
IE121	Asia	Russia	188	83	127	162
IE122	Asia	Russia	199	87	121	0
IE123	Europe	Faroe Islands	203	0	115	146
IE124	Europe	Faroe Islands	188	92	118	162
IE127	Europe	Russia	190	87	115	146
IE128	Europe	Russia	192	87	108	116
IE131	Europe	Russia	196	83	115	146
IE132	Europe	Russia	192	87	115	162
IE134	Europe	Ukraine	183	83	0	143
IE135	Europe	Ukraine	243	87	118	146
IE136	Europe	Scotland	201	83	112	139
IE137	Europe	Russia	192	87	118	162
IE138	Asia	Russia	194	0	115	162
IE139	Asia	Russia	190	87	121	116
IE140	Asia	Russia	205	87	115	149
IE141	Asia	Russia	192	83	121	187
IE142	Asia	Russia	240	80	115	146
IE143	Asia	Mongolia	190	87	121	146
IE145	Asia	China	192	83	115	116
IE146	Europe	Russia	240	83	115	143
IE147	Asia	Russia	192	87	115	149
IE149	Europe	Russia	210	87	115	146
IE150	Europe	Russia	203	87	103	146
IE151	Europe	Russia	192	87	115	146
IE152	Europe	Russia	190	83	135	162
IE153	Europe	Russia	199	83	118	162

ID#	Continent	Country	hyl6	30	9	8
IE154	Europe	Russia	205	80	115	143
IE155	Europe	Russia	188	87	115	116
IE157	Europe	Russia	201	80	129	0
IE158	Europe	Russia	201	83	112	143
IE159	Europe	Georgia	192	83	115	162
IE160	Europe	Russia	188	87	118	162
IE161	Europe	Russia	188	80	121	162
IE162	Asia	Russia	188	87	118	162
IE163	Asia	Russia	188	83	121	162
IE164	Asia	Russia	192	87	115	146
IE165	Asia	Russia	183	87	115	146
IE166	Asia	Russia	192	83	115	139
IE167	Asia	Russia	0	83	115	149
IE168	Asia	Russia	183	83	112	139
IE169	Asia	Russia	201	83	112	116
IE170	Asia	Russia	201	83	132	116
IE172	Asia	Russia	192	87	115	149
IE173	Asia	Russia	192	87	115	143
IE174	Asia	Russia	171	87	121	116
IE175	Asia	Russia	192	0	115	162
IE176	Asia	Russia	208	87	115	162
IE177	North America	USA	192	80	110	0
IE178	North America	USA	0	87	115	116
IE179	North America	USA	225	87	115	116
IE180	North America	USA	190	83	115	116
IE181	North America	USA	190	87	115	116
IE182	North America	USA	208	87	118	143
IE183	North America	USA	225	87	115	116
IE184	North America	USA	190	87	112	139
IE185	North America	Canada	208	87	115	149
IE186	North America	Canada	190	87	115	0
IE187	North America	Canada	205	87	115	146
IE188	North America	Canada	203	87	115	146
IE189	North America	Canada	203	83	115	146
IE190	North America	Canada	208	87	121	146
IE191	North America	Canada	192	0	87	115

ID#	Continent	Country	hyl6	30	9	8
IE192	North America	Canada	192	0	87	115
IE193	North America	Canada	192	175	83	110
IE194	North America	Canada	190	87	115	143
IE196	North America	Canada	192	87	0	0
IE197	North America	Canada	192	87	115	0
IE199	North America	USA	192	87	127	0
IE200	Europe	Norway	192	87	108	149
IE202	Europe	Norway	196	87	115	143
IE203	Europe	Finland	205	87	118	0
IE204	Europe	Norway	223	87	115	146
IE205	Europe	Norway	205	87	115	146
IE206	Europe	Norway	196	87	0	146
IE207	Europe	Norway	240	87	115	0
IE208	Europe	Norway	240	87	115	146
IE209	Europe	Norway	192	83	118	139
IE210	Europe	Norway	192	0	118	0
IE211	Europe	Norway	205	80	115	162
IE212	Europe	Norway	199	83	132	162
IE213	Europe	Norway	190	83	121	162
IE214	Europe	Norway	194	83	115	139
IE215	Europe	Norway	194	80	115	162
IE217	Europe	Norway	192	83	115	162
IE218	Europe	Norway	192	80	129	143
IE219	Europe	Norway	194	83	118	143
IE220	North America	Canada	203	87	118	0
IE221	North America	Canada	192	0	118	146