

# Comparison of genetic diversity in the two arctic–alpine plants *Diapensia lapponica* var. *obovata* (Diapensiaceae) and *Empetrum nigrum* var. *japonicum* (Empetraceae) between Sakhalin in Russian Far East and Jeju Island in Korea, the southernmost edge of their distribution range

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**Abstract** We compared allozyme variation in the two arctic–alpine plants *Diapensia lapponica* var. *obovata* and *Empetrum nigrum* var. *japonicum* between Sakhalin Island in Russian Far East, within their range core, and the Korean island of Jeju, their world’s southernmost distribution. For *D. lapponica* var. *obovata*, Sakhalin populations harbored moderate levels of within-population genetic variation and

low among-population divergence, whereas extremely low levels of within-population genetic diversity and high among-population differentiation were found in Jeju Island populations. In contrast, we found moderate levels of within-population variation and low among-population differentiation in *E. nigrum* var. *japonicum* in both northern populations (those of Sakhalin and an additional population from northern Japan) and Jeju Island populations. Under a similar scenario of immigration history of arctic–alpine plants on Jeju Island during the glacial periods of the Pleistocene and local persistence through glacial/interglacial cycles, the contrasting genetic structure between *D. lapponica* var. *obovata* and *E. nigrum* var. *japonicum* is mainly attributable to their different life-history, ecological, and demographic traits: (1) hermaphroditic versus monoecious, dioecious or polygamous, (2) seeds with no adaptations for long-distance dispersal versus berry-like drupes dispersed by animals and birds, and (3) a very small patch near the peak of Mt. Halla with a few hundred individuals versus a relatively continuous distribution around the peak of Mt. Halla with numerous individuals. From a conservation perspective, in situ and ex situ conservation measures should be strengthened for *D. lapponica* var. *obovata* on Jeju Island given their extreme rarity there.

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## Introduction

The Quaternary glacial–interglacial oscillations may have played an important role in shaping the current distribution of plant species and, thus, the amount and distribution of

genetic diversity within and among populations (Hewitt 1996, 2004; Fujii and Senni 2006; Hu et al. 2009; Ohsawa and Ide 2011; Qiu et al. 2011). Arctic-alpine plant species are widely distributed throughout the world, extending through the circum-Arctic region and southwards into the high mountains of Europe, North America and Asia. During the Quaternary period, these species experienced drastic changes in their distribution, tracking the climatic changes (Hewitt 2004). In fact, the genetic imprints of the Quaternary climatic oscillations have been clearly detected for a number of arctic–alpine plants in Europe, North America, and Asia (DeChaine and Martin 2005; Albach et al. 2006; Fujii and Senni 2006; Skrede et al. 2006; Birks and Willis 2008 and references therein; Ikeda et al. 2008 and references therein; Marr et al. 2008; Shimonon et al. 2010; Ohsawa and Ide 2011 and references therein). Analyses of genetic structure of arctic–alpine plants along with pollen records have been employed to infer the location of glacial refugia, post-glacial migration routes, and the history of past distribution changes (Hewitt 1999, 2000; Abbott et al. 2000; Arbogast and Kenagy 2001; Birks and Willis 2008 and references therein; Ikeda et al. 2008 and references therein; Semerikova et al. 2011).

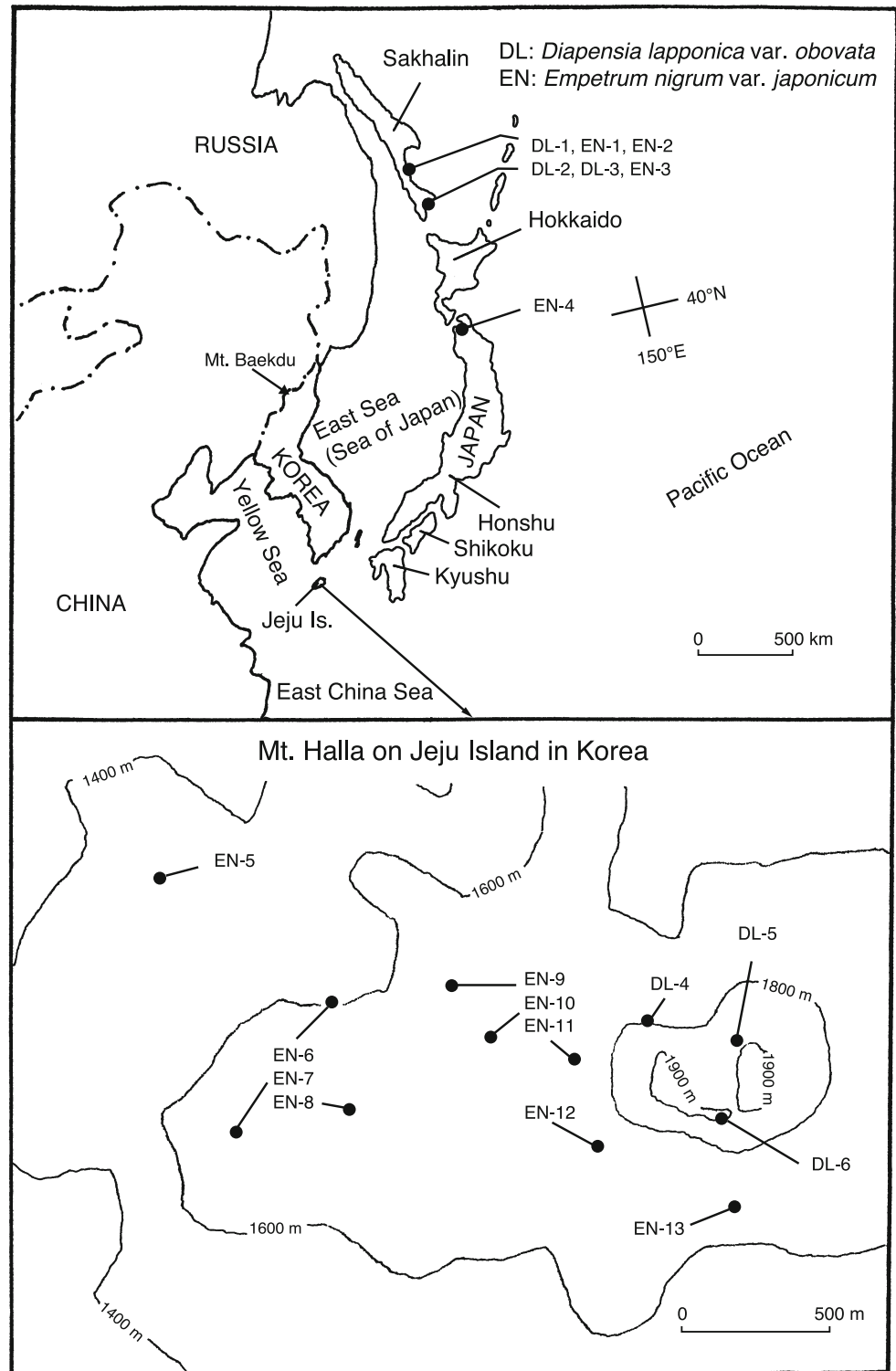
Although many studies on population genetic structure of arctic–alpine plants are available in the literature, little is known about levels of genetic diversity in populations located at the southernmost edge of their range with the notable exception of populations within the European mountain systems (Schönswetter et al. 2003, 2005; Birks and Willis 2008; Kropf et al. 2008; Schmitt et al. 2010). Southern populations of arctic–alpine plants are, however, very significant both in evolutionary and conservation terms, as (1) they would served as ‘interglacial’ refugia and even for some cases ‘long-term’ refugia (sensu Stewart and Dalén 2008; i.e., both during the glacial and interglacial periods) and (2) most species in these southern refugia constitute clear disjunctions from their northern populations occurring in ‘polar refugia’ (Birks and Willis 2008; Stewart et al. 2010). In other words, and taking Europe as the best-studied geographic area (e.g., Birks and Willis 2008; Schmitt et al. 2010), many cold-adapted species such as arctic-alpine species (e.g., *Dryas octopetala*) that during glacial periods would have been widespread on the open habitats throughout most of the continent, today occur disjunctly in northern latitudes and then scatteredly in some southern mountains (the Pyrenees, the Alps, or the Carpathians) at high elevations, forming ‘relict’ populations (Bennett and Provan 2008; Birks and Willis 2008; Stewart et al. 2010). A population-genetics study in highly isolated populations of arctic–alpine species could, thus, provide insights into genetic consequences of long-term habitat fragmentation and isolation of populations for plant species in general (Young et al. 1996).

The two small shrubs *Diapensia lapponica* L. var. *obovata* F. Schmidt (Diapensiaceae) and *Empetrum nigrum* L. var. *japonicum* K. Koch (Empetraceae) are widely distributed in both arctic and alpine regions of north-eastern Asia (Kim J-H 2007; Kim K-J 2007; Ikeda et al. 2008). The two taxa are relatively common in Russian Far East (including Sakhalin Island), north-eastern China (Manchuria), and central and northern Honshu and Hokkaido in the Japanese Archipelago. Interestingly, these two arctic–alpine plants reappear in a single disjunct location on Mt. Halla (its peak is 1,950 m above sea level, a.s.l.) on the subtropical Jeju Island off the southern coast of the Korean Peninsula (Fig. 1). Jeju populations constitute the world’s southernmost limit of their distribution range, and the island has been suggested to be a putative Quaternary refugium for arctic–alpine plants (Kong 1998a, b, 1999; Dolezal et al. 2012). Populations of these taxa on Jeju Island are highly isolated from their nearest conspecific ones, which are those of Mt. Baekdu (2,744 m a.s.l., located very close to the border between northern Korea and north-eastern China; Fig. 1), the only known occurrences in mainland Korea, and those from central Honshu in Japan; both distribution areas are separated from Jeju by a linear distance of ca. 1,000 km (Fig. 1).

It has been hypothesized that the distribution range of these two taxa, along with other arctic–alpine elements, would have extended downhill and southward in the Korean Peninsula during the Pleistocene glacial periods, in a similar fashion to what has been described for Japan (Ikeda et al. 2008); subsequently, their former continuous range would have been divided into fragments (i.e., by species’ retreat to mountaintops) as the climate ameliorated during the post-glacial warming (Kong and Watts 1993; Kong 1998a, b, 1999). Recent molecular phylogeographic studies have revealed that high mountains in central Honshu (Japan) were the most important refugia for many arctic–alpine plants along glacial/interglacial climatic oscillations thanks to altitudinal shifts (Fujii and Senni 2006; Ikeda et al. 2008 and references therein). Thus, it is highly likely that the disjunctly distributed *D. lapponica* var. *obovata* and *E. nigrum* var. *japonicum* at the top of Mt. Halla on Jeju Island were also present, together with other arctic–alpine plants, in the mountain during the glacial periods, probably at lower elevations (Kong 1998a, b, 1999).

In addition to historical factors (e.g., past climatic oscillations), arctic–alpine plant species’ life-history and ecological traits could influence contemporary levels of within-population genetic variation and among-population genetic divergence (Loveless and Hamrick 1984; Hamrick and Godt 1989). Species with high rates of outcrossing, wind pollination, wind- or animal-mediated seed dispersal, high fecundities, and long generation times usually maintain high intra-population genetic variation and low

**Fig. 1** Locations of sampled populations of *Diapensia lapponica* var. *obovata* and *Empetrum nigrum* var. *japonicum* from Sakhalin Island in Far East Russia and Jeju Island in Korea. Note that Jeju Island is the southernmost limit of the two alpine taxa range, and that one population of *E. nigrum* var. *japonicum* from northern Japan (EN-4) has also been sampled



inter-population differentiation. Geographically central populations within a species' range tend to harbor higher levels of within-population genetic variation than marginal or highly isolated populations, whereas among-population differentiation shows a reverse trend (Eckert et al. 2008). In

addition, large populations with contiguous distribution generally harbor higher levels of within-population genetic variation and lower among-population genetic differentiation than small populations with highly isolated distribution (Chung and Kang 1994). Nevertheless, it should be

noted here that current distribution ranges and population sizes of plant species are highly dependent on past events (Davis and Shaw 2001; Hewitt 2004; Hu et al. 2009).

*Diapensia lapponica* var. *obovata* is characterized by having bisexual flowers (hermaphroditic), insect-pollination by bumblebees and members of Diptera, and seeds with no adaptations for long-distance dispersal (Bergman et al. 1996; Suh et al. 2001; Aiken et al. 2007). On Jeju Island, it is extremely restricted to the peak of Mt. Halla at elevations from 1,800 to 1,900 m, and grows in rather inaccessible crevices on steep cliffs. The number of estimated individuals is only ca. 400 within an area of 0.003 km<sup>2</sup> (Suh et al. 2001). In contrast, *E. nigrum* var. *japonicum* is a monoecious, dioecious or polygamous small shrub with unisexual or bisexual flowers, wind-pollinated (i.e., outcrossing rates should be high), and with their seeds dispersed by animals and birds (Bell and Tallis 1973; Guitián et al. 1994; Lee et al. 1997). On Jeju Island, it is common and continuously distributed around the peak of Mt. Halla at elevations from 1,500 to 1,800 m, with an the estimated occurrence area of ca. 8 km<sup>2</sup>.

The effect of Quaternary climatic oscillations and the role of Mt. Halla populations as Pleistocene refugia on the genetic make-up of both taxa are difficult to predict. Populations of the two taxa on Jeju Island are highly isolated and marginally distributed regarding their neighboring occurrences, and their post-glacial retreat to high elevations (i.e., mountaintops) would have caused genetic drift and genetic isolation. Given this, a first prediction is that they should harbor lower levels of genetic variation within populations and a higher degree of among-population genetic differentiation compared to the populations in northern East Asia, where both taxa are relatively common, with large and continuously distributed localities. Alternatively, one may expect relatively high levels of genetic diversity for populations of the two taxa on Jeju Island, as they would constitute refugia during the Quaternary climatic oscillations. On the basis of the different life-history, ecological and demographic traits for the two taxa, a second prediction would be that levels of within-population genetic variation and degree of among-population differentiation in *D. lapponica* var. *obovata* on Jeju Island are lower and higher than those in *E. nigrum* var. *japonicum* on the same Island, respectively.

In this study, we surveyed the levels and distribution of genetic diversity in these two taxa both on Jeju Island and on Sakhalin Island in Russian Far East (i.e., a location within the core distribution area) to test our predictions. We have also used this genetic information to draw up recommendations for the recovery and management of the rare, endangered *D. lapponica* var. *obovata* on Jeju Island.

## Materials and methods

### Study plants

*Diapensia lapponica* var. *obovata* occurs on dry slopes and rocky cliffs in arctic, subarctic, and alpine regions of Kamchatka, the Kuril Islands, Sakhalin Island, east Siberia, north-eastern China (Jilin Province), Japan (central and northern Honshu, and Hokkaido), the northernmost parts in high-elevation mountains (Mt. Baekdu and adjacent mountains) in mainland Korea, and Jeju Island off the southern coast of the Korean Peninsula; out of Asia, this taxon is also found in Alaska and north-western Canada (Scott and Day 1983; Kim K-J 2007). It is a hermaphroditic, dwarf, evergreen, and densely matted subshrub (1–3 cm high). Peduncles are short (1–2 cm high) and solitary, and bear a white flower (1.0–1.5 cm long in diameter), produced during June and July. Fruits (capsules, ca. 3 mm long) contain many small seeds (70–90 per capsule).

*Empetrum nigrum* var. *japonicum* also occurs on Kamchatka, Sakhalin Island, east Siberia, north-eastern China (Heilongjiang and Inner Mongolia provinces), Japan (central and northern Honshu, and Hokkaido), Mt. Baekdu and adjacent mountains in northern Korea, and Jeju Island (Min and Anderberg 2005; Kim J-H 2007). It is a monoecious, dioecious or polygamous, small, evergreen, procumbent shrub (10–20 cm high), forming dense mats. Small purple flowers are axillary, sessile, unisexual or bisexual, and produced between June and July. Fruits (berry-like drupes, ca. 6 mm long in diameter) contain 6–9 nutlets.

### Population sampling

On Sakhalin, *D. lapponica* var. *obovata* and *E. nigrum* var. *japonicum* commonly occur on open places along ridgelines at elevations from 100 to 900 m, and grow together with the two shrubs *Vaccinium vitis-idaea* and *Thymus quinquecostatus*, the fern ally *Lycopodium complanatum*, and several herbaceous perennials. On Jeju Island, however, *D. lapponica* var. *obovata* is restricted to north, south, and north-west cliffs near the peak of Mt. Halla, and grows together with the shrub *Rhododendron mucronulatum* var. *ciliatum* and several herbaceous perennials. *Empetrum nigrum* var. *japonicum* on Jeju Island occurs commonly on relatively open habitats with *Abies koreana*, several shrubs (*Ilex crenata*, *Juniperus chinensis* var. *sargentii*, *R. mucronulatum* var. *ciliatum*, *R. yedoense*, and *Vaccinium japonicum*), and several herbaceous perennials.

In total, 157 leaf samples of *D. lapponica* var. *obovata* were collected from three populations from Sakhalin Island (DL-1 to DL-3,  $n = 51$ ; Fig. 1; sampling date: September 2008) and three more from Jeju Island (DL-4 to DL-6,

$n = 106$ ; Fig. 1; sampling date: October 2006). Populations DL-2 and DL-3 are geographically very close (the linear distance between the two populations is ca. 300 m). However, we considered these two as separate populations because there are no individuals between them. For *E. nigrum* var. *japonicum*, a total of 573 leaf samples were collected from three populations from Sakhalin (EN-1 to EN-3,  $n = 78$ ; Fig. 1; sampling date: September 2008), one from northern Japan (on an open rocky area dominated by *Gaultheria pyroloides* near Hakkoda Botanical Garden, at the northern tip of Honshu Island, EN-4,  $n = 30$ ; Fig. 1; sampling date: September 2009), and nine populations (EN-5 to EN-13,  $n = 465$ ; Fig. 1; sampling date: August 2008 and October 2009) from Jeju Island. Although *E. nigrum* var. *japonicum* also reproduces by procumbent stems, each distinct patch represents a different genet (a genetically different individual) based on a previous clonal analysis (Choi et al. 2004). Thus, each sample was randomly collected from spatially separated distinct patches to avoid collection of the same ramets (at 2–5 m intervals). To minimize the damage to these plants, we used leaf materials taken from one leaf for *D. lapponica* var. *obovata* and just 1 cm cut from the tip of the stem for *E. nigrum* var. *japonicum*.

#### Enzyme electrophoresis

Leaf samples were wrapped in damp paper towels, placed in plastic bags, returned to the laboratory, and then stored at 4 °C until protein extraction. For extraction, leaf samples were crushed using chilled mortars and pestles by adding a crushing buffer (Mitton et al. 1979) and enzyme extracts were absorbed onto 4 × 6 mm paper wicks (Whatman 3MM chromatography paper). Starch gels (13 %) were stained for 14 enzyme systems, which were alcohol dehydrogenase (ADH), cathodal peroxidase (CPX), diaphorase (DIA), fluorescent esterase (FE), fructose-1,6-diphosphatase (F1,6), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), and triosephosphate isomerase (TPI). Stain recipes were taken from Soltis et al. (1983), except for DIA (Cheliak and Pitel 1984). Putative loci were designated sequentially, with the most anodally migrating isozyme designated as 1, the next 2, and so on. Alleles were also designated sequentially with the most anodally migrating allele designated as superscript *a*, the next *b*, and so on. Banding patterns of each enzyme were interpreted on the basis of the known quaternary structure of enzymes (Weeden and Wendel 1989). Two buffer systems were used to resolve a total of 23 putative loci for each taxon (Table 1).

**Table 1** The allozyme loci assayed for *Diapensia lapponica* var. *obovata* and *Empetrum nigrum* var. *japonicum* and the buffer systems used to resolved them

| Taxon  | Buffer system | Loci resolved   |
|--|---------------|---|
| <i>Diapensia lapponica</i> var. <i>obovata</i> | 6             | <i>Adh</i> , <i>Cpx</i> , <i>Dia-1</i> , <i>Dia-2</i> , <i>Fe-1</i> , <i>Fe-2</i> , <i>Fe-3</i> , <i>Lap</i> , <i>Me</i> , <i>Pgi-1</i> , <i>Pgi-2</i> , <i>Pgm-1</i> , <i>Pgm-2</i> , <i>Pgm-3</i> , <i>Tpi-1</i> , <i>Tpi-2</i> |
|  | MC            | <i>Idh-1</i> , <i>Idh-2</i> , <i>Mdh-1</i> , <i>Mdh-2</i> , <i>Mdh-3</i> , <i>6Pgd-1</i> , <i>6Pgd-2</i>  |
| <i>Empetrum nigrum</i> var. <i>japonicum</i>   | 6             | <i>Adh-1</i> , <i>Adh-2</i> , <i>Cpx</i> , <i>Dia-1</i> , <i>Dia-2</i> , <i>Fe-1</i> , <i>Fe-2</i> , <i>Lap</i> , <i>Me</i> , <i>Pgi</i> , <i>Pgm-1</i> , <i>Pgm-2</i> , <i>Tpi-1</i> , <i>Tpi-2</i>                              |
|  | MC            | <i>F1,6-1</i> , <i>F1,6-2</i> , <i>Idh</i> , <i>Mdh-1</i> , <i>Mdh-2</i> , <i>Mdh-3</i> , <i>6Pgd-1</i> , <i>6Pgd-2</i> , <i>Skdh</i>   |

The meaning of the abbreviations for the loci are given in the “Materials and methods” section. We used a the modification of Haufler (1985) of the system 6 of Soltis et al. (1983), and the morpholine-citrate system, MC (pH 6.1) was taken from Clayton and Tretiak (1972)

#### Data analysis

To estimate genetic diversity and structure, we considered that a locus was polymorphic when two or more alleles were observed, regardless of their frequencies. We estimated the following genetic diversity parameters using the programs POPGENE (Yeh et al. 1999) and FSTAT (Goudet 1995): percent polymorphic loci (% *P*), mean number of alleles per locus (*A*), allelic richness (*AR*) using a rarefaction method that compensates uneven population sample sizes (Hurlbert 1971; El Mousadik and Petit 1996), mean number of alleles per polymorphic locus (*AP*); observed heterozygosity (*H<sub>o</sub>*), and Nei’s (1978) unbiased gene diversity or Hardy–Weinberg (*H–W*) expected heterozygosity (*H<sub>e</sub>*). To test for differences between Sakhalin and Jeju islands for observed statistics *OSx* (e.g., *AR*, *H<sub>o</sub>*, and *H<sub>e</sub>*), we used a permutation scheme (15,000 replicates) by randomly allocating whole samples to the different groups, keeping the number of samples in each group constant and calculating differences between Sakhalin and Jeju islands for randomized statistics *RSx*. We then obtained the *P* value of the test as the proportion of randomized data sets giving a larger *RSx* than the observed *OSx*. These calculations were performed using FSTAT (Goudet 1995). To test for recent decreases in effective population size (bottlenecks), we evaluated differences across loci between the *H–W H<sub>e</sub>* and the equilibrium heterozygosity (*H<sub>eq</sub>*) expected from the number of alleles assuming mutation-drift equilibrium. These differences were evaluated using a sign test and a Wilcoxon sign-rank test conducted under an infinite allele model using the program BOTTLENECK (Cornuet and

Luikart 1996). Since allelic diversity is generally lost more rapidly than  $H_e$  (Nei et al. 1975), recently bottlenecked populations will exhibit an excess of H–W  $H_e$  relative to  $H_{eq}$  (Cornuet and Luikart 1996; Luikart et al. 1998).

Mean fixation indices ( $F_{IS}$ ) across polymorphic loci were estimated for each population, and their levels of significance ( $P$  values) were calculated by gene permutation tests (999 replicates) under the null hypothesis ( $F_{IS} = 0$ ) using the program SPAGeDi (Hardy and Vekemans 2002). To measure the average level (grand mean) of inbreeding within populations and genetic differentiation among them, Wright's (1965)  $F_{IS}$  and  $F_{ST}$  were respectively estimated, according to the method of Weir and Cockerham (1984). We used the FSTAT (Goudet 1995) to construct 95 % CI by bootstrapping over loci (999 replicates) around mean of the  $F_{IS}$  and  $F_{ST}$ , and considered their observed estimates to be significant when 95 % CI did not overlap zero. To test for differences between groups (Sakhalin Island vs. Jeju Island) for means of  $F_{IS}$  and  $F_{ST}$ , we also used the permutation approach (15,000 replicates) outlined above. To test for the influence of individuals within populations, populations within regions, and regions on the observed genetic variation, we conducted an analysis of molecular variance (AMOVA) for each taxon using the program GenAlEx (Peakall and Smouse 2006).

To determine the degree of genetic divergence among populations of each taxon, we calculated Nei's (1978) unbiased genetic identity ( $I$ ) and distance ( $D$ ) between pairs of populations. In addition, a unweighted pair-group method using arithmetic averages (UPGMA) phenogram was generated from Nei et al.'s (1983) genetic distance matrix with branch support produced by 1,000 bootstrapping over loci, utilizing POPULATIONS v. 1.2.30 (Langella 1999) and TREEVIEW 1.6 (Page 1996). Finally, we conducted a correlation analysis between pairwise  $F_{ST}$  values (Weir and Cockerham 1984) and linear geographic distances (km) of populations to determine the relative importance of gene flow and genetic drift at a regional scale (Hutchison and Templeton 1999). If a positively significant linear relation exists, it suggests that populations are at regional equilibrium between gene flow and drift (Hutchison and Templeton 1999). Using the program PERMUTE! (Casgrain 2001), we tested a linear regression model using a Mantel test (by making 999 replicates) under the null hypothesis of no spatial genetic structure (regression slope,  $b = 0$ ).

## Results

### Genetic diversity in *D. lapponica* var. *obovata* and *E. nigrum* var. *japonicum*

Of the 23 putative loci surveyed for *D. lapponica* var. *obovata*, 13 loci (*Dia-2*, *Fe-1*, *Fe-2*, *Fe-3*, *Idh-1*, *Idh-2*,

*Mdh-3*, *6Pgd-1*, *6Pgd-2*, *Pgm-1*, *Pgm-2*, *Tpi-1*, and *Tpi-2*) were polymorphic across six populations. Except for *Fe-3* and *6Pgd-1*, the remaining 11 loci were polymorphic at Sakhalin, resulting in moderate levels of average genetic variation within populations (%  $P = 36.2$ ,  $AR = 1.41$ ,  $A = 1.42$ , and  $H_e = 0.119$ ; Table 2). In contrast, only two loci (*6Pgd-1* and *6Pgd-2*) were polymorphic at Jeju Island, resulting in extremely low population-level allozyme variation (%  $P = 8.7$ ,  $AR = 1.09$ ,  $A = 1.09$ , and  $H_e = 0.035$ ; Table 2). On average, populations from Sakhalin maintained higher estimates for  $AR$ ,  $H_o$ , and  $H_e$  than those from Jeju Island (all  $P = 0.049$ ; Table 3). Higher levels of genetic variation were found when the samples from the two islands were pooled (Table 2).

For *E. nigrum* var. *japonicum*, 15 of the 23 surveyed loci (*Adh-2*, *Dia-1*, *Dia-2*, *Fe-1*, *Fe-2*, *F1,6-1*, *Idh*, *Mdh-2*, *Mdh-3*, *6Pgd-2*, *Pgi*, *Pgm-1*, *Skdh*, *Tpi-1*, and *Tpi-2*) were polymorphic across 13 populations. Only slightly lower levels of average genetic variation were detected in populations from Jeju Island compared to those from Sakhalin, whereas the northern Japanese population also maintained moderate levels of genetic variation (Table 2). On average, populations from Sakhalin harbored greater  $AR$  than those from Jeju Island ( $P = 0.004$ ), but there was no significant difference for  $H_o$  and  $H_e$  between the groups ( $P = 0.568$  and  $P = 0.583$ , respectively; Table 3). Again, levels of genetic variation for pooled samples were higher than those averaged in 13 populations (Table 2).

There was no significant indication of recent bottlenecks in the three populations from Sakhalin of *D. lapponica* var. *obovata* and in the three populations from Sakhalin and the northern Japanese population (EN-4) of *E. nigrum* var. *japonicum* (Table 4). However, we detected significant indications of recent bottlenecks in three populations (EN-6, EN-7, and EN-11) of *E. nigrum* var. *japonicum* from Jeju Island based on both sign (EN-7) and Wilcoxon sign-rank tests (EN-6, EN-7, and EN-11) conducted using BOTTLENECK (Table 4).

### Inbreeding and population genetic structure

Except for DL-1 in Sakhalin, population-level  $F_{IS}$  estimates in the remaining five populations of *D. lapponica* var. *obovata* were significantly positive at the 0.05 level (Table 2). These results, as well as the significant multi-population-level  $F_{IS}$  ( $F_{IS} = 0.480$ ; Table 2 and 95 % CI = 0.124–0.682), indicate a substantial deficit of heterozygotes within populations. About three-fold greater  $F_{IS}$  value was detected for Jeju Island populations ( $F_{IS} = 0.834$ ; Table 2 and 95 % CI = 0.831–0.837) relative to Sakhalin ones ( $F_{IS} = 0.258$ ; Table 2 and 95 % CI = 0.047–0.432), and the difference between the two groups of populations was significant ( $P = 0.045$ ;

**Table 2** Levels of genetic diversity in six populations of *Diapensia lapponica* var. *obovata* and in 13 populations of *Empetrum nigrum* var. *japonicum* both from Sakhalin and Jeju islands

| Taxon/region/population                        | <i>n</i> | ALT (m) | % <i>P</i> | <i>AR</i> | <i>A</i> | <i>H<sub>o</sub></i> (SE) | <i>H<sub>e</sub></i> (SE) | <i>F<sub>IS</sub></i> |
|--|----------|---------|------------|-----------|----------|---------------------------|---------------------------|-----------------------|
| <i>Diapensia lapponica</i> var. <i>obovata</i> |          |         |            |           |          |                           |                           |                       |
| Sakhalin Island                                |          |         |            |           |          |                           |                           |                       |
| DL-1   | 14       | 300     | 30.4       | 1.35      | 1.35     | 0.084 (0.032)             | 0.099 (0.040)             | 0.153                 |
| DL-2   | 19       | 700     | 39.1       | 1.41      | 1.43     | 0.071 (0.025)             | 0.109 (0.041)             | 0.355*                |
| DL-3   | 18       | 900     | 39.1       | 1.47      | 1.48     | 0.114 (0.036)             | 0.149 (0.045)             | 0.242*                |
| Average  | 17       | 633     | 36.2       | 1.41      | 1.42     | 0.090 (0.013)             | 0.119 (0.015)             | 0.258 <sup>a,b</sup>  |
| Jeju Island                                    |          |         |            |           |          |                           |                           |                       |
| DL-4   | 21       | 1800    | 8.7        | 1.08      | 1.09     | 0.008 (0.006)             | 0.025 (0.022)             | 0.686*                |
| DL-5   | 70       | 1880    | 8.7        | 1.09      | 1.09     | 0.006 (0.004)             | 0.040 (0.028)             | 0.865*                |
| DL-6   | 15       | 1910    | 8.7        | 1.09      | 1.09     | 0.006 (0.004)             | 0.039 (0.027)             | 0.876*                |
| Average  | 35       | 1863    | 8.7        | 1.09      | 1.09     | 0.007 (0.001)             | 0.035 (0.005)             | 0.834 <sup>a,b</sup>  |
| Total average                                  | 26       |         | 22.5       | 1.25      | 1.26     | 0.048 (0.001)             | 0.077 (0.020)             | 0.480 <sup>a,b</sup>  |
| Pooled samples                                 | 157      |         | 56.5       | 1.48      | 1.65     | 0.033 (0.010)             | 0.131 (0.037)             |                       |
| <i>Empetrum nigrum</i> var. <i>japonicum</i>   |          |         |            |           |          |                           |                           |                       |
| Sakhalin Island                                |          |         |            |           |          |                           |                           |                       |
| EN-1   | 28       | 100     | 43.5       | 1.46      | 1.52     | 0.104 (0.039)             | 0.107 (0.034)             | 0.026                 |
| EN-2   | 24       | 250     | 47.8       | 1.52      | 1.57     | 0.134 (0.039)             | 0.131 (0.035)             | −0.025                |
| EN-3   | 26       | 800     | 52.2       | 1.60      | 1.70     | 0.086 (0.029)             | 0.104 (0.033)             | 0.197*                |
| Average  | 26       | 383     | 47.8       | 1.53      | 1.60     | 0.107 (0.014)             | 0.114 (0.009)             | 0.060 <sup>a</sup>    |
| Northern Japan                                 |          |         |            |           |          |                           |                           |                       |
| EN-4   | 30       | 936     | 43.5       | 1.54      | 1.65     | 0.136 (0.044)             | 0.152 (0.047)             | 0.104                 |
| Jeju Island                                    |          |         |            |           |          |                           |                           |                       |
| EN-5   | 54       | 1500    | 39.1       | 1.36      | 1.44     | 0.115 (0.041)             | 0.113 (0.040)             | −0.017                |
| EN-6   | 80       | 1600    | 34.8       | 1.34      | 1.39     | 0.108 (0.042)             | 0.111 (0.041)             | 0.023                 |
| EN-7   | 22       | 1640    | 26.1       | 1.30      | 1.30     | 0.140 (0.052)             | 0.124 (0.045)             | −0.130                |
| EN-8   | 50       | 1720    | 34.8       | 1.33      | 1.39     | 0.107 (0.041)             | 0.113 (0.041)             | 0.057                 |
| EN-9   | 94       | 1650    | 43.5       | 1.40      | 1.48     | 0.098 (0.036)             | 0.107 (0.037)             | 0.083*                |
| EN-10  | 70       | 1700    | 43.5       | 1.41      | 1.48     | 0.113 (0.040)             | 0.121 (0.040)             | 0.067                 |
| EN-11  | 20       | 1760    | 30.4       | 1.34      | 1.35     | 0.100 (0.037)             | 0.122 (0.042)             | 0.180*                |
| EN-12  | 60       | 1680    | 39.1       | 1.39      | 1.44     | 0.101 (0.037)             | 0.120 (0.040)             | 0.164*                |
| EN-13  | 15       | 1750    | 34.8       | 1.39      | 1.39     | 0.110 (0.039)             | 0.128 (0.040)             | 0.143                 |
| Average  | 52       | 1667    | 36.2       | 1.36      | 1.41     | 0.110 (0.004)             | 0.118 (0.002)             | 0.062 <sup>a</sup>    |
| Total average                                  | 44       |         | 39.5       | 1.41      | 1.47     | 0.110 (0.005)             | 0.119 (0.004)             | 0.065 <sup>a</sup>    |
| Pooled samples                                 | 573      |         | 65.2       | 1.54      | 1.96     | 0.109 (0.036)             | 0.122 (0.038)             |                       |

*n* the number of individuals, *ALT* (m) altitude (m) a.s.l., % *P* percentage of polymorphic loci, *AR* mean allelic richness (adjusted for a sample size of 14 and 15 plants for *D. lapponica* var. *obovata* and *E. nigrum* var. *japonicum*, respectively), *A* mean number of alleles per locus, *H<sub>o</sub>* observed heterozygosity, *H<sub>e</sub>* H–W expected heterozygosity or genetic diversity, *SE* standard error, *F<sub>IS</sub>* fixation index within populations

\* In individual populations denotes significance ( $P < 0.05$ ) based on permutation (999 replicates) under the null hypothesis of  $F_{IS} = 0$

<sup>a</sup> Weir and Cockerham (1984) estimate of  $F_{IS}$  over populations

<sup>b</sup> Denotes significance based on 95 % confidence intervals by bootstrapping over loci (999 replicates) around mean of  $F_{IS}$

Table 3). For *E. nigrum* var. *japonicum*, only four out of 13 populations were significantly positive for this parameter at the 0.05 level (Table 2). Accordingly, multi-population-level  $F_{IS}$  value for *E. nigrum* var. *japonicum* was low and not significantly different from zero (Table 2). In addition, the difference between groups (Sakhalin vs. Jeju islands) was not significant ( $P = 0.496$ ; Table 3).

For *D. lapponica* var. *obovata*, higher  $F_{ST}$  were found among the three populations from Mt. Halla on Jeju Island compared to those from Sakhalin ( $F_{ST} = 0.221$  vs.  $F_{ST} = 0.089$ ), although the difference was not significant (Table 3). When we consider all the six surveyed populations, a considerably high degree of allele frequency differences was detected ( $F_{ST} = 0.587$ , 95 % CI = 0.359–0.786),

**Table 3** Differences between groups (Sakhalin Island vs. Jeju Island) of populations of *Diapensia lapponica* var. *obovata* and *Empetrum nigrum* var. *japonicum* for the five statistics  $AR$ ,  $H_o$ ,  $H_e$ ,  $F_{IS}$ , and  $F_{ST}$ 

| Parameter | <i>Diapensia lapponica</i> var. <i>obovata</i> |             |                    | <i>Empetrum nigrum</i> var. <i>japonicum</i> |             |                    |
|-----------|--|-------------|--------------------|--|-------------|--------------------|
|           | Sakhalin Island                                | Jeju Island | $P$                | Sakhalin Island                              | Jeju Island | $P$                |
| $AR$      | 1.409  | 1.085       | 0.049 <sup>a</sup> | 1.532  | 1.364       | 0.004 <sup>a</sup> |
| $H_o$     | 0.090  | 0.009       | 0.049 <sup>a</sup> | 0.106  | 0.108       | 0.568 <sup>a</sup> |
| $H_e$     | 0.121  | 0.037       | 0.049 <sup>a</sup> | 0.114  | 0.118       | 0.583 <sup>a</sup> |
| $F_{IS}$  | 0.258  | 0.834       | 0.045 <sup>b</sup> | 0.060  | 0.062       | 0.496 <sup>b</sup> |
| $F_{ST}$  | 0.089  | 0.221       | 0.139 <sup>b</sup> | 0.077  | 0.022       | 0.681 <sup>b</sup> |

<sup>a</sup> The one-sided  $P$  values (Sakhalin Island > Jeju Island) obtained after 15,000 permutations

<sup>b</sup> The one-sided  $P$  values (Sakhalin Island < Jeju Island) obtained after 15,000 permutations

**Table 4** Results of statistical tests for evidence of recent population bottlenecks in *Diapensia lapponica* var. *obovata* in Sakhalin and *Empetrum nigrum* var. *japonicum* both in Sakhalin and Jeju islands

| Taxon/region/population                        | Sign test    | Wilcoxon sign-rank test |
|--|--------------|-------------------------|
| <i>Diapensia lapponica</i> var. <i>obovata</i> |              |                         |
| Sakhalin Island                                |              |                         |
| DL-1   | 0.582        | 0.656                   |
| DL-2   | 0.552        | 0.455                   |
| DL-3   | 0.293        | 0.125                   |
| <i>Empetrum nigrum</i> var. <i>japonicum</i>   |              |                         |
| Sakhalin Island                                |              |                         |
| EN-1   | 0.415        | 0.784                   |
| EN-2   | 0.211        | 0.449                   |
| EN-3   | 0.068        | 0.974                   |
| Northern Japan                                 |              |                         |
| EN-4   | 0.486        | 0.410                   |
| Jeju Island                                    |              |                         |
| EN-5   | 0.137        | 0.213                   |
| EN-6   | 0.223        | <b>0.037</b>            |
| EN-7   | <b>0.009</b> | <b>0.008</b>            |
| EN-8   | 0.069        | 0.125                   |
| EN-9   | 0.398        | 0.348                   |
| EN-10  | 0.443        | 0.161                   |
| EN-11  | 0.168        | <b>0.027</b>            |
| EN-12  | 0.140        | 0.125                   |
| EN-13  | 0.592        | 0.231                   |

Note that tests were not conducted in the three populations of *D. lapponica* var. *obovata* from Jeju Island because the number of polymorphic loci was only two. Numbers reported are  $P$  values of sign and Wilcoxon sign-rank tests conducted using the program BOTTLENECK, and significant  $P$  values (at 0.05 level) are boldfaced

suggesting a high degree of genetic divergence between groups (Sakhalin vs. Jeju) since values of  $F_{ST}$  for each of the two groups were much lower (Table 3). This was further confirmed by the AMOVA analysis (variation between regions contributed up to 70 % to the total genetic variance;

**Table 5** Analysis of molecular variance (AMOVA) of *Diapensia lapponica* var. *obovata* populations and *Empetrum nigrum* var. *japonicum* populations

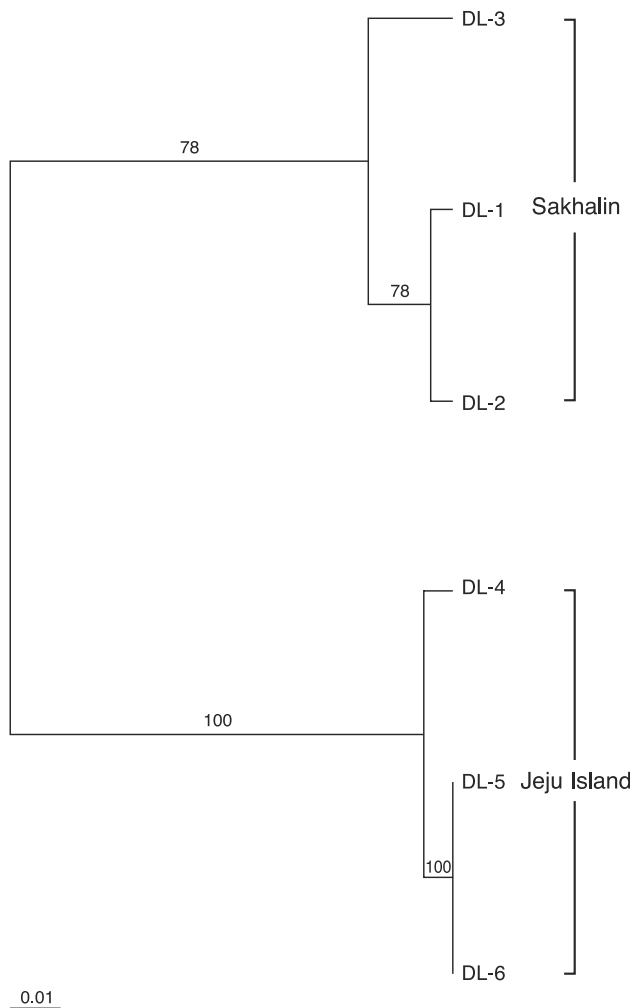
| Taxon/source                                   | $df$ | $SS$     | $MS$    | Variance component | % variation |
|--|------|----------|---------|--------------------|-------------|
| <i>Diapensia lapponica</i> var. <i>obovata</i> |      |          |         |                    |             |
| Between regions                                | 1    | 441.678  | 441.678 | 6.164              | 70          |
| Among populations                              | 4    | 54.322   | 13.581  | 0.523              | 6           |
| Within populations                             | 151  | 328.331  | 2.174   | 2.174              | 24          |
| Total  | 156  | 824.331  |         | 8.861              | 100         |
| <i>Empetrum nigrum</i> var. <i>japonicum</i>   |      |          |         |                    |             |
| Between regions                                | 1    | 116.770  | 116.770 | 0.816              | 22          |
| Among populations                              | 10   | 98.218   | 9.822   | 0.155              | 4           |
| Within populations                             | 531  | 1488.513 | 2.803   | 2.803              | 74          |
| Total  | 542  | 1703.501 |         | 3.775              | 100         |

$df$  the degrees of freedom,  $SS$  sum of squares,  $MS$  mean squares, % variation the percentage of total variance contributed by each component

Table 5) and by the pairwise Nei's (1978)  $I$  values. Whereas the mean  $I$  between populations was  $0.897 \pm 0.075$ , the average  $I$  value between groups (i.e., Sakhalin and Jeju islands) was  $0.839 \pm 0.015$ , estimates lower than those expected for conspecific plant populations (average  $I = 0.950 \pm 0.059$ ,  $n = 1,572$ ; van der Bank et al. 2001). The topology of the UPGMA phenogram (Fig. 2), together with the region specific alleles (nine unique alleles for Sakhalin and two for Jeju Island; Table 6) further confirm the high genetic divergence between the two groups.

Unlike *D. lapponica* var. *obovata*, a low degree of allele frequency differences was detected when the 13 populations of *E. nigrum* var. *japonicum* were taken as a whole ( $F_{ST} = 0.086$ , 95 % CI = 0.052–0.144), a slightly higher





**Fig. 2** UPGMA phenogram based on Nei et al.'s (1983) genetic distances between populations of *Diapensia lapponica* var. *obovata*. Numbers above branches represent bootstrap support for 1,000 replicates, and only values greater than 50 % are shown above the branches

value than those found at each group (0.077 at Sakhalin and 0.022 at Jeju; Table 3). Accordingly, AMOVA revealed that variation among individuals within populations contributed most to the total genetic variance (74 %; Table 5). In concordance with this, pairwise Nei's (1978)  $I$  values were high (mean  $I$  between populations =  $0.982 \pm 0.015$ ; average  $I$  value between groups =  $0.973 \pm 0.008$ ), well within those found for most conspecific plant populations (van der Bank et al. 2001). The UPGMA phenogram showed that all populations from Sakhalin clustered together, which were then followed by the northern Japanese population EN-4 and Jeju Island populations (Fig. 3). However, all the branches of the cluster formed by EN-4 and Jeju populations had less than 50 % bootstrap values (Fig. 3). Following Hutchison and Templeton (1999) method, we found a weak but significant

positive linear relationship between pairwise  $F_{ST}$  and linear geographic distance between populations of *E. nigrum* var. *japonicum* on Jeju Island ( $r = 0.309$ ,  $P = 0.031$ ), suggesting that populations are at regional equilibrium of gene flow and drift (Fig. 4).

## Discussion

### Genetic diversity and structure in *D. lapponica* var. *obovata*

As expected, the three populations of *D. lapponica* var. *obovata* from Jeju Island harbor lower levels of within-population genetic variation and higher among-population divergence than those from Sakhalin (i.e., in agreement with the first prediction regarding central-marginal population scheme; see "Introduction"). Results of AMOVA, the UPGMA phenogram, and relatively low average Nei's pairwise genetic identities indicate high genetic divergence between Sakhalin and Jeju. This pattern would be explained by historical factors. The current occurrences of the taxon on Jeju Island are likely the descendants of immigrants that would have arrived from Northeast Asia via the Korean Peninsula during the glacial periods of the Pleistocene, a scenario that has been proposed not only for the Korean native arctic-alpine plants (Im 1992; Kong 1998a, b, 1999) but also for the Japanese ones (Ikeda et al. 2008). Under this scenario, after the colonization of Korea, arctic-alpine plants including *D. lapponica* var. *obovata* would have tracked the glacial/interglacial cycles by means of latitudinal and altitudinal movements. During the cold periods, populations would have migrated southward and downhill, reaching a more or less former continuous distribution along the peninsula. Indeed, pollen records of the lowlands adjacent to Mt. Halla (Chung 2007) as well as those obtained from several lowland locations throughout the Korean Peninsula (e.g., Choi 1998, 2001; Chung et al. 2006; Yi and Kim 2010) show the presence of subalpine and cold-tolerant species—e.g., *Abies*, *Pinus*, and *Betula*, elements that at present co-occur with the two subshrubs studied in this paper—during the Last Glacial Maximum (LGM). This might suggest a glacial expansion of arctic-alpine elements during the Quaternary glacial periods, which would be common in the Korean lowlands. Moreover, it should be taken into account that during the LGM Jeju Island was connected to the Korean Peninsula by a land bridge due to the marine regression (e.g., Xu et al. 2010). As the climate ameliorated during the interglacial periods (such as the current Holocene), these plants would have retreated northward and also toward the mountaintops (such as the summit of Mt. Halla) looking for more optimum environments.

**Table 6** Allele frequencies for eight polymorphic loci with the highest degree of population differentiation found in *Diapensia lapponica* var. *obovata* both in Sakhalin and Jeju islands

| Locus         | Allele   | Sakhalin Island |      |      | Jeju Island |      |      |
|---------------|----------|-----------------|------|------|-------------|------|------|
|               |          | DL-1            | DL-2 | DL-3 | DL-4        | DL-5 | DL-6 |
| <i>Dia-2</i>  | <i>a</i> | 1.00            | 0.82 | 0.72 | 1.00        | 1.00 | 1.00 |
|               | <i>b</i> | 0.00            | 0.18 | 0.28 | 0.00        | 0.00 | 0.00 |
| <i>Fe-3</i>   | <i>a</i> | 0.00            | 0.00 | 0.00 | 1.00        | 1.00 | 1.00 |
|               | <i>b</i> | 1.00            | 1.00 | 1.00 | 0.00        | 0.00 | 0.00 |
| <i>Mdh-3</i>  | <i>a</i> | 0.86            | 0.90 | 0.69 | 1.00        | 1.00 | 1.00 |
|               | <i>b</i> | 0.14            | 0.11 | 0.31 | 0.00        | 0.00 | 0.00 |
| <i>6pgd-1</i> | <i>a</i> | 1.00            | 1.00 | 1.00 | 0.05        | 0.60 | 0.70 |
|               | <i>b</i> | 0.00            | 0.00 | 0.00 | 0.95        | 0.40 | 0.30 |
| <i>6pgd-2</i> | <i>a</i> | 0.00            | 0.00 | 0.50 | 0.38        | 0.69 | 0.70 |
|               | <i>b</i> | 1.00            | 1.00 | 0.42 | 0.62        | 0.31 | 0.30 |
|               | <i>c</i> | 0.00            | 0.00 | 0.08 | 0.00        | 0.00 | 0.00 |
| <i>Pgm-1</i>  | <i>a</i> | 0.39            | 0.47 | 0.50 | 1.00        | 1.00 | 1.00 |
|               | <i>b</i> | 0.61            | 0.53 | 0.50 | 0.00        | 0.00 | 0.00 |
| <i>Pgm-2</i>  | <i>a</i> | 0.18            | 0.21 | 0.22 | 0.00        | 0.00 | 0.00 |
|               | <i>b</i> | 0.54            | 0.42 | 0.72 | 1.00        | 1.00 | 1.00 |
|               | <i>c</i> | 0.29            | 0.37 | 0.06 | 0.00        | 0.00 | 0.00 |
| <i>Tpi-1</i>  | <i>a</i> | 0.00            | 0.03 | 0.06 | 1.00        | 1.00 | 1.00 |
|               | <i>b</i> | 1.00            | 0.97 | 0.94 | 0.00        | 0.00 | 0.00 |
| <i>Tpi-2</i>  | <i>a</i> | 0.39            | 0.55 | 0.36 | 1.00        | 1.00 | 1.00 |
|               | <i>b</i> | 0.61            | 0.45 | 0.64 | 0.00        | 0.00 | 0.00 |

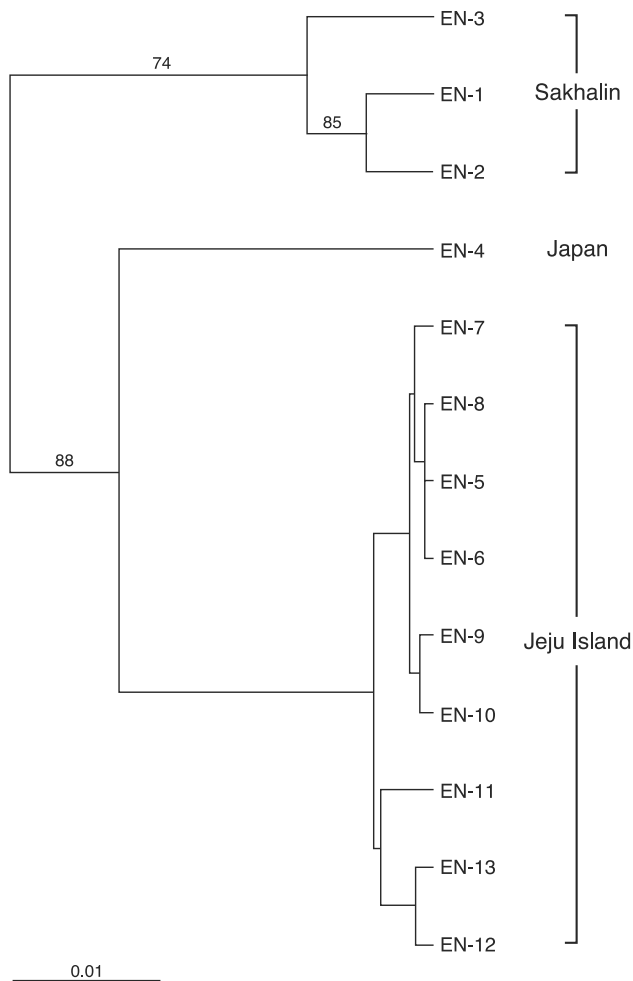
The drastic altitudinal and latitudinal shifts of alpine-plant vegetation should have been much less severe in those species and populations located at higher latitudes (e.g., Russian Far East) than those at lower altitudes (e.g., Mt. Halla on Jeju Island, currently the world's southernmost distributional limit for *D. lapponica* var. *obovata*) because of the species' climatic tolerances (e.g., Stewart et al. 2010). Currently, Jeju Island obviously does not provide the optimal conditions for alpine plants because of its subtropical climate, and thus contemporary populations on Jeju Island should be regarded as glacial relicts, representing a clear disjunction from central Honshu (Japan) and northernmost Korea. Under this scenario, populations of *D. lapponica* var. *obovata* from Jeju Island would have suffered much more severe random genetic drift (genetic bottlenecks) than those from Sakhalin. The allozyme frequencies provide clear indications that genetic drift have operated within Jeju populations, as many alleles present in Sakhalin (some at high frequencies) are absent in Jeju (Table 6). Moreover, we found no significant indication of recent bottlenecks in the three studied populations from Sakhalin. However, we did not conduct the bottleneck tests in the three populations of *D. lapponica* var. *obovata* from Jeju Island because the number of polymorphic loci was only two.

In addition to these historical factors, life-history and ecological traits such as insect-pollination, seeds with no specialized mechanism for long-distance dispersal, and the

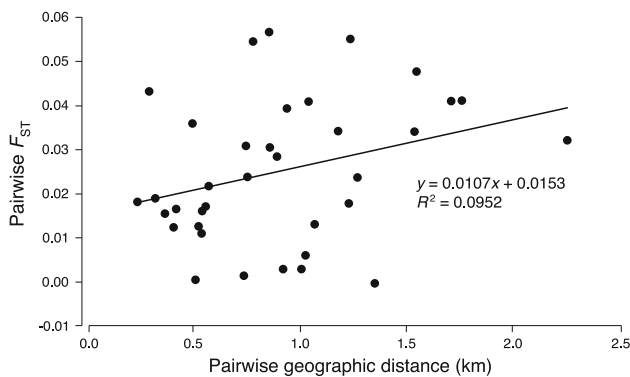
very small number of individuals (ca. 400 individuals within 0.003 km<sup>2</sup> near the peak of Mt. Halla at elevations from 1,800 to 1,900 m; Suh et al. 2001) may help to explain the extremely low allozyme variation for *D. lapponica* var. *obovata* on Jeju Island compared to Sakhalin, despite Jeju Island is thought to harbor suitable refugia for many alpine plants during both cold (i.e., 'glacial refugia', putatively located in low mountains and lowlands) and warm periods (i.e., 'interglacial refugia', located in high mountain summits) (Kong 1998a, b, 1999). Finally, the about three-fold greater  $F_{IS}$  value found on Jeju Island ( $F_{IS} = 0.834$ ) relative to Sakhalin ( $F_{IS} = 0.258$ ) suggests that more windy environments near the peak of Mt. Halla on Jeju Island would limit pollinators' flight distances across crevices on the steep cliffs.

#### Genetic diversity and structure in *E. nigrum* var. *japonicum*

As for *D. lapponica* var. *obovata*, we predicted that populations of *E. nigrum* var. *japonicum* from Jeju Island would harbor lower levels of within-population genetic variation and higher degree of among-population genetic differentiation than relatively common and continuously distributed populations in Sakhalin. Unlike *D. lapponica* var. *obovata*, our genetic results for *E. nigrum* var. *japonicum* do not support the first prediction. Instead, our results support the second prediction that levels of



**Fig. 3** UPGMA phenogram based on Nei et al.’s (1983) genetic distances between populations of *Empetrum nigrum* var. *japonicum*. Numbers above branches represent bootstrap support for 1,000 replicates, and only values greater than 50 % are shown above the branches



**Fig. 4** Relationship between geographic distance (km) and pairwise  $F_{ST}$  between populations of *Empetrum nigrum* var. *japonicum* on Jeju Island in Korea. A weak but significant positive linear relationship between pairwise  $F_{ST}$  and pairwise linear geographic distance has been found ( $r = 0.309$ ,  $P = 0.031$ )

within-population genetic variation and degree of among-population differentiation in populations of *E. nigrum* var. *japonicum* on Jeju Island would be higher and lower than those of *D. lapponica* var. *obovata* on the same island, respectively.

Populations of *E. nigrum* var. *japonicum* from Sakhalin Island had greater allelic richness than those from Jeju Island ( $AR = 1.53$  vs.  $1.36$ ; Table 3), but we found similar average expected heterozygosity in both islands ( $H_e = 0.114$  vs.  $0.118$ ; Table 3). We detected several low frequency alleles ( $<5\%$ ; *Dia-2<sup>b</sup>*, *F1,6-1<sup>b,c</sup>*, *Idh<sup>c</sup>*, *Mdh-3<sup>b</sup>*, and *Tpi-2<sup>b</sup>*; data not shown) in populations from Sakhalin, which explains their greater allelic richness compared to Jeju populations. Since the frequency of common alleles has a major effect on the magnitude of  $H_e$ , we found similar  $H_e$  estimates for both islands. In parallel with this, we found no significant indication of recent bottlenecks for any of the populations of *E. nigrum* var. *japonicum* from Sakhalin, whereas we detected significant indication of recent bottlenecks in three of the nine studied populations from Jeju Island. This may reflect the fact that current populations in Sakhalin are large and are continuously distributed from lowlands to mountainous regions, whereas the species has a much more restricted distribution in Jeju Island as a consequence of the Holocene upslope retreat. Interestingly, the population from northern Japan (EN-4) maintains the highest genetic diversity ( $H_e = 0.152$ ) among the populations examined, which may support the existence of long-term refugia for arctic–alpine plants in central Honshu mountains (Fujii and Senni 2006; Ikeda et al. 2008) if a higher within-population genetic variation than that of EN-4 exists in central Japan.

Under the same scenario of immigration history of arctic–alpine plants on Jeju Island during the glacial periods of the Pleistocene and local persistence through glacial/interglacial cycles (Im 1992; Kong 1998a, b, 1999), the considerably higher levels of genetic variation found for *E. nigrum* var. *japonicum* compared to *D. lapponica* var. *obovata* ( $AR = 1.36$  and  $H_e = 0.118$  vs.  $AR = 1.09$  and  $H_e = 0.035$ ; Table 2) would be attributable to their different life-history and ecological traits, with a special significance of their local demography in Mt. Halla. First, individual plant sexuality is different from each other. *Diapensia lapponica* var. *obovata* is hermaphroditic, whereas *E. nigrum* var. *japonicum* is monoecious, dioecious or polygamous. Thus, outcrossing rate of *E. nigrum* var. *japonicum* should be higher than that of *D. lapponica* var. *obovata* (multi-population-level  $F_{IS} = 0.065$  vs.  $0.480$ ). Indeed, Jeju Island populations of *D. lapponica* var. *obovata* seem to be strongly inbred (multi-population-level  $F_{IS} = 0.834$ ) whereas those of *E. nigrum* var. *japonicum* are close to H–W equilibrium (multi-population-level  $F_{IS} = 0.062$ ). Second, seed dispersal mechanisms are also

very different from each other. *Diapensia lapponica* var. *obovata* has seeds with no adaptations for long-distance dispersal, whereas fruits (berry-like drupes) of *E. nigrum* var. *japonicum* contain 6–9 nutlets and are dispersed by animals and birds. Thus, degree of among-population genetic divergence of *E. nigrum* var. *japonicum* should be lower than that of *D. lapponica* var. *obovata* (multi-population-level  $F_{ST} = 0.022$  vs. 0.221). Nevertheless, Choi et al. (2004) reported a significant fine-scale genetic structure in a population (60 m × 80 m,  $n = 189$ ) of this taxon on Jeju Island and we also found a weak but significant positive linear relationship between pairwise  $F_{ST}$  and linear geographical distance. These data suggest that most seeds of *E. nigrum* var. *japonicum* fall around the maternal plant and populations are at regional equilibrium between gene flow and drift, following an isolation-by-distance pattern. Third, the occupation area of the two taxa on Jeju Island is significantly different. Populations of *D. lapponica* var. *obovata* are restricted to a very small patch (of ca. 0.003 km<sup>2</sup>) near the peak of Mt. Halla (alt. 1,800–1,900 m a.s.l.) with just a few hundred individuals, whereas populations of *E. nigrum* var. *japonicum* have a relatively continuous distribution around the peak of Mt. Halla (alt. 1,500–1,800 m), occupying an area of ca. 8 km<sup>2</sup>. These, together with other unknown factors, might be associated with the higher levels of within-population genetic variation and lower degree of among-population differentiation in *E. nigrum* var. *japonicum* compared to *D. lapponica* var. *obovata* on Jeju Island.

#### Implications for conservation

*Empetrum nigrum* var. *japonicum* on Jeju Island harbors moderate levels of genetic variation, a low degree of divergence, and it is relatively common with large population sizes. In addition, it is located within the borders of a nature reserve (Hallasan National Park, which has been also designated as a UNESCO Biosphere Reserve and World Natural Heritage Site) and thus does not require the implementation of urgent management or recovery measures. In contrast, *D. lapponica* var. *obovata* is extremely rare and threatened on Jeju Island, and the Ministry of Environment in the Republic of Korea has listed it as one of the most critically endangered plant taxa in the country (<http://www.me.go.kr/kor/index.jsp>).

*Diapensia lapponica* var. *obovata* on Jeju Island is facing at present several threatening factors. First, the taxon occurs on steep cliffs (with a slope of ca. 70°) on Mt. Halla where individuals can be easily lost by soil erosion of steep habitats. Second, illegal collections have been taking place, although the authority of Hallasan National Park has strictly closed trails to prevent trampling and illegal collection of alpine plants. It should also be noted here that the

summit of Mt. Halla represents one of the world's southernmost edges for other cold-adapted species in addition to the two studied here, such as *Juniperus chinensis* L. var. *sargentii* A. Henry, *Pedicularis verticillata* L., *Ranunculus borealis* Trautv., or *Vaccinium uliginosum* L. Third, the current population size is extremely small (ca. 400 occurring in just 0.003 km<sup>2</sup>; Suh et al. 2001). Finally, levels of within-population genetic variation are significantly lower than populations in Sakhalin, which suggests that populations on Jeju Island have suffered severe genetic erosion and are unlikely to regain genetic variation without human intervention (e.g., Godt et al. 1996). Thus, in situ and ex situ conservation efforts should be of particular importance for this taxon. Most importantly, we recommend that all known subpopulations should be strictly protected in situ by law to prevent further decreases in population size despite the recent efforts of the Hallasan National Park in protecting the local flora. A method of seed germination should be developed as a short-term conservation measure and also for the ex situ conservation of the taxon, as artificial propagation and transplantation are very difficult (Suh et al. 2001). As a long-term conservation effort, we suggest to introduce seeds from northernmost Korea (e.g., Mt. Baekdu) or central Honshu in Japan into Jeju Island to increase levels of genetic diversity. However, this should be done with extreme care as the mixing of genetically divergent individuals may suppose the disruption of co-adapted gene complexes, producing outbreeding depression (Fenster and Dudash 1994). Moreover, these conservation strategies would require additional data of the ecology of the taxon, such as demographic dynamics, pollination biology, and seedling establishment, among others.

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