ISSR analysis of two founding plant species on the volcanic island Surtsey, Iceland: grass versus shrub

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ABSTRACT

Prior to the present study there was limited knowledge about the genetic basis of plant colonization on the 50-year-old island of Surtsey, South Iceland. The aim here was to compare genetic structure of two contrasting species, *Festuca rubra* (arctic fescue) and *Empetrum nigrum* (crowberry), which have colonized Surtsey since 1973 and 1993, respectively. Inter-simple sequence repeat (ISSR) markers were used to assess genetic diversity and population structure. Two census periods were compared: 1996-1997 and 2005-2006. Using six ISSR primers, we obtained 103 and 139 discernible DNA fragments from *F. rubra* and *E. nigrum* respectively. Although the two species displayed similarly high genetic diversity indices (h = 0.238 and 0.235; I = 0.384 and 0.380, respectively), they differed significantly in their genetic profiles. *Festuca* was genetically structured at the subpopulation level (F_{ST} = 0.034, p = 0.007), whereas *Empetrum* showed a lack of genetic differentiation. A Bayesian STRUCTURE computation further revealed temporal and spatial genetic structure of the species. The early arrival grass *F. rubra* has expanded from a local genepool. The population was however initially established from different sources, forming a genetic melting pot on Surtsey. On the other hand, the late arrival shrub *E. nigrum* probably derived from a common source of immigrants.

Keywords: AMOVA, *Empetrum*, *Festuca*, founder effect, genetic diversity, island biogeography, ISSR, phenetic analysis

INTRODUCTION

Some theoretical and empirical studies seem to suggest that founder effects and bottlenecks can be both strong and frequent (Avise & Hamrick 1996) and can consequently determine the fate of species colonization in a new habitat (Barton & Charlesworth 1984; Carson & Templeton 1984). Studies on oceanic islands have often shown that colonizing plant species that experience these effects tend to show reduced genetic diversity (e.g. Westerbergh & Saura 1994; Affre et al. 1997; Yamada & Maki 2012). Reduced variation within a population, due to inbreeding and increased homozygosity, often leads to loss of fitness or evolutionary potential and, in extreme cases, species depression and extinction (Charlesworth & Charlesworth 1987; Ellstrand & Elam 1993; Frankham 2005; Dostálek et al. 2009; Triantis et al. 2010). However, many invasive or introduced species seem either not to have gone through a genetic bottleneck or not to have suffered

much loss of fitness or evolutionary potential as a result of colonization (Hollingsworth & Bailey 2000; Sakai et al. 2001; Fernández-Mazuecos & Vargas 2011). In light of such consequential variability, the study of organisms which colonize recently formed islands could be an essential step towards a better understanding of the genetic behaviour of colonizing populations (Emerson 2002; Franks 2010).

Oceanic islands have long been thought of as natural laboratories for the study of evolutionary processes (MacArthur & Wilson 1967). The recent (1963) origin of the island of Surtsey just south of Iceland (lat. 63° 18' 22'' N, long. 20° 36' 5'' W) as well as the meticulously well-documented history of plant colonization on the island since its inception (Fridriksson 1966; Baldursson & Ingadóttir 2007; Magnússon et al. 2009, 2014) provide an unprecedented opportunity to study the colonization of a species in terms of its establishment, distribution and dispersal by natural means, devoid of all human influence. The place to actually see a founder effect in island archipelagos is in populations of newly established species, and in this context the young island of Surtsey provides an ideal study stage.

In this study, we investigate genetic diversity in populations of two plant species with different taxonomic statuses, life histories, modes of reproduction and histories of colonization on Surtsey: the arctic fescue, *Festuca rubra* L. subsp. *richardsonii* (Hook.) Hultén (Synonym: *Festuca rubra* subsp. *arctica* (Hack.) Govor., Poaceae), a grass species which was found on the island for the first time in 1973, only ten years after Surtsey was formed; and the crowberry, *Empetrum nigrum* L. (Empetraceae), an evergreen dwarf shrub which became established on the island 20 years later, in 1993. While arctic fescue has become one of the most common vascular plant species on the island (Magnússon et al. 2014), crowberry is still rare (authors' own observation). The creeping runner grass, *F. rubra,* is common and has a widespread distribution over all regions of Iceland (Kristinsson 2008) as well as on islands of the Vestmannaeyjar archipelago where it thrives on the fertile soils of seabird colonies (Fridriksson & Johnsen 1967; Magnússon et al. 2014). Based on flower/fruit and leaf morphology, the *E. nigrum* plants on Surtsey belong to the wind-pollinated, dioecious subspecies *nigrum*, which is found only in lowland areas of Iceland (Kristinsson 2005, 2008). The more common crowberry in Iceland belongs to subspecies *hermaphroditum* (Basionym *Empetrum hermaphroditum* Hagerup), which has bisexual flowers and is found more inland and at higher altitudes than subspecies *nigrum* (Kristinsson 2005, 2008). The two subspecies are also distinctively different in their chromosome number: subspecies *nigrum* is diploid but subspecies *hermaphroditum* is tetraploid (Löve & Löve 1975; Suda et al. 2004) and this has been confirmed with material collected in Iceland using flow cytometry (Jacobsen 2005) and by direct chromosome counting (K. Anamthawat-Jónsson, unpublished).

Both *Festuca* and *Empetrum* species were genetically screened in the present study using the PCR-based fingerprinting method "inter-simple sequence repeat (ISSR, or anchored SSR)" originally described by Zietkiewicz et al. (1994), to detect variable sites in the microsatellite regions of the genome (Stepansky et al. 1999). The ISSR method has proven useful not only in detecting clonal and somaclonal diversity in crop plants as well as in the fingerprinting of closely related genotypes and cultivars (e.g. Li & Ge 2001; Bairu et al. 2011; Mukherjee et al. 2013), but also in resolving genetic relationships among related and hybridizing taxa in natural habitats (Chokchaichamnankit et al. 2008; Słomka et al. 2011, Sutkowska et al. 2013).

The aim of this study was to compare the genetic structure of the current populations of the grass *F. rubra* versus the shrub *E. nigrum* on the island in relation to previously collected data on species distribution dating to the timing of the species colonization of the island. The following questions were addressed: (1) are there genetic differences among populations at the temporal and spatial levels; and (2) what are the links between the multivariate-phenetic, Bayesian and population genetic characteristics of the species?

MATERIALS AND METHODS

Plant material and site description

The island of Surtsey, a UNESCO World Heritage Site since 2008, had an area above sea level of about 1.4 km^2 above sea level at that time (Baldursson & Ingadóttir 2007). In order to obtain material suitable for DNA analysis, fresh leaf samples, represented by 16 individual plants of *Festuca rubra* and 14 individuals of *Empetrum nigrum* (Fig. 1), were collected from the entire range of the two species distribution on the island of Surtsey during the month

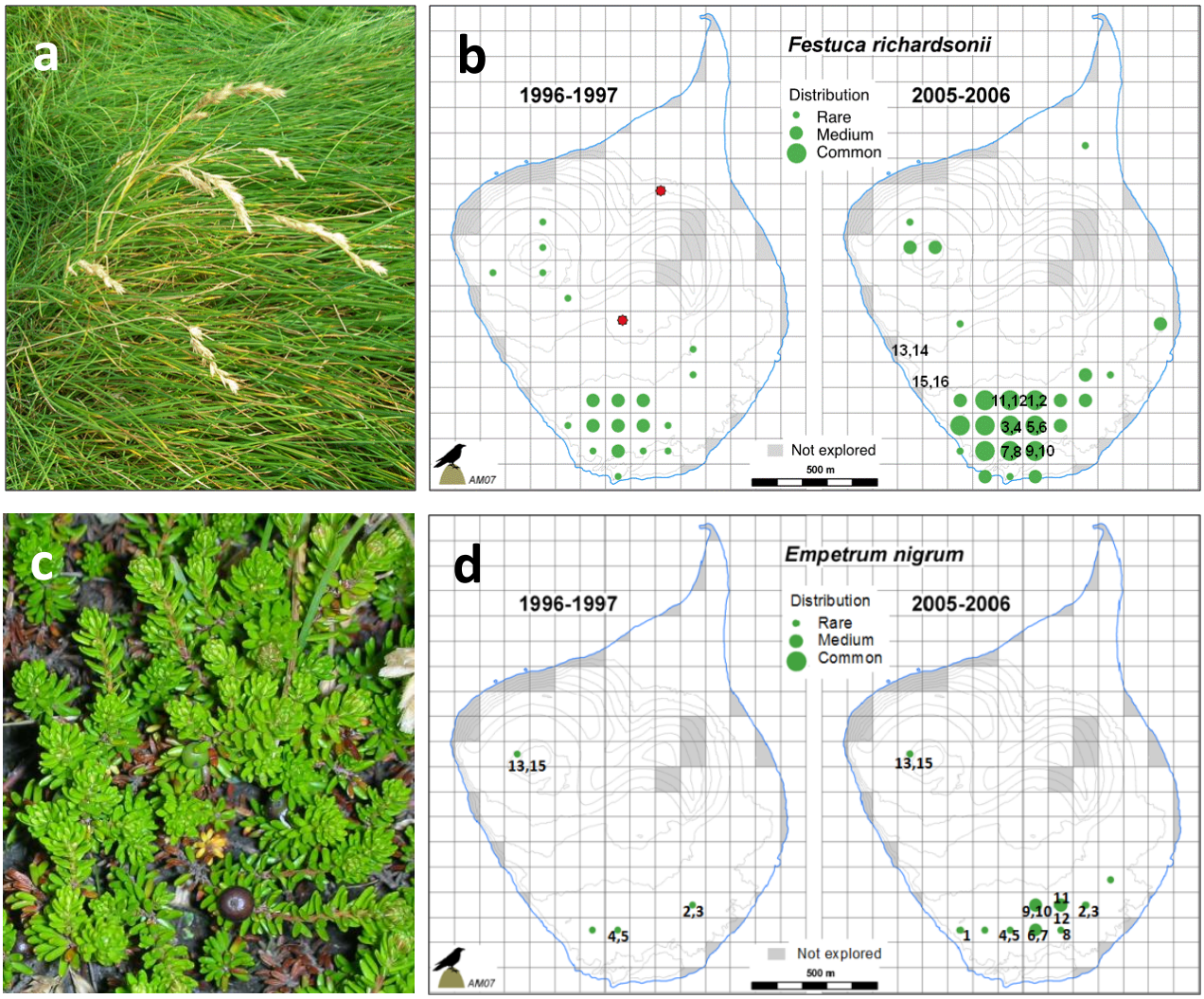

Figure 1. Distribution of *Festuca rubra* (a & b) and *Empetrum nigrum* (c & d) on the island of Surtsey. Photographs of *F. rubra* and *E. nigrum* plants (a & c), taken by author KAJ on 14 July 2010. Location of individual plants investigated (b & d), superimposed on the vegetation map showing distribution and abundance of the species in two different census periods: 1996-1997 and 2005-2006. Red dots indicate location of the first appearance on the island. The vegetation maps were kindly provided by Sigurdur H Magnússon from the Icelandic Institute of Natural History.

of June, 2010. The ID numbers from one to 12 were assigned to samples of both species collected from the most vegetated site on the island's south-western lava field known primarily as "the gull colony". This area, initially a barren lava field with sparse vegetation, became the nesting place for a few pairs of seagulls (*Larus* spp.) around 1985, and by 2008 the vegetation in this area had reached about 10 ha, providing the main nesting area for hundreds of pairs of gulls and other seabirds (Petersen 2009). The gull colony has been particularly important in the development of plant community on Surtsey, due to the positive effects of bird nesting on soil fertility (Sigurdsson & Magnússon 2010, Magnússon et al. 2014). Higher sample ID numbers represent the sampling areas located furthermost from the centre of the gull colony: *F. rubra* samples 13-16 were collected from newly established sites located near the western cliff of the island and *E. nigrum* samples 13 and 15 were collected from inside the crater *Surtungur,* at about 150 masl.

The island is visited every summer to record colonization and plant succession. Permanent plots (10x10 m^2) were set up: the first five plots in 1990, additional 14 plots in 1994 and six plots in 1995 (Magnússon et al. 1996). Due to ground erosion or a revision of methods, a few plots were abandoned, new ones established, and by 2012 there were 25 plots operational on the island (Magnússon et al. 2014). Samples collected for the present study were

mostly from within the oldest area of vegetation, i.e. in the gull colony which is the location of the first ten permanent plots. Two floristic census periods, i.e. 1996-1997 and 2005-2007 (Fig. 1), were used in the present study to implement the molecular analysis of population structure on Surtsey.

DNA isolation and ISSR

Genomic DNA was isolated from dehydrated leaf tissue using Genomic Mini AX Plant extraction kit (A&A Biotechnology, Gdynia, Poland). Six (15-18 nucleotides) ISSR primers were used (Table 1). The primer sequences followed Stepansky et al. (1999). Amplification was carried out with a 25 µl reaction mixture, consisting of 2.5 µl 10-fold concentrated reaction buffer, 1.5 mM $MgCl_2$, 0.19 mM of each dNTP (Fermentas), 27 pmol primer, 100 ng template DNA and 1.4 units of Taq polymerase (Fermentas). Reactions were conducted in a 2720 thermal cycler (Applied Biosystems®). The annealing temperature for primers ISSR2, ISSR4 and ISSR7 was 44°C, but was 47°C for ISSR1, ISSR3 and ISSR6. Optimal conditions for the reaction were as follows: initial denaturation at 94°C 5 min; 42 amplification cycles of 94°C 59 s, 44°C (47°C) 59 s and 72°C 59 s; and final extension of 7 min at 72°C. A negative control reaction without DNA template was included in all amplifications. In order to confirm the results, 50% of the samples were amplified twice. ISSR reproducibility tests (Bonin et al. 2004) included within-plate (n=12) and between-plate (n=9) replicates. The products were subjected to electrophoresis in 1.5% agarose gel stained with ethidium bromide (50 µl/100 ml) at 100V for about 1.5 h. Bands were observed and digitized using an ImageMaster VDS (Amersham Pharmacia) and Liscap Capture version 1.0 software. For analysis of band patterns, GelScan software version 1.45 (Kucharczyk, Poland) was used. Molecular weight of the resulting amplification products was determined using a calibration curve based on the bend pattern of the length of the markers (GeneRuler TM 100 bp, Fermentas).

Table 1. Primers used in the PCR-ISSR reactions.

Primer	Primer sequence
ISSR1	$(TC)_8C$
ISSR2	$(AG)_8T$
ISSR3	$(GGGTG)_3$
ISSR4	$(ATG)_6$
ISSR6	$(AC)_8G$
ISSR7	$(AC)_8T$

Data analysis

All the analyses performed were based on the following assumptions: ISSR markers behaved as dominant markers; co-migrating fragments were considered homologous loci; and populations were at Hardy-Weinberg equilibrium, in which case allele frequencies were estimated from the square root of the frequency of the null (recessive) allele (Yeh et al. 1999) or Bayesian method with non-uniform prior distribution of allele frequencies, to calculate expected heterozygosity *Hj* (Vekemans 2002). The among-population diversity was estimated using Wright's fixation index F_{ST} (Wright 1965). Statistics of genetic diversity and population genetic structure were computed after estimating allele frequencies, including percentage of polymorphic bands (PPB) at 95% criterion and expected heterozygosity or Nei's gene diversity (*h*). The calculations were performed with AFLP-SURV (Vekemans 2002).

Additionally, Shannon's diversity index (*I*) was calculated to provide a relative estimate of the degree of genetic variation within each population using POPGENE version 1.31 (Yeh et al. 1999), based on the formula $I = -\sum$ Pi log2 PI, where Pi was the frequency of each ISSR band. As an additional diversity marker, the rarity index *DW*, corresponding to "frequency-down-weighted marker values" per individual (Schönswetter & Tribsch 2005) was computed using AFLPdat (Ehrich 2006). Fixed private fragments were searched for in all investigated individuals of each species (Schönswetter et al. 2002). Analysis of molecular variance (AMOVA, Excoffier et al. 1992) was carried out using the program Arlequin version 3.11 (Excoffier et al. 2005).

The genetic division among individuals in populations of both species was estimated by means of STRUCTURE, version 2.3.3 (Pritchard et al. 2000), applying a Bayesian model-based clustering algorithm for the use of dominant markers (Falush et al. 2003). The numbers of K = 1-5 groups were tested in ten replications per each K. A burn-in period 200 000 was applied, followed by a procedure using 1 million Markov chain Monte Carlo (MCMC) repetitions (Gilbert et al. 2012). Every individual collected (excluding one of *Festuca* with missing bands, plant ID no. F4) was included in the analysis and an admixture model with uncorrelated allele frequencies was applied. The dominant ISSR data

were analysed by treating each class of genotypes as being, effectively, haploid alleles, according to the software documentation. The estimation of the optimal number of groups was based on the likelihood of partitions, estimates of posterior probability provided in STRUCTUTRE output, examined as a function of increasing K (Pritchard et al. 2000) and ΔK values, estimating the change in the likelihood function with respect to K and estimated as an indicator of the most reliable clustering structure (Evanno et al. 2005). Similarity between runs was estimated using the symmetric similarity coefficient (Nordborg et al. 2005) with the R-script Structure-sum-2011 (Ehrich et al. 2007). The function *Clones* in AFLPdat (Ehrich 2006) was used to check the clonality in the species. No clonal ramets within ISSR error rate 3-5% were found.

In the phenetic analyses, Dice distances (Nei & Li 1979) were obtained using PhylTools software (Buntjer 1997). The distance matrices were imported into the software package PHYLIP version 3.6 (Felsenstein 2005) to produce UPGMA trees using the NEIGHBOUR program. The bootstraps were then calculated in CONSENSE. The tree was displayed using MEGA version 5 (Tamura et al. 2011). Principal coordinates analysis (PCoA) was conducted to ordinate relationships among populations with Nei's distance matrix, and a minimum spanning tree (MST, Gower & Ross 1969) was calculated using NTSYS-pc version 2.11a (Rohlf 2002).

RESULTS

Festuca rubra

A total of 139 polymorphic ISSR markers were obtained from 16 plants using six primers (Table 1). These primers produced clear and reproducible fragments (error rate within 5%). Calculated from these fragments (Table 2), the polymorphism among all individuals, i.e. the percentage of polymorphic bands (PPB) for this species, was 99.3%. In four individuals that appeared after the later census period of 2005-2006, the PPB amounted to 51.8%, whereas in 12 individuals established earlier (from 1996-1997) the index reached 85.6%. At the species level, Nei's gene diversity *h* calculated over all loci was 0.238 and Shannon's diversity (*I*) index equalled 0.384. However, when comparing between the two periods the gene diversity indices (*h* and *I*) were generally lower in the recent, secondary population after 2005-2006 in comparison with the earlier period from 1996-1997, i.e. $h = 0.180$ vs. 0.231, and $I = 0.272$ vs. 0.365. The expected heterozygosity *Hj* was similar in both subpopulations and ranged within 0.271-0.275. Only the rarity index *DW* was almost the same (9.78-9.79) in the subpopulations from two periods of time.

The results of the Bayesian STRUCTURE (Fig. 2) indicated $K = 2$ as the most appropriate clustering of the individuals based on the mean *L (K)* and *ΔK* criteria (the analysis is available upon request). The average similarity coefficient among runs for $K = 2$ was 0.992 (SD = 0.004). Other *K* values were characterized by lower similarity coefficients (from

Table 2. Genetic diversity of *Empetrum nigrum* and *Festuca rubra* on Surtsey Island. Abbreviations: *n* – sample size, PPB – percentage of polymorphic bands, *I* – Shannon's information index, *h* – Nei's gene diversity (POPGENE), *Hj* - Nei's gene diversity (AFLP-SURV), *SD* – standard deviation, and *SE* – standard error.

Species	*Festuca*		*Empetrum*	
	From 1996-1997	After 2005-2006	1996-1997	2005-2006
n	12	4	6	8
PPB	85.61	51.80	77.64	78.64
h (SD)	0.231 (0.160)	0.180 (0.192)	0.290 (0.179)	0.285 (0.175)
Hj (SE)	0.271 (0.012)	0.275 (0.016)	0.303 (0.013)	0.290 (0.014)
I (SD)	0.365 (0.219)	0.272 (0.278)	0.432 (0.252)	0.428 (0.246)
DW	9.789 (1.824)	9.777 (1.007)	5.760 (1.271)	4.669 (1.017)
	$F_{ST} = 0.034$, $p = 0.007$		$F_{ST} = -0.003$, $p = 0.913$	
Total	Festuca		Empetrum	
n	16		14	
PPB	99.28		94.17	
h (SD)	0.238 (0.146)		0.235 (0.136)	
I (SD)	0.384 (0.188)		0.380 (0.180)	
DW	9.786 (1.609)		5.214 (1.271)	

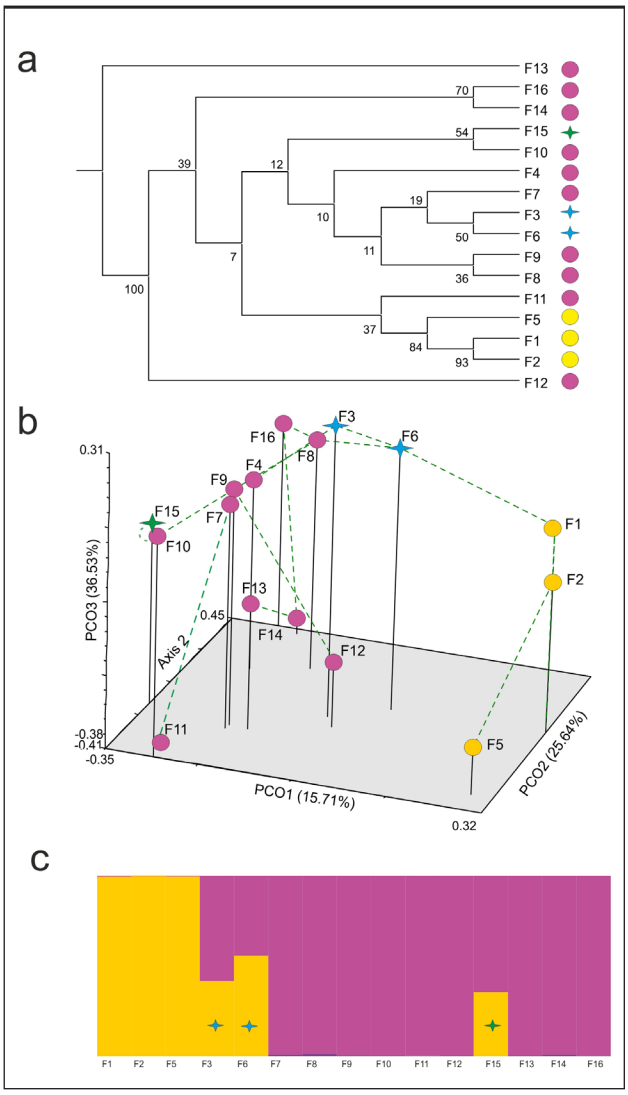

Figure 2. Phenetic and genetic variability of *Festuca rubra* (plant ID numbers F1-F16) based on ISSR data. (a) UPGMA clustering on Dice's distance matrix, bootstrap values based on 1000 runs. (b) PCoA ordination on Nei's distance matrix with Minimum Spanning Tree imposed. (c) Estimated population structure of 15 individuals (one individual removed due to missing bands) by STRUCTURE "admixture" model.

0.285 to 0.809). The first cluster included three individuals F1, F2 and F5 (Fig. 2c: genetic group 1, colour code yellow) while the second contained nine individuals F7-F16 (genetic group 2, colour code purple). Three individuals, i.e. F3, F6 and F15, could be regarded as genetic hybrids. The phenetic analyses (Figs. 2a and 2b) also showed individuals F3 and F6 as intermediates between the two genetic groups. In the UPGMA clustering, the group of putative hybrids is supported with 50% bootstrap analysis. One of their parental genotypes could be F8 (PCoA, Fig. 2b). Also, individual F15 could be of hybridogenous origin and one of its parental individuals could be F10 (UPGMA, 54% support, Figs. 2a and 2b).

The individuals F1, F2 and F5 (genetic group 1) had one fixed private band, whereas all individuals in the genetic group 2 had two fixed private bands (Table S1). The four newly established individuals in the second genetic group, F13-F16, had two additional private bands (Table S1).

The level of genetic differentiation among temporal subpopulations is low but statistically significant, as reflected in the fixation F_{ST} index of 0.034 for *Festuca* (Table 2). The AMOVA analysis contributed a statistically significant 10.53% of the total variance to the among-population component, i.e. between the two census periods (Table 3).

Empetrum nigrum

A total of 103 polymorphic ISSR markers were obtained from 14 plants with six primers (Table 1). These primers produced clear and reproducible fragments (error rate 3%). Calculated from these bands (Table 2), the percentage of polymorphic bands (PPB) for this species was 94.2%. There were no clear differences between the two subpopulations from the first (1996-1997) and second census period (2005-2006), in terms of the percentage of

Table 3. Analysis of molecular variance (AMOVA) of *Festuca rubra* and *Empetrum nigrum* on Surtsey Island. The population level includes the species occurrence in two periods: 1996-1997 and 2005-2006.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	p
Festuca rubra					
Among populations	1	37.180	2.589	10.53	0.002
Within populations	13	285.886	21.991	89.47	
Total	14	323.067	24.580		
Empetrum nigrum					
Among populations	1	16.089	0.279	1.93	0.208
Within populations	12	170.125	14.177	98.07	
Total	13	186.214	14.456		

polymorphic bands PPB (77.7 vs. 78.6), the expected heterozygosity *h* and *Hj* (0.290 vs. 0.285 and 0.303 vs. 0.290) and Shannon's diversity index *I* (0.432 vs. 0.428), respectively. The rarity index *DW* was slightly lower in 2005-2006 subpopulation and amounted to 4.67, as compared to 5.76 in the earlier period.

The results of the Bayesian STRUCTURE (Fig. 3) indicated K = 3 as the most appropriate clustering of the individuals based on the mean L (K) and ΔK criteria (the analysis is available upon request). The average similarity coefficient among runs for K = 3 was 0.996 (SD = 0.001). Other K values were characterized by lower similarity coefficients (from 0.64 to 0.96) and higher SD values. Of the three genetic groups, the first group (colour code purple)

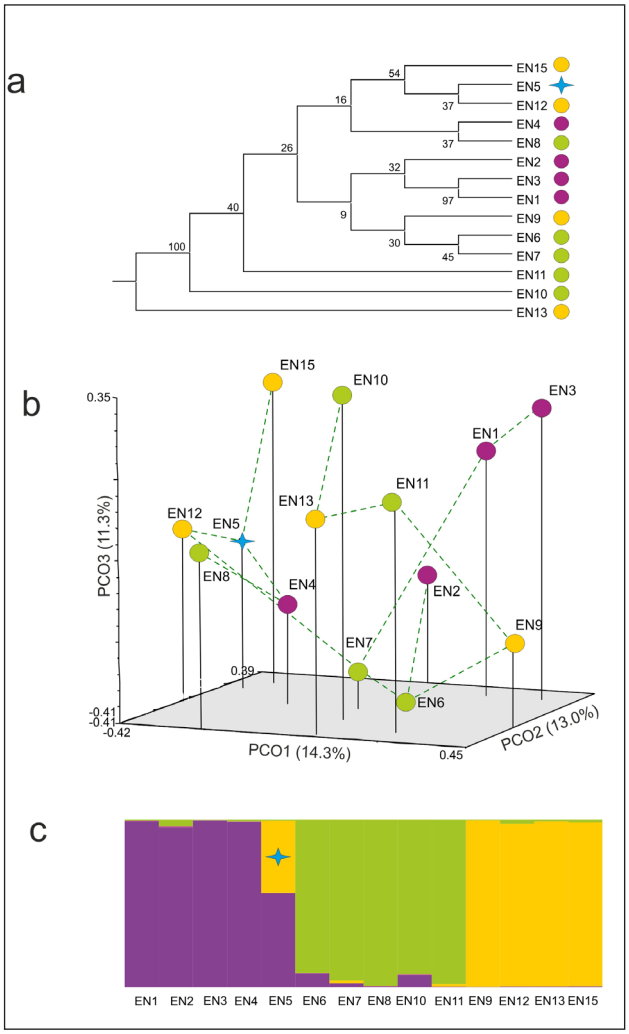

Figure 3. Phenetic and genetic variability of *Empetrum nigrum* (plant ID numbers EN1-EN15) based on ISSR data. (a) UPGMA clustering on Dice's distance matrix, bootstrap values based on 1000 runs. (b) PCoA ordination on Nei's distance matrix with Minimum Spanning Tree imposed. (c) Estimated population structure of 14 individuals by STRUCTURE "no admixture" model. Plant ID number EN14 did not exist.

included four individuals EN1-EN4 and one putative hybridogenous EN5 (Fig. 3c). However, only EN1 and EN3 were highly supported with 97% bootstrap values (Fig. 3a). The putative hybrid EN5 was genetically close to EN12 and EN15 (54% support), but also to EN4 (Fig. 3a). The next two groups (colour codes green and yellow) consisted of genetically pure individuals with a negligible admixture of alien alleles (Fig. 3c). The phenetic analyses UPGMA (Fig. 3a) and PCoA (Fig. 3b) pointed to weak genetic signals as some individuals (EN4, EN8, EN9) were intermingled within different genetic groups, however with a weak support (30-37%). Two individuals from inside the crater far from the main vegetated gull area, EN13 and EN15, belonged to the same genetic group (Fig. 3c).

There were no fixed private bands in the three genetic groups, but two individuals EN4 and EN5 shared one private band (Table S2). These two samples represented the population established early, i.e. at least in 1996-1997. The AMOVA analysis (Table 3) showed only 1.93% and statistically insignificant (p = 0.208) part of the total genetic variance that was attributed to among-population diversity.

DISCUSSION

Genetic structure of the grass Festuca rubra *on Surtsey*

The results of Bayesian analysis (STRUCTURE) of the grass species on the island of Surtsey are in accordance with its temporal and spatial organization. Two genetic groups from genetically different stocks were distinguished. The spatial and temporal subdivision of the population was supported by AMOVA analysis and F_{ST} index. The results also indicated hybridization between genetic groups. Significant population subdivision and the presence of some unique ISSR bands are in general attributed to selfing (Culley & Wolfe 2001). But for the wind-pollinated *Festuca* the subdivision is more likely to be associated with the founder effect from various genetic pools, and in this case from sources outside the island.

The first genetic group can be attributed to the early colonization phase, i.e. before 1996-1997, and the individuals in this group (F1, F2 and F5) were actually from the area of the earliest successful colonization of the species (Fridriksson 1978), the area which was part of the permanent plot no. 1

established in 1990 (Magnússon et al. 1996). The second and more extensive genetic group consists of individuals from both inside and outside the vegetated gull area. The samples from the marginal habitat outside the gull area (F13-F15) were collected from small and newly established (after the census period 2005-2006) but spatially separated (at least 20m apart) individual clusters of *F. rubra* scattering over almost barren lava by the sea. The STRUCTURE analysis indicated that this marginal subgroup most probably originated *in situ* (on the island) from the older and denser population in the gull area. The newly established group showed lower values of the genetic diversity indices, and especially the percentage of polymorphic bands PPB, than the earlier group, but no difference in the rarity index *DW* was found. This reduced genetic diversity is a clear sign of the genetic bottleneck effect occurring on the island.

According to the vegetation monitoring records, the spread of *Festuca* coincided with the expanding range of sea gulls nesting in the area, especially in the period 1985-1994 defined in Baldursson & Ingadóttir (2007) as "new wave of colonization and succession facilitated by breeding gulls" as opposed to the earlier period (1965-1974) of "the invasion of coastal species on the empty island". Initially the ground substrate in this gull area was barren lava with some tephra sand, but the architecture of lava sheets is believed to have provided shelter for nesting of the first breeding pairs of sea gulls (Magnússon et al. 1996). Although *Festuca* was spotted on the island as early as 1973, the founders during the first six years were only very few seedlings, most of which did not survive in the following years, either due to the windy and cold conditions or the first gulls gathering all of the grass for use as nesting material (Fridriksson 1982). It was not until 1978 that the *Festuca* population became established and began flowering on the island. However, what followed was still a slow progress of vegetation development, possibly due to the lack of essential soil nutrients. But as the gull colony increased drastically in size from ten nests in 1986 to 180 nests by the summer of 1990, new founders as well as old survivors became established and the grass expanded into dense turfs. The vegetation cover on Surtsey was found to be strongly related to density of gull nests, driven by the significant increase in soil respiration, soil carbon, nitrogen and the C: N ratio (Sigurdsson & Magnússon 2010, Magnússon et al. 2014). The first census period shown in the present study (1995-1996) reflects the rapid expansion of *Festuca* from the initial phase of colonization inside the gull area. The population then became dense in the second period (2005-2006), after which *F. rubra* had spread out of the gull area and colonized marginal habitats.

Studies of *Festuca* species from subarctic-alpine regions show highly variable levels of genetic diversity. Average gene diversity *h* based on AFLP analysis of 81 local populations and cultivars of *F. pratensis* from Norway and Lithuania varied from as low as 0.090 in a population near agricultural fields with active gene flow from meadows to a high level of 0.254 in an isolated population in a wooded area (Fjellheim et al. 2009). At the species level, the population of *Festuca* on Surtsey falls in the upper limit of the genetic diversity index ($h = 0.180 - 0.231$). The study of *F. pratensis* growing on the islands of Lake Onega, Russia (Fedorenko et al. 2009), reveals lower levels of genetic diversity based on 64 RAPD loci ($h = 0.093$) than those found in *F. rubra* on Surtsey and much less loci polymorphism (PPB only 30% vs. 51.8 - 85.6% for Surtsey). Although it is not ideal to compare levels of genetic diversity among populations based on different methodology and marker types, there is a clear indication that genetic diversity of *Festuca* in the Surtsey populations is on the higher end of the spectrum. A recent study on spatial genetic diversity of the coastal plant species, *Honckenya peploides,* in relation to populations from a nearby island and from the southern coast of Iceland revealed a high level of gene diversity on Surtsey compared to its source populations (Árnason et al. 2014). Together with the Bayesian analysis of the species, the authors have interpreted the results as an indication of multiple origins of immigrants and active colonization especially during the early stage of population establishment on an empty island. This may be the case for *Festuca* as well, particularly regarding the early colonization and establishment on Surtsey. Successful invaders, especially grasses and herbaceous weeds, can be characterized as "gap grabbers" (early germinators with fast initial growth), competitors (for resources) and survivors (Sakai et al. 2001). *Festuca rubra* has been spreading relatively fast on Surtsey, especially in the nutrient-rich gull area.

Colonization of the late arrival shrub Empetrum nigrum *on Surtsey*

Empetrum nigrum was found on the island for the first time in 1993, right at the end of the period known as "gull invasion and vegetation enhancement" and at the beginning of the period "secondary plant colonization and first breeding of land birds" with other shrubs such as *Salix* species following suite (Fridriksson 2000; Magnússon et al. 2009). At the time of the sampling for the present study in 2010, only one *E. nigrum* individual (EN7) produced berries for the first time on the island. All individuals on the island found that year were therefore first generation immigrants.

The genetic diversity of *E. nigrum* on Surtsey was generally high (h = 0.235) and to some extent comparable to the range of the grass *F. rubra*. But unlike *Festuca*, almost 100% of the genetic variability of this evergreen shrub species was attributed to the within-population genetic component, a feature characteristic of highly out-crossing, wind- or mixed-wind pollinated, late-successional and long-lived perennials (Hamrick & Godt 1989). Populations of *E. nigrum* subsp. *hermaphroditum* in northern Sweden also showed high levels of genetic variation (Szmidt et al. 2002). The percent of polymorphic loci and diversity indices were the highest in the youngest population (c. 150 year-old), with the clonal fraction also being the lowest there. This, along with the large number of unique genotypes observed, indicated that clonal propagation had minor significance in comparison to seedling recruitment in the initial colonization. In other early successional communities, rapid population expansion of *Empetrum* from seedling recruitment has been documented (Boudreau et al. 2010). But island colonization of Japanese crowberry (*E. nigrum* subsp. *japonicum*) showed the opposite, i.e. very low gene diversity (Chung et al. 2013), a typical situation of genetic depauperation expected among isolated oceanic islands when compared with their mainland conspecifics (Wright 1940; Whittaker et al. 2008). As for the case of Surtsey reported here, *Empetrum* had just begun its sexual propagation on the island and therefore nearly all of its genetic diversity should be similar to that of the source population(s), therefore not depauperated. Indeed the level of gene diversity in *E. nigrum* on Surtsey is similar to, or even higher, not lower, than that found by Jacobsen (2005) for populations on mainland Iceland or elsewhere across the North Atlantic regions. Furthermore, the history of colonization on Surtsey was so short for this long-lived perennial, merely 17 years, that the species had not as yet experienced any founder or bottleneck effects, selection or adaptation. *Empetrum* is a slow-growing plant and in boreal forest ecosystems both sexual and asexual propagation allow colonization of vacant sites, though it can be 100 years or more before it dominates the site (Szmidt et al. 2002).

In *E. nigrum* virtually no clear differences in genetic diversity indices were found in either the temporal or spatial population subdivisions. Neither the results of AMOVA analysis nor F_{ST} index were statistically significant. The spatial genetic structure of *Empetrum* population on Surtsey appeared to be more uniform when compared to *Festuca*. Although the admixture analysis produced three main genetic subpopulations from *Empetrum* samples, this genetic division based on Bayesian inference was not supported by the genetic analysis of variance, as the among-population differentiation revealed only 1.93% of the total variance, in contrast to the highly significant 10.53% variation among *Festuca* subpopulations. Even so, it is an indication that the genetic groups derived from different colonization episodes. The first colonization episodes (seen in the census period 1996-1997) involved two spatially separated genetic groups. One of these groups includes individuals from inside the western crater about 600 m northwest of the gull area where all other *Empetrum* samples in this study were taken. An enigma is the appearance of a hybrid or a mixed type (EN5) before the arrival of one of the putative parental groups (the third group, which appeared in the period 2005-2006). The hybridisation must have taken place in the original, source population. As the spatial subdivision of the population on the island into the older and younger subpopulations was not statistically supported, the existence of the genetic groups can only be a relic of the source population(s). Thus, no founder effect was found.

The most likely source locations are along the southern part of Iceland as well as from the nearby island of Heimaey. This dioecious subspecies of *E. nigrum* is common in lowland and coastal areas, especially in the south of Iceland (Kristinsson 2005). Surtsey is only 30 km away from the nearest point of the southern coast of mainland Iceland. This plant is believed to have colonized Surtsey by means of seed dispersal most probably via ingestion by birds such

as snow buntings and ravens (passerines), which became more common among breeding birds during the secondary phase of plant colonization on the island (Petersen 2009). The AFLP analysis of bird-dispersed *E. nigrum* by Jacobsen (2005) indicated that the species colonized Svalbard in the first place from East Greenland, which in turn was colonized from West Siberia source populations. Popp et al. (2011) used molecular tools to demonstrate that long-distance dispersal by birds (i.e. from northwest N-America to the southern hemisphere) could explain the extreme bipolar disjunction in *Empetrum*. Seed dispersal by birds is highly effective and can reach farther than previously thought.

Grass versus shrub

Both *Festuca rubra* and *Empetrum nigrum* probably colonized Surtsey via bird-facilitated seed dispersal, directly (bird as seed carrier) or indirectly (site amelioration by breeding gulls), and the initial seedling (sexual) recruitments seem to have been highly successful. About 75% of the plant species colonizing Surtsey were brought to the island by birds, the rest by sea currents and wind (Magnússon et al. 2009, 2014). As a result of colonization and rapid expansion on the island of Surtsey, although at different time periods, both species are genetically divergent at the species level. At the time of study the grass species *Festuca* had formed a genetic melting pot, i.e. a place where various genetic lineages met, whereas the shrub *Empetrum* could be characterized as near-panmictic populations, however still reflecting the genetic structure of the source population.

No signs of reduced genetic variability are found in the two species under study apart from the secondary subpopulation of *Festuca* deriving from the *in situ* genetic material. The limited sample size and the lack of reference materials do not allow a conclusive statement to be made regarding genetic diversity in the source populations, which are presumably from mainland Iceland and other islands in the Vestmannaeyjar archipelago. It should be noted that *Festuca* is a dominant species on all islands of the archipelago, whereas *Empetrum* is only found on Surtsey and the largest island, Heimaey (Magnússon et al. 2014). In any case, it may be reasonable to assume that genetic diversity of both *Festuca* and *Empetrum* on Surtsey is not depleted when compared to the source populations, a case similar to that which was found for the early colonizing coastal species *Honckenya peploides* on Surtsey (Árnason et al. 2014). Active colonization of a species, i.e. by multiple or repeated introductions of genetic material, is thought to be one of the main factors ameliorating losses of gene diversity in founding populations (Dlugosch & Parker 2008). But in the case of the long-lived shrub species *Empetrum*, the relatively high level of genetic diversity discovered in the present study is clearly a relic of the gene diversity brought by the initial immigrants. Due to Surtsey's small size, young age and fast-eroding nature (Jakobsson & Gudmundsson 2003), we predict that the species will in the near future suffer from reductions in genetic diversity and adaptive potential. Both physical dimensions and biological characteristics are important factors influencing levels of genetic variation in plant species found on oceanic islands (Whittaker et al. 2008; Stuessy et al. 2014). The present study shows that genetic diversity is already drastically reduced in the secondary subpopulation of the grass *Festuca*, due to founder and bottleneck effects.

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Appendix

Table S1. Block-structure of ISSR bands across genetic groups delimited by STRUCTURE in *Festuca rubra.*

population	2	1	5	7	3	6	9	0	1	1	8	2	5	3	4	6
band no.					x	x							x			
76	*	*	*		*	*						*	*	*		
127	*	*	*		*	*	*					*				
124	*	*	*			*		*								*
48	*	*	*		*									*	*	
52	*	*	*													
55				*	*	*	*	*	*	*	*	*	*	*	*	*
53				*	*	*	*	*	*	*	*	*	*	*	*	*
33				*	*	*	*			*			*			
47				*		*	*					*	*		*	
30				*	*	*						*	*	*		
114								*	*			*		*		*
121									*				*	*		*
50												*		*	*	
111														*	*	
39				*			*	*	*	*						
120						*	*	*								*
29							*	*		*						*
131									*							*
102													*	*	*	*
69													*	*	*	*

Table S2. Block-structure of ISSR bands across genetic groups delimited by STRUCTURE in Empetrum nigrum.

	1	9	3	1	2	5	8	4	5	1	3	2	6	7	1	1	0
									x								
3 5								*	*								
3 3								*	*	*							
2 8					*	*											
4 2					*		*										
5 8					*	*	*	*									
1 4		*		*	*	*											
9 9	*			*	*	*		*	*				*	*			
7 3					*	*		*	*				*	*			
2					*		*	*	*				*	*			
5 2					*	*			*					*			
8 7					*	*	*	*	*			*	*				
1 0 2						*				*	*	*					
1 0 0						*				*	*	*					*
3						*				*	*						
3 1						*				*	*	*	*	*			
3 0							*	*		*							
5 6	*							*		*	*						
2 6	*							*		*	*				*		
4 9	*	*								*	*		*		*		
6 9		*			*	*		*	*							*	*
2 9	*	*				*		*	*	*	*	*	*	*	*	*	*
8 1	*	*		*	*	*		*	*			*	*	*	*	*	*
6 4	*	*		*	*	*		*	*			*	*	*	*		
1 0 3	*	*		*		*		*					*	*	*	*	*
1 0 1	*	*		*		*		*	*				*	*	*	*	*
3 7		*													*	*	
2 2	*	*											*	*			
7 4	*											*			*		
5 1	*											*	*		*		