Marjut Kreivi

CONSERVATION GENETICS AND PHYLOGEOGRAPHY OF ENDANGERED BOREOARCTIC SEASHORE PLANT SPECIES
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**Abstract**

The amount and distribution of genetic diversity are likely to affect the evolutionary potential of a species. When feasible and cost-effective policies for management and monitoring of endangered populations or species are planned, knowledge of the spatial genetic structure and the type of population dynamics is of great concern. In this thesis the genetic diversity and population structures of two endangered arctic plant species was examined on different geographical scales in Northern Europe. The species were Siberian primrose (*Primula nutans*) and pendant grass (*Arctophila fulva*), which both grow in a seashore habitat and have similar distribution patterns on the shores of the Arctic Ocean and the Bothnian Bay.

The goal of the present study was to provide basic population genetic information for the study species using microsatellite and AFLP markers. Both markers were used for the first time in these species, and species-specific microsatellite markers were developed during the study. A further aim was to interlink the population genetic processes of the species into distribution patterns at the regional and population levels and to compile a synthesis of the impact of hierarchical spatiotemporal processes and autocorrelation in genetic variation at different levels.

The results of this thesis provided new information on the diversity and population structure of the endangered study species and new markers that are useful in future genetic studies of primrose species. The diversity of Siberian primrose was low, and there was no dispersal between the study regions. In pendant grass, a relatively high amount of variation was found considering the evident clonal reproduction and gene flow that was detected between populations connected by waterways. The results suggested that both clonal and sexual reproduction are important in this species. On a local scale, pendant grass populations had characteristics of “stepping stone” and classical metapopulation models.

The results indicated that on a long time scale, both species will continue to decline without efficient management efforts. Most critical for the persistence of the species is the conservation of suitable habitats. Translocations could be considered in order to enhance the diversity of existing populations and establish new populations.

By examining the present day structure of Siberian primrose, it was possible to make inferences regarding the colonisation history of the species in the North European area. The current distribution of Siberian primrose seemed to result from a vicariant process that took place after the last ice-age, when the species colonised the area. It spread first to the White Sea area, probably from the east, and subsequently colonised the Bothnian Bay and the Barents Sea in the west. The isostatic land uplift after the retreat of the Eurasian ice sheet uncovered large areas of land from the Baltic Sea basin that previously were under water. These geological changes resulted in the current disjunct distribution of Siberian primrose.

**Keywords:** AFLP, conservation genetics, genetic diversity, microsatellite, phylogeography, population structure, Primula sibirica group, seashore meadow
To Ilkka

“If this is a refugium, why are my feet so bloody cold?”

(Buckland & Dugmore 1991)
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AFLP</td>
<td>amplified fragment length polymorphism</td>
</tr>
<tr>
<td>AMOVA</td>
<td>Analysis of Molecular Variance</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>BP</td>
<td>before present</td>
</tr>
<tr>
<td>cpDNA</td>
<td>chloroplast DNA</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>FCA</td>
<td>factorial correspondence analysis</td>
</tr>
<tr>
<td>IBD</td>
<td>isolation by distance</td>
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<tr>
<td>MLG</td>
<td>multilocus genotype</td>
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<tr>
<td>$N_e$</td>
<td>effective population size</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>SSR</td>
<td>simple sequence repeat</td>
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List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


In addition, unpublished data have been included.
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1 Introduction

Habitat loss, fragmentation and degradation are pervasive processes threatening several species all over the world. Many species are endangered due to direct or indirect human-related factors, such as loss and alteration of habitat and introduction of species. Conservation genetics aims to minimize the risk of extinction, taking genetic factors into consideration. Maintenance of genetic diversity is the primary goal in the management of threatened populations or species (Frankham et al. 2002). Genetic diversity in natural populations is of great concern because the amount and distribution of genetic diversity are likely to affect the evolutionary potential of species and populations (Futuyma 1998). Knowledge of the genetic structure of a species is also necessary because it can be applied to preservation of the evolutionary potential of the species, which is one of the goals of conservation (Godt & Hamrick 1998). Management recommendations can vary significantly depending on the population structure of the species (Frankham et al. 2002). Empirical assessment of genetic variability is therefore essential for the successful management of endangered plants (e.g., Escudero et al. 2003).

Genetic diversity, including the whole variety of alleles and genotypes, is commonly used to describe the heritable variation in a population or species. The genetic diversity of plant species reflects their breeding systems. Also, fluctuations in the number and size of populations and their biogeographic history may play critical roles in determining the current genetic composition of species (Hamrick & Godt 1996). The genetic diversity of a population can be structured by spatial factors and by the genetic backgrounds of species. Structuring can exist at different scales, for example, among populations, subpopulations or neighbouring individuals (Escudero et al. 2003). The spatial distribution of plants is a product of environmental influences, including human activities, life-history traits and past demographic histories of species (Knowles et al. 1992, Frankham et al. 2002).

1.1 Disjunct distributions and phylogeography

One of the major theoretical problems in biogeography is the origin of disjunct distributions of animals or plants. Understanding how species, whose distributions are characterised by significant gaps, colonised their present distribution areas is important, especially when anticipating possible extinctions
and the possibility of natural ‘genetic rescue’ for endangered populations (e.g., Ingvarsson 2001). The contemporary distributions of species are determined by several factors, such as the dispersal ability of the species and the availability of suitable habitats. Recent phylogeographic studies also revealed the important role of post-glacial recolonisation events in the geographical distribution of northern species (reviewed in Abbott & Brochmann 2003 and Brochmann et al. 2003). Phylogeographic patterns of sequence divergence across geographic regions are concordant in a surprisingly large number of species (Avise 2000). By comparing the genetic and geographic structures of co-distributed species we can make inferences about their history. This comparative phylogeography approach can contribute significantly to our understanding of the relationship between earth history and biotic diversification (e.g., Taberlet et al. 1998, Arbogast & Kenagy 2001).

1.1.1 Phylogeographic processes

Two processes can cause discontinuities in species dispersal on a large geographical scale: (i) jump dispersal and (ii) vicariance. Dispersal over heavy barriers is a rare stochastic process and is unlikely to cause parallel distribution patterns among ecologically divergent organisms. Vicariance, in turn, is a process in which a large, continuous population is fragmented into smaller populations by a geological or environmental event, causing a barrier to dispersal. Vicariance can lead to the evolutionary emergence of two or more closely related forms of animals or plants in different geographical areas (Avise 2002). A large-scale vicariant event is likely to simultaneously affect a great number of organisms that may show similar distribution patterns long after the event.

1.1.2 Early history of the North European area

During the last Weichselian glaciation that occurred 18,000 – 16,000 years before present (BP), an ice sheet covered western Eurasia (e.g., Clark & Mix 2002, Velichko et al. 1997). However, during the same period, large parts of Eastern Europe and Asia remained ice-free, including parts of the Siberian arctic (Clark & Mix 2002, Velichko et al. 1997). During the coldest period, most animal and plant species found in Europe today receded into refugia located in the peninsulas of southern Europe and possibly near the Caucasus and the Caspian Sea (e.g., Hewitt 1999). North of these refugia, the landscape consisted of tundra or cold steppe
that extended eastward to the Ural Mountains (Skinner & Porter 1987). It has also been proposed that Beringia was a major northern refuge for arctic plants throughout the Quaternary (Hultén 1937, see also Abbott & Brochmann 2003).

The ice started to retreat around 16,000 BP, followed by a rapid advance northwards by plants and animals. During the Younger Dryas period (11,000 – 10,000 BP), an ice sheet covered Scandinavia, but parklands and forests had already reached the Baltic Sea basin (Baltic Ice Lake) from the southeast. During this phase and later when the Baltic Sea experienced a salt-water phase (Yoldia Sea), the Baltic Sea and the White Sea were connected by waterways or were separated by only a narrow land isthmus. After the final retreat of the ice, land gradually rose and the isthmus between the White Sea and the Baltic Sea broadened and was occupied by forests (Mäkinen & Mäkinen 1964, Jantunen 2004). By 6000 BP the distribution of fauna and flora was close to that of the present situation.

1.2 Population structure

The genetic structure of plant populations is largely shaped by factors such as selection, spatial habitat structure, isolation by distance, social organization, mating system, gene flow, genetic drift, evolutionary history, life history, and other ecological and evolutionary factors at a wide variety of spatial and temporal scales (Loveless & Hamrick 1984, Avise 2004). When dispersal between populations is restricted, gene flow between them is reduced, resulting in high genetic structuring at the population level. Populations of nearly all species exhibit at least some degree of genetic differentiation across geography (Ehrlich & Raven 1969).

The distribution of a plant species can be viewed as a hierarchy of spatial entities. Physical individuals comprise groups of close relatives or clones and these, in turn, are aggregated in patches of suitable habitats. Each patch may contain a single or several genetic or physical individuals. Inhabited patches may form regional clusters that can be further grouped to describe the distribution of the species. Therefore, there is also a hierarchy of entities within which genetic structure of populations can be described and there can be genetic differentiation at all levels of this hierarchy (e.g., Hewitt & Butlin 1997, Hewitt 1999).

It is a continuing challenge for scientists to describe population genetic architectures within species and identify the biological forces responsible for them. These forces may include migration or gene flow, random genetic drift,
selection, mutational divergence, genetic recombination mediated by organismal behaviours, and mating systems (Avise 2004). In general, outcrossing species usually exhibit higher levels of genetic diversity and lower differentiation between populations compared with selfing and clonal species (Loveless & Hamrick 1984, Rossetto et al. 1995, Nybom 2004).

1.2.1 Clonal vs. sexual reproduction

The relative roles of sexual and clonal recruitment are likely to have important consequences for the local genetic structure in species that use both of these alternative methods of propagation. Two different processes may underlie the local genetic population structure (Ennos 2001, Kalisz et al. 2001). In sexually reproducing populations, restricted pollen and seed dispersal may produce microgeographic isolation by distance after a few generations. However, most aquatic and coastal plant species show clonal propagation or perennation and many only rarely reproduce sexually (Barrett et al. 1993, Silvertown & Charlesworth 2001). Spatial genetic structure may also be generated in clonal or sexually reproducing plant populations as a consequence of “sampling” events that occur when the population is founded. Spatial genetic structure generated in this fashion will have a distinguishable genetic structure where regeneration sites are colonised with a limited number of propagules (Whitlock & McCauley 1990, Ennos 2001, Kalisz et al. 2001).

The clonal reproduction of species may have several population genetic consequences, both within and between populations (e.g., Balloux et al. 2003, Bengtsson 2003). High rates of clonal reproduction will positively affect allelic diversity, whereas genotypic diversity decreases with increasing rates of clonal reproduction. Asexual populations thus maintain higher genetic diversity at each single locus, but a lower number of distinct genotypes. Furthermore, population differentiation is strongly decreased in purely clonal populations compared with purely sexual ones (Balloux et al. 2003). With increasing clonal reproduction, allelic effective population size first increases slowly and then rises towards extreme values when the reproductive system approaches strict clonality (Balloux et al. 2003).

An increasing amount of literature deals with the question of whether particular organisms are clonal or sexual (see Brookfield 1992, Anderson & Kohn 1998, Halkett et al. 2005). Molecular markers are often applied to infer the predominant mode of reproduction (Tibayrenc et al. 1991, Burt et al. 1996, Gräser et al. 1996). Basically, two different population genetic criteria could be
used to distinguish between sexual and clonal reproduction (Tibayrenc et al. 1991, Avise 2004). Clonal reproduction in nature could be inferred if there is 1) absence of meiotic segregation at particular marker loci and 2) absence of recombination among multiple marker loci. If dominant markers are used, it is not possible to confirm the absence of segregation. In that case, several criteria could be used to reveal the absence of recombination. Often, the presence of particular multilocus genotypes in great excess is the most robust and significant evidence of clonal reproduction, especially when the same genotypes appear in excess in various localities. However, non-random association of alleles or significant deficits of expected recombinant genotypes have also been used to infer whether organisms recombine (Mes 1998).

1.3 Study species

The two study species, Siberian primrose (*Primula nutans*, formerly *P. sibirica*) and pendant grass (*Arctophila fulva*), are arctic plant species growing on seashore meadows. Both species belong to a so-called *Primula sibirica* group.

1.3.1 Primula sibirica group

Several arctic seashore plants have a similar disjunctive distribution pattern along the coastal habitats of both the Baltic Sea and the White Sea. Most also have a more or less continuous circumpolar distribution along the coasts of the Arctic Ocean. In the Baltic Sea area, the populations of these plants are spatially fragmented, whereas in their main distribution area, the populations are largely continuous. By the Baltic Sea, some of the species are restricted to the Bothnian Bay.

The disjunctive distribution pattern was first recognized by Fries (1865), who identified twelve unrelated taxa sharing the same distribution pattern. Later, the term “*Primula sibirica* group”, according to one of the species (currently *P. nutans*), was introduced by Erlandsson (1939). Subsequently, plant biographers included a variable number of species in the group. Ericson and Wallentinus (1979) listed altogether four moss and 29 vascular plant species, some of them (*e.g.*, *Salix triandra*, *Catabrosa aquatica*) with a disjunct distribution in Northern Europe and a continuous southern distribution, with a number of peripheral northern populations at a larger geographical scale. Eurola (1999) has named six ‘core’ species of the *Primula sibirica* group: *Carex halophila*, *Carex paleacea*, *Carex nocherti*, *Carex alba*, *Carex petraea*, and *Carex arenaria*. These species have been studied in detail in the Baltic Sea area.
Hippuris tetraphylla, Potentilla anserina ssp. eddeii, Puccinellia phryganodes, and P. nutans.

Several species belonging to the Primula sibirica group are considered endangered in Finland (Rassi 2001). For example, S. triandra and C. aquatica are near threatened, Moehringia lateriflora is vulnerable, P. nutans var. jokelae, P. phryganodes and Salicornia europaea are endangered, A. fulva var. pendulina is critically endangered and Stellaria humifusa is regionally extinct.

1.3.2 Phylogeography of the Primula sibirica group

In order to determine how the species in the Primula sibirica group dispersed to the Baltic Sea area, both classical processes of Avise (2002) (see 1.1.1) have been suggested to explain the discontinuities in the distribution of the species. According to the jump dispersal hypothesis, (i) long-distance dispersal by wind or migrating birds would explain the present distribution of the species (Havas 1961). On the other hand, the vicariance hypothesis (ii) suggests that the species dispersed to the Baltic Sea basin in the end of the last ice age, when the Baltic Sea and the White Sea were connected by waterways or separated by only a narrow land area (Lovén 1862, Fries 1865, Ericson & Wallentinus 1979). In addition to these classic models, it has also been suggested that (iii) the present occurrences of the species are the remains of a much larger distribution area covering the shores of the whole Fennoscandia (Eurola 1999).

Several areas have been suggested for the location of the ice age refugia. The oldest hypothesis, proposed by Hultén (1937, see also Abbott & Brochmann 2003), suggests that many arctic plants may have survived in Siberian or Beringian refugia, and this “eastern refugia” hypothesis is thought to be appropriate also for Siberian primrose (as well as for other species in the Primula sibirica group) (Mäkinen & Mäkinen 1964). On the other hand, Christophersen (1941) and Mäkinen and Mäkinen (1964) suggested that the variety finmarchica of Siberian primrose growing at the Barents Sea may have survived the latest glaciation in northern coastal refugia has then spread to the surrounding areas. On the basis of recent phylogeographic studies, some arctic-alpine plants in Europe seem to have colonised arctic areas from source populations situated south of the North European ice sheet (Abbott & Brochmann 2003, Schönswetter et al. 2003, Skrede et al. 2006). Among the Primula sibirica group, the populations of Siberian primrose, C. paleacea, and pendant grass in Norway differ morphologically from the populations of the White Sea and the Bothnian Bay,
which resemble each other (e.g., Mäkinen & Mäkinen 1964, Rautiainen 2002). This pattern may suggest that although Siberian primrose or pendant grass currently do not grow in central Europe, they may have survived in southern refugia during the latest ice age, spread to the north and later disappeared from the southern areas.

1.3.3 Siberian primrose

Siberian primrose is a perennial rosette hemicryptophyte, which grows mainly on seashore meadows. The species grows on low-lying clayey meadows mixed with sand and is often inundated both by the rising sea water and by the flooded rivers. It prefers open habitats, where it starts to grow early in the spring, when it is not suppressed by the shading effect of other, larger plants (Mäkinen & Mäkinen 1964, Björnström 2006). It has a circumpolar distribution ranging from North America to Europe and Asia. The type subspecies *P. nutans* ssp. *nutans* grows on moist riverside meadows in eastern Asia and North America (Christophersen 1941, Fedorov 1952, cited by Mäkinen & Mäkinen 1964), whereas *P. nutans* ssp. *finmarchica* (Löve & Löve 1961) is a seashore plant and grows only in Northern Europe. There is a gap in distribution between these two subspecies in middle Russia.

In Northern Europe, the Siberian primrose subspecies *finmarchica* has a disjunctive, tripartite distribution (Fig. 1). It grows in the northern part of the Bothnian Bay in Finland and Sweden, in the Barents Sea part of the Arctic Ocean, in Northern Norway and Russia, and on the western shores of the White Sea in Russia. These regions are located more than two hundred kilometres apart. According to Mäkinen and Mäkinen (1964), the Bothnian Bay and the White Sea populations differ morphologically from the populations at the Norwegian coast of the Barents Sea. Accordingly, they classified the White Sea and Bothnian Bay morphotype as *P. nutans* ssp. *finmarchica* variety *jokela* and the morphotype at the Norwegian coast of the Arctic Ocean as *P. nutans* ssp. *finmarchica* var. *finmarchica*.

Siberian primrose can reproduce both sexually and vegetatively. White root-like runners originate from axils of lower leaves. Vegetative growth takes place in late August to early September (Björnström 2006).

Siberian primrose is a heterostylic plant that has two floral types, pin and thrum (distyly). Heterostyly is thought to have evolved as a means of avoiding inbreeding and ensuring the effective exchange of pollen between the different
mating types (Barrett & Kohn 1991). Heterostylous self-incompatible plants are particularly sensitive to habitat fragmentation and disruption of pollination processes due to their need for intermorp cross-pollination to produce seeds. Heterostyly in the genus *Primula* is characterized by two floral morphs that differ in the reciprocal positioning of stigma and anthers, usually coupled with a self-incompatibility system that prevents self and intramorph fertilization (Wedderburn & Richards 1992). There is often variation in the pin-thrum morph distribution between Siberian primrose populations (Degerman-Fyrsten 2001). In this study, however, the morph distributions were unknown. Pollination experiments in Siberian primrose have revealed that the species is not fully self-incompatible (Degerman-Fyrsten 2001). The flowers are pollinated by insects (Lepidoptera and *Bombus*: Mäkinen & Mäkinen 1964) and the seeds mature and are shed in early August. Seeds are dispersed mainly by water currents (Ulvinen 1997).

Siberian primrose is endangered in Finland (Rassi *et al.* 2001) and is near threatened in Sweden and Norway (Gärdenfors 2005, Kålås *et al.* 2006), whereas it is not considered endangered in the White Sea region (Konstantinova 2003).

### 1.3.4 Pendant grass

Pendant grass is a tall, clonal grass that usually grows in shallow water along seashores, rivers and even lakes (Väre 1997). It grows from perennial underground rhizomes and the life span of an individual shoot is generally two years (Dobson 1989). Because of its strong clonal rhizomatous growth, pendant grass usually forms dense, almost pure one-species plant stands (Väre 1997).

Although pendant grass flowers regularly, its sexual reproduction seems to be unsuccessful (Rautiainen *et al.* 2004). The mechanism of sterility in pendant grass is unknown. In general, sterility of a species is often a result of a mechanism such as self-incompatibility or pollen limitation. Other mechanisms could also be responsible, such as mutation affecting embryo and endosperm development (Hong *et al.* 1995), fungal endophytes (Groppe *et al.* 1999) or even unsuitable environmental stochasticity, such as high water level during flowering. Even if pendant grass may occasionally reproduce sexually, it can be concluded that its main means of dispersal is clonal growth based on rhizomes.

Because of the rare or absent seed formation, the species seems to lack a specialised means of long-distance dispersal (Dobson 1989, Rautiainen *et al.* 2004). Rhizome fragments carried by water can, however, live for a long time and...
may occasionally be carried over long distances, but usually they end up close to existing patches (Rautiainen et al. 2007a).

Pendant grass has an almost circumpolar distribution in the arctic shores. It grows from Kola Peninsula through Siberia to arctic Canada (Väre 1997). It has a few clearly separate populations on the shores of the Bothnian Bay and River Tornionjoki in Finland and Sweden. Plants in these populations belong to the variety *pendulina*, which is endemic to the Bothnian Bay area. Another variety, *lapponica*, grows on the north-eastern shores of the Kola Peninsula (Väre 1991). Pendant grass also has a known occurrence in Kautokeino Norway, along the River Kautokeino in Finnmark (Council of Europe 2007). It was first found in Kautokeino in 1981 (Elven & Johansen 1981). However, the finders did not report whether the plants belong to the variety *pendulina* or *lapponica*. It is assumed that the plants in Kautokeino are a different variety than the populations in Finland and Sweden, because they differ morphologically, being taller and more robust than the var. *pendulina* found in the Bothnian area (Rautiainen 2002). In addition, hybrids of pendant grass and *Dupontia fisheri* have been found in Svalbard (Brysting & Elven 2005).

The variety *pendulina* in the Bothnian area has suffered a serious decline in the number of populations and in the size of the remaining populations during the past few decades. At present, only eight clearly separate, small populations exist in Sweden and Finland (Ericson & Wallentinus 1979, Siira 1994) and the species is considered critically endangered according to the IUCN classification (Rassi et al. 2001, Gärdenfors 2005). The largest population at Liminka Bay in Finland consists of 186 patches that cover an area of approximately 3500 m$^2$, whereas the areas of the other populations vary from less than one square metre to some tens of square metres. The species is also considered critically endangered in Norway (Kålås et al. 2006).

### 1.4 Threats for seashore plants at the Bothnian Bay

The two study species are adapted to seashore habitats and exhibit a more or less patchy population structure in the Bothnian Bay area of the Baltic Sea. In recent decades, the number of their populations in Finland and Sweden and the area they cover has markedly decreased. This is thought to result from a change in agricultural practices, which ended the traditional cattle grazing and hay making that used to keep seashore habitats open. In addition, enhanced eutrophication (Siira 1994), which has increased competition for space, has led to the demise of
some species, such as Siberian primrose and pendant grass (Markkola 1993, Rautiainen et al. 2004).

As a result, the risk of genetic erosion (i.e., the loss of genetic diversity in these species) has increased. Because genetic diversity is the raw material for adaptive evolutionary change, genetic erosion diminishes the capacity of populations to respond to environmental change and, in the long term, increases the risk of extinction (Keller & Weller 2002). Additionally, genetic erosion is often associated with reduced reproductive fitness (Crnokrak & Roff 1999). Maintenance of genetic diversity is a principal objective in the management of populations of threatened species (Frankham et al. 2002). The risk of genetic erosion is highest in small and isolated populations. This can particularly be the case where a single continuous population has been fragmented into smaller sub-populations.

The study species grow in a temporally and spatially narrow area between the sea and the dense vegetation of the upper shore (Siberian primrose in upper geolitoral and pendant grass in hydrolitoral), created by the post-glacial isostatic land uplift (relative land uplift ca. 6.9 mm year\(^{-1}\); Johansson et al. 2004). Fluctuations in the sea water level, together with movements of ice during the winter, destroy vegetation and create new patches suitable for colonisation. The potential for fast dispersal is crucial for these early successional species. Many seashore species rely predominantly on clonal growth when occupying virgin soil, which is reflected both in their population dynamics (Rautiainen et al. 2004, Rautiainen 2006) and in the genetic structure of populations.

The often unpredictable impact of the environmental stochasticity of sea forces poses a special problem when considering seashore species. The dynamics and reproduction of pendant grass, for example, are largely affected by disturbances caused by ice scouring during the winter and flooding in autumn (Rautiainen 2006). Flooding in the spring can also prevent the sexual reproduction of Siberian primrose (Björnström 2006).

Climate change may also inflict problems due to habitat decrease. One main prediction in climate models (e.g., ACIA 2005) is the future predicted loss of winter ice cover in the Bothnian Bay. This would mean that ice scouring would no longer expose soil for secondary colonisation. In addition, the rising sea water level is counteracting geological isostatic land uplift (Johansson et al. 2001), slowing the emergence of virgin land for early successional plant colonisers. In recent years, the mean sea water level in the Bothnian Bay has been ca. 5 cm
higher than predicted by the historical trend (Johansson et al. 2004), which
denotes a considerable reduction in the rate of new land formation.

1.5 Aims of the study

It is broadly accepted that knowledge of the genetic variation of a species is
essential for managing a comprehensive conservation plan (Falk & Holsinger
1991). Genetic diversity in natural populations is important because the amount
and distribution of genetic diversity are likely to affect evolutionary potential.
When feasible and cost-effective policies for management and monitoring of
endangered populations or species are planned, knowledge of the spatial genetic
structure and the type of population dynamics is of great concern.

Here, I focus on the genetic diversity and population structure of two
endangered seashore plant species, Siberian primrose (*P. nutans*) and pendant
grass (*A. fulva*), which both belong to the so called *Primula sibirica* group. The
species have similar distribution patterns and both grow on a seashore habitat.
Björnström (2006) and Rautiainen (2006) studied population viability and life
history traits of these species, whereas my aim is to examine their population
structure, population differentiation and post-glacial colonisation. The main goal
of the present study is to provide basic population genetic information of the
study species. The results, combined with the earlier knowledge, can then be used
for efficient management planning of the species. Efficient management efforts
are needed for both Siberian primrose and pendant grass, as well as other
endangered species.

The overall objectives in the project were as follows. First, I developed new
species-specific microsatellite markers (SSR, simple sequence repeat) for
Siberian primrose (I) and pursued the transfer of cross-species microsatellite
markers from related species. Then, using these microsatellite loci, I investigated
the population structure of Siberian primrose on the Northern European scale (II)
and examined the post-glacial phylogeographic history and processes underlying
the present distribution pattern of the species. I also examined the levels of genetic
diversity in pendant grass and Siberian primrose populations, as well as the
distribution of genetic variation among and within populations of the species, using
AFLP markers (III-V). My further aim has been to interlink the population
genetic processes of the species into the distribution patterns at the regional and
population levels and compile a synthesis of the impact of hierarchical
spatiotemporal processes and the autocorrelation in genetic variation at different levels.
2 Materials and methods

2.1 Sampling and populations studied

2.1.1 Siberian primrose (I, II, IV)

Siberian primrose samples \((n = 549)\) were collected from 15 populations representing the two varieties (\(P.\) nutans ssp. finnarchica var. jokelae and var. finnarchica) growing in Fennoscandia, Northern Europe. Seven populations were located in the Bothnian Bay (Finland), four in the Barents Sea (Norway) and four in the White Sea (Russia; Fig. 1). Sampling of the populations is described in more detail in papers II and IV.
Fig. 1. The map in the lower right corner illustrates the distribution of Siberian primrose subspecies *finmarchica* in Northern Europe (after Hultén 1937 and Ulvinen 1997), with the known populations marked in black and the sampled populations in grey. The sampled populations are named in the magnifications of the three study areas.
2.1.2 Pendant grass (III, V)

Samples of pendant grass \((n = 265)\) were collected in Kautokeino (Northern Norway), in Kainuunkylä (Northern Finland) and in Liminka Bay (Finland; Fig. 2). The Kautokeino (Kautokeinoelva River) and Kainuunkylä (Tornionjoki River) populations were both small and located in a riparian habitat. In Kautokeino, the population was approximately \(10 \times 20\) m in size and the samples \((n = 32)\) were taken from a \(2 \times 2\) metre grid. In Kainuunkylä, the population covered an area of approximately \(10 \times 300\) m (Juntunen 2003) and samples \((n = 36)\) were taken every 2 metres along the transect. The Liminka Bay population was large (approximately \(3500\) m\(^2\)) and consisted of several subpopulations (see Fig. 1 in paper III). Shoot material was collected from nearly all of the existing pendant grass patches from six subpopulations. Sampling of the Liminka Bay population is described in more detail in paper III. In addition, dried samples were obtained from Sweden and from the Russian Kola peninsula area. However, those samples could not be used for analysis because the DNA was degraded.

![Fig. 2. Map of the sampled pendant grass populations in Finland and Norway. 1: Liminka Bay population, 2: Kainuunkylä population and 3: Kautokeino population.](image-url)
2.2 DNA isolation

Genomic DNA of both pendant grass and Siberian primrose samples was isolated from 0.1 g of frozen or dried leaves of an individual plant using the cetyl trimethyl ammonium bromide (CTAB) method (Rogers & Bendich 1985) (I-V). Plant tissue was ground in liquid nitrogen in a mortar and approximately 0.1 g of the powder was weighed in an Eppendorf tube. The tube was placed immediately on ice and stored at -20°C prior to DNA extraction. The extraction was performed as described by Rogers and Bendich (1985), with the exception of slightly longer incubation and centrifugation times than suggested.

2.3 Molecular methods: Neutral molecular markers

The amplified fragment length polymorphism (AFLP) method was used to study genetic variation in (predominantly) nuclear DNA of pendant grass (III, V). Eleven microsatellite loci (I, II, IV) were used to study the genetic variation in Siberian primrose nuclear DNA. Both AFLPs and microsatellites are considered good marker systems and are commonly used for measuring population genetic structure and diversity. Because microsatellite markers were available for primrose species, this marker type was selected for the Siberian primrose studies. Because there was no prior knowledge available for pendant grass, AFLP markers were used. Recent comparative analyses of the performance of both marker types in population genetic studies have indicated that microsatellites often give higher estimates of genetic diversity compared with AFLPs. However, Nybom (2004) indicated that AFLP markers may be as effective as codominant markers for the estimation of intraspecific genetic diversity in plants, assuming that a sufficiently large amount of loci is scored. AFLPs can have higher discrimination power in spatial genetic structure studies and perform better in cluster analysis and assignment. No hard incongruence between the marker systems has been detected (e.g., Woodhead et al. 2005, Garoia et al. 2007, Jump & Penuelas 2007, Sonstebo et al. 2007).

2.3.1 Microsatellite isolation, optimization and fragment analysis

Microsatellite loci are DNA sequences with very short motifs of 1–6 bases repeated in a tandem fashion. They are abundant in the genomes of higher organisms and usually show high levels of polymorphism (Ellegren 2004). Due to
their frequency within most genomes and their high variability, microsatellites are important tools for studying the genetic structure of populations.

Variation in Siberian primrose was studied using microsatellites specifically developed for the species [PN2, PN3, PN4, PN5, PN6, PN9, PN10 (I, II, IV)] and cross-species amplified microsatellites originally developed for *P. modesta* Bisset et Moore, a common alpine plant distributed throughout Japan (Shimono *et al.* 2004) [PM175, PM801, PM850 and PM901 (II, IV)]. The microsatellite isolation procedure for Siberian primrose is described in paper I and the optimization of microsatellite loci originally developed for *P. modesta* is described in papers II and IV. Microsatellites originally developed for *P. sieboldii* (Isagi *et al.* 2001, Ueno *et al.* 2003) were also tested, but they did not appear to be transferable to Siberian primrose.

Siberian primrose microsatellite genotypes were assayed by polymerase chain reaction (PCR) with fluorescent labelling, followed by capillary electrophoresis on an ABI 3730 DNA Analyzer (Applied Biosystems) with GENEMAPPER® 3.7 software (Applied Biosystems) using an internal size standard, GeneScan 500 LIZ (Applied Biosystems). Gel analysis was performed using the software packages GeneScan 3.1 and Genotyper 2.0 (Applied Biosystems). In order to minimize possible scoring errors, some samples were re-genotyped up to three times. The microsatellite data for Siberian primrose (I, II, IV) were checked using the program MICRO-CHECKER (Van Oosterhout *et al.* 2004) to identify possible null alleles, large allele dropout, scoring errors due to stutter peaks and possible typographic errors.

### 2.3.2 AFLP fragment analysis

AFLPs are generated by complete restriction endonuclease digestion of total genomic DNA. Digestion is followed by selective PCR amplification and electrophoresis of a subset of the fragments (Vos *et al.* 1995). AFLP analysis results in a unique, reproducible fingerprint for each individual. The fragments that make up the fingerprint for each sample are widely distributed throughout the genome, allowing an assessment of genome-wide variation. These anonymous fragments consist largely of non-coding DNA (Wong *et al.* 2001, Shirasawa *et al.* 2004).

The AFLP technique has many advantages compared with other marker types. When using AFLP, prior sequence knowledge is not necessary, the repeatability is generally good, quantity and quality requirements of DNA are small, and the resulting DNA fingerprints provide a large number of genetic markers. The
drawback is that the markers are dominant and therefore heterozygotes cannot be distinguished from homozygotes.

Variation in pendant grass (III, IV) was investigated using the AFLP™ Plant Mapping kit (Applied Biosystems). The samples were analysed with a ROX-500 internal size standard using an automated DNA sequencer ABI Prism® 377 (Applied Biosystems). AFLP electropherograms were analysed using GeneScan software (Applied Biosystems). AFLP genotypes were scored for the presence or absence of certain fragments between 50 and 500 bp using Genotyper software (Applied Biosystems). Each band in the AFLP profile was treated as an independent locus with two alleles, and a binary matrix based on the bands was thus generated. The protocol in described in more detail in paper III.

2.3.3 Chloroplast DNA analysis

Organelle genomes are typically nonrecombinant, uniparentally inherited and effectively haploid. In angiosperms, chloroplast DNA (cpDNA) is generally maternally inherited and does not disperse by pollen, whereas nuclear DNA migrates both by pollen and by seeds. The detection of useful polymorphism at the population level with cpDNA is often difficult because of the low level of mutation rate associated with the chloroplast genome. Chloroplast microsatellites represent potentially useful markers that can circumvent this problem. To date, studies have demonstrated high levels of intraspecific variability (Provan et al. 2001). A cpDNA analysis for the pendant grass and Siberian primrose samples was conducted using chloroplast microsatellite markers developed by Weising and Gardner (1999). Because no variation was found, cpDNA microsatellites were not useful in the analysis of genetic structure in the study species.

2.4 Statistical methods

Both classical population genetic and Bayesian methods were used to analyse within- and between-population variations in the study species. Recently, Bayesian methods have revolutionised statistical thinking in genetics (Beaumont & Rannala 2004). Bayesian analysis is based on the notion of posterior probabilities, or probabilities estimated on prior expectations. Bayesian analysis offers a more direct approach to many questions, the incorporation of prior information into the analysis, and sometimes more straightforward interpretation of results (Shoemaker et al. 1999).
2.4.1 Genetic variation within populations

In papers II and IV, the genetic variability for Siberian primrose was estimated as observed and expected heterozygosities, observed number of alleles, effective number of alleles, observed polymorphism and the estimator $F_{IS}$ of inbreeding were determined according to Weir and Cockerham (1984) using GENETIX ver. 4.0 (Belkhir et al. 2004) software. Allelic richness and allele frequencies for polymorphic loci in each population were estimated using FSTAT v.2.9.3.2 (Goudet 2001). For pendant grass AFLP data (III), the population genetic parameters were estimated as the number and percentage of polymorphic loci and Nei’s unbiased expected gene diversity, assuming both HW-equilibrium and total inbreeding using POPGENE (Yeh et al. 1997).

For Siberian primrose, an online version of GENEPOP (Raymond & Rousset 1995) was used in testing for deviations from Hardy-Weinberg equilibrium. Linkage disequilibrium at each microsatellite locus in Siberian primrose was checked with FSTAT (Goudet 2001), whereas for pendant grass the analysis was conducted using LIAN software, version 3.1 (Hauboldt & Hudson 2000).

2.4.2 Clonal vs. sexual reproduction

The presence of an excess of particular multilocus genotypes is the most robust and significant evidence of clonal reproduction (e.g., Gregorius 2005). To characterize genotypic diversity in paper IV, the number of genotypes per population was estimated using GenAlEx (Peakall & Smouse 2006) and the proportion of distinguishable genotypes of plants sampled per population was calculated (Ellstrand & Roose 1987). Clones were assessed in the 15 Siberian primrose populations on the basis of $P_{SEX}$-values (i.e., the likelihood of finding at least as many identical multilocus genotypes (MLG) from the allele frequencies of each population as those observed in a panmictic population) (Ivey & Richards 2001). MLGsim (Stenberg et al. 2003) was used to compute the $P_{SEX}$-values and test for their significance.

Non-random association of alleles has also been used to infer whether organisms recombine (e.g., Gregorius 2005). The presence of incompatibilities in a genetic dataset can only be explained by recurrent mutations or recombination. In paper III, the number of multilocus genotypes and the genotypic diversity ($G_o$) of pendant grass subpopulations were determined using AFLP multilocus data, and in paper V an analysis of character compatibility was used to probe the
absence of recombination (Meacham 1981, 1984, 1994, Mes 1998, van der Hulst et al. 2000, Wilkinson 2001). Asexual lineages are assumed to accumulate unique somatic mutations, which results in an arrangement of genotypes in a tree-like structure. Thus, none of the loci, or only a small fraction (resulting from parallel mutations), are expected to be incompatible in a clonally reproducing species. The sum of incompatible loci over all pairwise comparisons (matrix incompatibility [MI]; Wilkinson 2001) can thus be used as a measure of recombination (Mes 1998, van der Hulst et al. 2000). The total number of incompatible character states, MI, within each AFLP-gnotype was calculated using the PICA software package (Wilkinson 2001).

2.4.3 Effective population size

The effective population size ($N_e$) is the size of an idealized population that would lose genetic diversity at the same rate as the actual population. The $N_e$ of a population is usually much smaller than the census size and it is affected by fluctuations in population size, relatedness, inbreeding, unequal sex ratios and a wide variety in reproductive success (Frankham et al. 2002). Compared with large populations, small populations lose diversity at a greater rate. Genetic diversity in populations is lost primarily because of sustained restrictions in the population $N_e$. Populations with recently reduced $N_e$ can also suffer increased risk of fitness loss or extinction (e.g., Allendorf & Luikart 2007). In paper IV, ONeSAMP (Tallmon et al. 2008) was used to estimate $N_e$ of the Siberian primrose populations, along with 95% credible limits. ONeSAMP is an effective population size ($N_e$) estimator that requires a single sample of microsatellite data from a single population. The method uses summary statistics calculated from the data in an approximate Bayesian framework to infer the effective size of the population that generated those data (Tallmon et al. 2008).

2.4.4 Spatial genetic structure within populations

In Paper V, a spatial autocorrelation analysis was conducted for each pendant grass subpopulation. Spatial structure was quantified using the kinship coefficient between individuals versus distance at a logarithmic scale (Hardy 2003) implemented in SPAGeDi, ver. 1.1 (Hardy & Vekemans 2002, 2003). Spatial genetic structure was also quantified as ‘neighbourhood size’ ($Nb$) and the ‘patch width’ for each subpopulation.
2.4.5 Genetic differentiation between populations and regions

*F- and R- statistics*

In paper IV, the level of genetic differentiation among Siberian primrose populations in three different regions was quantified using both $F_{ST}$ (Weir & Cockerham 1984) and $R_{ST}$. In contrast to $F_{ST}$, which is based on an infinite allele model, $R_{ST}$ is an estimator of gene differentiation that accounts for differences in allele size and is defined for genetic markers that undergo a stepwise-mutation model (Slatkin 1995). GENETIX was used to estimate the overall $F_{ST}$ and FSTAT was used to estimate the overall $R_{ST}$ estimates of the three regions. Pairwise $F_{ST}$ and $R_{ST}$ differentiation between each pair of populations within each region were estimated using ARLEQUIN, ver 3.1 (Excoffier et al. 2005) (1000 permutations).

In paper III, the approach of Holsinger et al. (2002) was used to calculate the pairwise $F_{ST}$ estimates for a Mantel test in an analysis of the genetic structure of pendant grass population in Liminka Bay using a Bayesian method in HICKORY, ver 0.7 (Holsinger et al. 2002).

*AMOVA and SAMOVA*

The method of Analysis of Molecular Variance (AMOVA) was first developed by Excoffier et al. (1992). It is a method of estimating population differentiation directly from molecular data and testing hypotheses about population differentiation. AMOVA is based on the calculation of a genetic distance matrix and it assesses the variance apportioned within and between predefined groups (Lowe et al. 2004). AMOVA produces estimates of variance components and $F$-statistic analogues, designated as $\Phi$-statistics, reflecting the correlation of haplotypic diversity at different levels of hierarchical subdivision (Excoffier et al. 1992). $\Phi$-statistics express the correlation of a pair of individuals drawn at random from a particular subgroup of the data set relative to that of a pair of individuals drawn from a wider grouping, indicating the relative partitioning of diversity between the hierarchical levels being analyzed (Lowe et al. 2004).

The presence of genetic structure at different scales was tested by an analysis of variance framework using AMOVA with the program ARLEQUIN. Version 2.0 (Schneider et al. 2000) was used for pendant grass subpopulations in paper III and version 3.1 (Excoffier et al. 2005) was used for Siberian primrose populations in
paper IV. The latest version was also used for the AMOVA analysis with three pendant grass populations.

To examine larger regional structures in Siberian primrose (II) we adopted the approach of SAMOVA (Spatial Analysis of MOlecular VAriance; Dupanloup et al. 2002) to define groups of populations that are geographically homogeneous and maximally differentiated from each other. Unlike AMOVA, this method does not require an a priori definition of population groups, but instead searches for emergent group structures based only on the genetic and geographic data. As a byproduct, it also leads to the identification of genetic barriers between the defined groups. The method is based on a simulated annealing procedure that aims at maximizing the proportion of total genetic variance due to differences between groups of populations. The method uses latitude and longitude for each population to create a user-defined number (K) of maximally differentiated groups of geographically proximate populations. Good results are obtained when the Nm value between groups is 0.01 or lower (Dupanloup et al. 2002).

**Clustering and multivariate methods**

An unrooted neighbour-joining dendrogram was constructed with the NEIGHBOR program of the PHYLIP-package (Felsenstein 1993) from a genetic distance matrix of Siberian primrose populations (II).

The program TFPGA (Miller 1997) was used to construct Reynolds genetic distance matrices (Reynolds et al. 1983) between populations. Then, an UPGMA tree was constructed from the distance matrices and visualized as an unrooted phylogram using TreeView software (Page 1996). The bootstrapping option of the TFPGA was selected, in which the program constructs multiple new data sets (1000) by resampling with replacement over the loci and reports the proportion of permuted data sets that result in the formation of a node seen in the original data set.

The spatial population structure of Siberian primrose at a local scale was studied using the program GenAlEx (Peakall & Smouse 2006) (II) and the extent of regional differentiation at a local and a larger scale was visualized by factorial correspondence analysis (II, IV) (FCA; Benzécri 1973, She et al. 1987). Principles of FCA have been described by Benzécri (1973). Briefly, correspondence analysis maximizes the correspondence between allele scores and individual scores. This computation is done by transforming the allele frequency data into a contingency table, where a chi-squared distance measures the
relatedness between any two samples in the $k$-dimensional ($k =$ number of alleles) space. The resulting factorial axes can be ordered by their eigenvalues, and projection of all individuals in a space defined by the axis can be used to express which individuals are most different or similar.

**Isolation by distance between and within populations**

The dispersal of the organism has a limit, which leads to a geographic pattern. The population genetic pattern "isolation by distance" (IBD) results from limited dispersal ability and spatially limited gene flow and it is a commonly observed phenomenon in natural populations (Wright 1943). The isolation by distance model assumes that geographical distance and genetic distance correlate. When there is IBD between populations, the genetic distance between them increases with increasing geographic distance. IBD measures the regression of pairwise estimates of population structure ($F_{ST}$) on pairwise geographical distance (km), and is used to infer the genetic connectivity within and between populations. The genetic relationships among populations and the extent of regional differentiation can be visualized by a factorial correspondence analysis.

In paper IV, the Mantel test option in the program ARLEQUIN was used to test for IBD in Siberian primrose populations within regions. The spatial population structure of Siberian primrose on a local scale was analysed using GenAlEx (Peakall & Smouse 2006).

To test for IBD between pendant grass subpopulations (III) and among the three populations (Liminka Bay, Kainuunkylä and Kautokeino), the assumptions of association between pairwise genetic and geographic distances were tested and the significance was determined with a Mantel test (Manly 1985) using GENEPOP (Raymond & Rousset 1995) and ARLEQUIN, respectively. Assignment analyses for the Liminka Bay subpopulations (III) and for three populations were conducted using AFLPOP ver. 1.1 (Duchesne & Bernatchez 2002). The loci were filtered and 201 and 206 loci, respectively, were used for the assignment analysis.

**Indirect and direct methods to estimate migration between populations and regions**

Possible gene flow between pendant grass subpopulations was studied using 2MOD (Ciofi et al. 1999), which compares the relative likelihoods of a gene flow
model versus a genetic drift model (III). The program uses a coalescent theory and a Markov chain Monte Carlo simulation approach with Metropolis–Hastings sampling to explore the alternative drift and immigration models. In order to analyse whether there has been any gene flow between the subpopulations, the program estimates the relative likelihoods of drift vs. immigration models since a certain time. In the drift model it is assumed that an ancestral panmictic population is separated into several independent units that start diverging purely by genetic drift, whereas the gene-flow model assumes that the gene frequencies within subpopulations are determined by a balance between genetic drift and immigration.

In paper II, a traditional $F$-statistic method (Wright 1969) was used to estimate the average effective number of migrants exchanged per generation between the regions. GENEPOP (Raymond & Rousset 1995) was used to estimate genetic distances ($F_{CT}$) between regions and corresponding estimates of the average effective number of migrants ($N_m$) exchanged per generation using Wright’s (1969) formula $N_m = (1 - F_{CT}) / 4 F_{CT}$. In paper II, migration rates in Siberian primrose were estimated and the proportion of migrants between regions was calculated using a Bayesian method implemented in the program BAYESASS, version 1.3 (Wilson & Rannala 2003). An assignment analysis was also conducted to identify possible first-generation migrants among the different regions using the Bayesian individual assignment method (Rannala & Mountain 1997) as implemented in GENECLASS2 (Piry et al. 2004).

**Past population history**

Historical data on population sizes are not often available, but inferences can be made from available genetic data. In paper II, a Bayesian coalescent-based approach developed by Beaumont (1999) was used to assess long-term changes in historical population size. The method implements a coalescent simulation-based Bayesian likelihood analysis and estimates the posterior probability distribution of population parameters using a Markov chain Monte Carlo simulation based on the observed distribution of microsatellite alleles and their repeat number. The most important parameters are $N_0$, $N_1$, and $t$, where (1) $N_0$ is the mean current effective population size; (2) $N_1$ is the mean ancestral population size in time $t$, and (3) $t$ is the time since the population started to decline. This method is implemented in the MSVAR program (Beaumont 1999).
3 Results

I examined the genetic diversity and population structure of two endangered seashore plant species, Siberian primrose and pendant grass, at different spatial scales. The present study provides important information for species conservation and management in terms of the genetic data that are needed for a better understanding of the species’ biology and dynamics in a constantly changing environment. The results of the study can be used in planning the management of the study species and give insight for other *Primula sibirica* group species as well, although each species requires its own study for efficient management.

3.1 Siberian primrose (I, II, IV)

3.1.1 Microsatellite markers (I, IV)

Seven polymorphic microsatellite loci were developed for Siberian primrose (I). These markers are the first microsatellite loci reported for this species. In addition, four cross-species amplified loci were successfully transferred from *P. modesta* (IV). These eleven microsatellite loci were used in the characterization of 549 Siberian primrose individuals from 15 Northern European populations.

3.1.2 Genetic variation and effective population size (IV)

No signs of null alleles or large allele dropouts were found in the microsatellite data. The genetic and allelic diversities were remarkably low in the North European Siberian primrose populations (see Table 1 in paper IV). For the 11 microsatellite loci, 36 alleles were detected among the 549 individuals sampled from 15 natural populations. The mean number of polymorphic loci was 4.57 among the Bothnian Bay populations, 3.25 among the Barents Sea populations and 10.0 among the White Sea populations. $H_{\text{exp}}$ averaged over 11 loci varied between 0.051 and 0.325 and $H_{\text{obs}}$ varied between 0.052 and 0.259.

No spatial structuring within the populations was found, nor was there evidence of extensive clonal reproduction on the study scale. Private alleles with allele frequency >0.05 were found in all three regions, one each in the Bothnian Bay and the Barents Sea and eight in the White Sea. There was no significant linkage disequilibrium found in any population.
Two White Sea populations (Lodeiny Island and Kolvitsa) showed significant heterozygote deficiency (see Table 1 in paper IV) and the Wright’s inbreeding coefficient ($F_{IS}$) values for all populations ranged from -0.21 to 0.25 in the Bothnian Bay, from -0.31 to 0.15 in the Barents Sea and from 0.13 to 0.45 in the White Sea region. The average values of $F_{IS}$ over all loci and populations of each region were 0.115 for the Bothnian Bay, -0.086 for the Barents Sea and 0.294 for the White Sea (see Table 3 in paper IV). Estimates were significantly different from zero in the White Sea and Bothnian Bay regions.

The mean $N_e$ estimates for Siberian primrose populations in all regions were very low and no significant differences between the regions were detected (IV). The Bayesian coalescent simulations indicated a long-term population decline in each region.

3.1.3 **Spatial genetic structure within populations (IV)**

No spatial structuring within the populations was found. The combined spatial autocorrelation analysis did not suggest any clear spatial structuring in the studied Siberian primrose populations. Spatial genetic structure was not detected within individual populations or combined populations in any region or with any of the used distance classes (data not shown).

3.1.4 **Clonal vs. sexual reproduction**

No evidence of extensive clonal reproduction at the study scale was found. Altogether, 55 repeated multilocus genotypes were found. However, according to the simulated critical values of $P_{SEX}$ (program MLGsim by Stenberg et al. 2003), only three of those were likely to be clonally produced ($P<0.05$; data not shown). One evident clone was detected in one of the populations in each region: populations Parhanlahti (Bothnian Bay), Talvik (Barents Sea) and Ryshkov Island (White Sea).

3.1.5 **Differentiation between populations within regions (IV)**

In the Bothnian Bay and the Barents Sea regions, FCA analysis did not suggest local population structure. In the analysis of the White Sea region, Luvenga was separated from the other three populations (see Fig. 2 in paper IV).
In the AMOVA analyses, a highly significant structure was detected, with two hierarchical levels in each region (see Table 2 in paper IV). Variation between populations explained 11.6% of the total variation in the Bothnian Bay, 16.6% in the Barents Sea and 27.2% in the White Sea region.

A significant differentiation between populations within each region was found. Most of the pairwise comparisons of populations within regions were significant under both infinite allele and stepwise mutation models (see Table 4 in paper IV). Isolation by distance was found only in the Bothnian Bay region (with between population distances over 121 km).

3.1.6 Disjunct distribution and post-glacial recolonisation (II)

Siberian primrose has a disjunctive, tripartite distribution in Northern Europe, where it grows along the coasts of the Bothnian Bay, the White Sea, and the Barents Sea at the Arctic Ocean.

Large genetic differences between the disjunctive regions were found. The White Sea region had significantly more genetic and allelic diversity than the other two regions. The difference in average genetic or allelic diversity between the Bothnian Bay and Barents Sea regions was not significant for any index. The genetic composition of plants from the White Sea region appeared to be intermediate between the individuals from the Barents Sea and the Bothnian Bay.

The results of SAMOVA analysis suggested that there are genetic barriers between each disjunctive distribution region and that there are three population groups (see Table 3 in paper II). Similarly, in the neighbour joining tree the populations tended to cluster according to their geographical origin (see Fig. 2 in paper II). Existence of the three geographical regions (Bothnian Bay, Barents Sea and White Sea) in the dendrogram was supported with high bootstrap values, and geographically closely located populations tended to cluster together.

The FCA analysis suggested that there was much more genetic variation in the White Sea than in other regions. The individuals from the Bothnian Bay and Barents Sea populations seemed to be clearly differentiated. Although individuals from both regions formed rather tight clusters, both clusters were still adjacent to the less aggregated White Sea individuals (see Fig. 3 in paper II). Correspondingly, the pairwise estimates of genetic differentiation between regions suggested that the Barents Sea and Bothnian Bay regions were distant from each other ($F_{CT} = 0.788$) and that the White Sea region populations were as closely
related to the Barents Sea ($F_{CT} = 0.490$) populations as to the Bothnian Bay ($F_{CT} = 0.491$) ones.

The traditional $F$-statistics method suggested a low level of migration among the three regions. The Bayesian approach and the assignment analysis also indicated high self-recruitment in each region and very low, virtually non-existing migration rates between the Siberian primrose populations in Northern Europe (see Table 4 in paper II). The MSVAR estimates of population expansion in the different regions, assuming a generation time of 15 years, suggested that the Bothnian Bay was colonised most recently, about 8500 years ago, the Barents Sea about 9300 years ago, and the White Sea earlier, about 13,400 years ago (II).

3.2 Pendant grass (III, V)

In this section, in addition to the results presented in the original papers (III, V), I present new results of pendant grass populations in Kainuunkylä and Kautokeino (Fig. 2).

3.2.1 Genetic variation (III)

The distribution of genetic variation within and between six pendant grass subpopulations was investigated in the largest remaining population at the Liminka Bay, Finland, using AFLP markers. Each examined shoot had a unique AFLP pattern and no repeated genotypes were found. The proportion of polymorphic loci in different subpopulations varied between 65% and 82%. The mean genetic diversity within subpopulations, assuming Hardy-Weinberg equilibrium ($H_{El}$), was 0.267. Assuming total inbreeding ($H_{En}$), the estimate was 0.197. Both estimates varied only slightly between subpopulations.

Relatively high amounts of variation were found in the subpopulations. The mean Nei expected heterozygosity was typical (0.267) for an outcrossing species (Nybom 2004). The average values of the proportion of polymorphic loci and Nei’s gene diversity appeared to be higher in the two central subpopulations than in the other, more peripheral subpopulations. However, this difference was significant only for $H_{El}$. No significant association was detected between the estimated subpopulation area and genetic variation, suggesting that drift and migration are not in equilibrium within the subpopulations. Significant linkage disequilibrium between loci was found in each subpopulation.
3.2.2 Spatial genetic structure within subpopulations (III)

A distinct spatial genetic structure was found in the studied Liminka Bay subpopulations of pendant grass (see Fig. 2 in paper V). On average, individuals located spatially close to each other were more likely to be genetically related than individuals separated by large distances.

The dominant spatial pattern within the subpopulations did not correspond with the shape of autocorrelation correlograms produced by theoretical models of spatially restricted gene flow, but indicated that clonal or sexual founding events could produce the spatial genetic structure of pendant grass. The Sp statistic varied between the subpopulations (see Table 1 in paper V), as did the neighbourhood size. No obvious association was found between the Sp statistic or the neighbourhood size and the subpopulation size. However, a relatively high positive correlation between population area and the “patch size” was found.

3.2.3 Clonal vs. sexual reproduction (V)

The matrix incompatibilities of subpopulations were high, which suggested sexual reproduction (see Table 1 in paper V). However, they were significantly lower than expected under random mating in all subpopulations, which indicated clonality. The incompatibility excess ratios varied slightly among the Liminka Bay subpopulations. Thus, the subpopulations departed from random mating to different degrees and the extent of sexual reproduction probably differed among them. Nearly all genotypes contributed some incompatibility in the character compatibility of pendant grass, but the contribution of each genotype varied.

Successive deletion of 86–92% of genotypes was necessary to remove matrix incompatibility in the data from different subpopulations (see Fig. 3 in paper V). In most subpopulations, a small group of individuals made the greatest contribution to the total incompatibility. After removal of those genotypes, incompatibility declined continuously. Contribution of conservative markers to character incompatibility differed markedly from site to site, which, on the other hand, is not easily explained as a result of recombination. In the Kainuunkylä population, the character incompatibility count was ten times as high as that in the Kautokeino population or the Liminka Bay subpopulations (Fig. 3, see also Fig. 3 in paper V). In Kainuunkylä population, one individual sample contributed most to the high incompatibility count. After removing this individual sample, incompatibility declined to the same level as in the other populations and...
subpopulations. After this, incompatibility declined linearly also in Kainuunkylä population (Fig. 3). In Kautokeino, character incompatibility declined linearly from the first removed individual.

![Graphs showing incompatibility count vs. individuals removed for Kainuunkylä and Kautokeino populations.](image)

**Fig. 3.** Character compatibility analysis of Kainuunkylä and Kautokeino populations of pendant grass.

### 3.2.4 Spatial genetic structure among subpopulations (V)

In the Liminka Bay population, the Bayesian analysis of population structure gave strong evidence that $F_{ST}$ was higher than 0. This suggests that there are significant genetic differences among the subpopulations, although the level of differentiation is not very high.

In the AMOVA analysis, the variance components were highly significant at all hierarchical levels. Most of the variation (76%) was found within patches (see Table 3 in paper III). The variation between subpopulations explained only a small amount of the total variation (4.57%), and the small $\Phi_{ST}$ estimate between subpopulations suggested low genotypic differentiation between subpopulations. However, all of the pairwise exact tests between subpopulations were significant, suggesting dissimilar genotype frequencies in different subpopulations. Much more variation was found among patches within subpopulations, and the reasonably large estimate of differentiation between patches also suggested significant genotypic substructuring within subpopulations.
Results of the 2MOD simulations indicated that the subpopulations of pendant grass are not totally isolated units, but that there is some migration between the subpopulations.

In the assignment analysis, the total assignment success was low, indicating that migration between subpopulations is high and/or that the subpopulations are so similar that the resolving power of the analysis is not very good. Only in the most isolated subpopulation did the assignment success exceed 50%. There was mixing between adjacent subpopulations, but also between subpopulations that were not located especially close to each other.

The scatterplot and the Mantel correlation analysis suggested isolation by distance because there was a relatively strong positive association between pairwise $F_{ST} / (1-F_{ST})$ values and linear geographical distance between subpopulations (see Fig. 2 in paper III).

### 3.2.5 Differentiation between populations

The UPGMA-dendrogram of pendant grass populations and subpopulations (Fig. 4) was supported with high bootstrap values at all nodes (88–100%), suggesting a high reliability of the pattern found. Clustering clearly separated the six pendant grass Liminka Bay subpopulations, and Kainuunkylä, and Kautokeino populations into three clusters. Cluster one contained the Liminka Bay subpopulations, cluster two represented the Kainuunkylä population, and cluster three contained the Kautokeino population. In cluster one, the subpopulations of Liminka Bay located geographically close to each other were clustered together in most cases (see Fig. 1 in paper III, Fig. 4).
Fig. 4. A neighbour-joining dendrogram that shows the genetic relationships among the three pendant grass populations and subpopulations. This tree is based on Reynolds genetic distance. The percentage of trees sharing the node after 1000 iterations by bootstrapping was 88% for the node marked with * and 100% for all other nodes.

In the assignment analysis of pendant grass, individual samples from three populations 27% of samples collected from Liminka Bay were allocated to the Kainuunkylä population and 8% of the Kainuunkylä samples were allocated to Liminka Bay subpopulations. All of the samples collected from Kautokeino were allocated to Kautokeino and none of the samples from the Kainuunkylä or Liminka Bay populations were allocated there (Table 1).

Table 1. Results of the assignment analysis of 265 pendant grass individuals. The numbers of individuals assigned to their sampling location are given in *italics*.

<table>
<thead>
<tr>
<th>Given population</th>
<th>Inferred population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liminka Bay</td>
</tr>
<tr>
<td>Liminka Bay</td>
<td>143</td>
</tr>
<tr>
<td>Kainuunkylä</td>
<td>3</td>
</tr>
<tr>
<td>Kautokeino</td>
<td>0</td>
</tr>
</tbody>
</table>

In an AMOVA analysis with three hierarchical levels, pendant grass populations from Liminka bay, Kainuunkylä, and Kautokeino were first grouped by population and then by their connectivity by waterways (Table 2). In the analysis with populations as groups, most of the variation was found within subpopulations (56%, $F_{ST} = 0.44$, P<0.001) and among regions (40%, $F_{CT} = 0.40$, P = 0.061). Only 4% of the variation was found among populations within regions.
When groups were chosen by connectivity by waterways, most of the variation was found among groups (52%, $F_{CT} = 0.053$, $P = 0.129$). The regional structure was not significant in either of the groupings.

Table 2. AMOVA results of pendant grass populations in Liminka Bay, Kainuunkylä and Kautokeino.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance component</th>
<th>% of total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three groups: Liminka Bay; Kainuunkylä; Kautokeino</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>2</td>
<td>1702.81</td>
<td>14.17</td>
<td>39.92</td>
<td>0.06</td>
</tr>
<tr>
<td>Among subpopulations within populations</td>
<td>5</td>
<td>356.15</td>
<td>1.58</td>
<td>4.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within subpopulations</td>
<td>257</td>
<td>5077.54</td>
<td>19.76</td>
<td>55.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Two groups: Liminka Bay and Kainuunkylä; Kautokeino</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among groups</td>
<td>1</td>
<td>1472.48</td>
<td>24.47</td>
<td>52.53</td>
<td>0.13</td>
</tr>
<tr>
<td>Among populations within groups</td>
<td>6</td>
<td>586.48</td>
<td>2.35</td>
<td>5.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within populations</td>
<td>257</td>
<td>5077.54</td>
<td>19.76</td>
<td>42.42</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The Mantel test revealed significant isolation by distance between all of the populations (Correlation coefficient 0.93, $P_{\text{mantel}} = 0.00067$, 100 000 permutations), as well as between the Kainuunkylä and the Liminka Bay populations (Correlation coefficient 0.75, $P_{\text{mantel}} = 0.0068$, 100 000 permutations). However, when linearised pairwise $F_{ST}$ estimates were plotted against geographic distances, there seemed to be a linear correlation between the Liminka Bay and the Kainuunkylä populations, which are connected by waterways, whereas the Kautokeino population seemed more isolated from the other populations (Fig. 5). This result suggests that there might not be much gene flow between the Bothnian Bay and Barents Sea regions in this species either.
Fig. 5. Pairwise genetic ($F_{ST}$ / $(1-F_{ST})$) and logarithmic geographical distances (metres) between the three pendant grass populations in Liminka Bay, Kainuunkylä and Kautokeino. The dashed line represents linear regression between these two distances at a scale of less than 200 km.
4 Discussion

4.1 Siberian primrose

4.1.1 Microsatellite markers for Siberian primrose

Despite of the great advances in genomic technology that have been observed in the last years, the availability of molecular tools such as microsatellite markers has been limited in the genus *Primula*. A few years ago, microsatellites were available for *Primula sieboldii* (Isagi *et al.* 2001, Ueno *et al.* 2003, 2005) and *P. modesta* (Shimono *et al.* 2004). I tested the cross-species transferability of microsatellites developed for *P. sieboldii* (Isagi *et al.* 2001, Ueno *et al.* 2003) and *P. modesta* (Shimono *et al.* 2004) using a range of reaction and cycling conditions.

Cross-species transfer of nuclear microsatellite markers in plants has been demonstrated in numerous taxa. The success of cross-species amplification of any DNA sequence by PCR depends on the source and characteristics of the genomic library and on the evolutionary distance of the species (Dayanandan *et al.* 1997). Different transfer success of loci can be explained by the relationships of the species in question. Both Siberian primrose and *P. modesta* are classified in the subgenus *Aleuritia* (Duby) Wendelbo (although different sections of Siberian primrose are classified in *Armerina* Lindley and *P. modesta* in *Aleuritia* (Duby)), and cluster in the same clade in a cpDNA phylogeny (Mast *et al.* 2001). The species were not too divergent, since four of the microsatellites developed for *P. modesta* were successfully transferred to Siberian primrose (II, IV).

The microsatellites developed for *P. sieboldii* were however not transferable to Siberian primrose, probably because they were too distantly related; *P. sieboldii* is classified in a different subgenus, *Aughantus*, and section *Cortusoides*. Similarly, Cotti (2008) was unable to transfer the microsatellites developed for *P. nutans*, *P. sieboldii* and *P. vulgaris* to *P. apennina*, which belongs to the section *Auricula*. On the other hand, five of the microsatellite primers developed for *P. sieboldii* by Ueno *et al.* (2003, 2005), have been successfully transferred to *Primula kisoana* (Ohtani *et al.* 2005). These two species are more closely related and classified in the same subgenus and section.

For a majority of species, cross-species microsatellites are not available or not applicable. Consequently, microsatellite markers have to be specifically isolated for each species in question. Since no microsatellite markers were
available specifically for Siberian primrose, I developed new microsatellite markers for it (I). Primers were designed for ten loci. Three of them were monomorphic, and therefore not useful in the genetic studies. Seven loci were polymorphic, and they were used in the genetic studies of Siberian primrose in papers II and IV, together with the four cross-species loci transferred from *P. modesta*.

4.1.2 Genetic diversity, *N_0*, and population differentiation of Siberian primrose populations

The overall variability of the studied Siberian primrose populations using microsatellite markers was low in each study region, especially in the Bothnian Bay and the Barents Sea populations. At a regional scale both the *F_{ST}* and *R_{ST}* estimates suggested moderate genetic structuring of the populations. The results of AMOVA analysis with two hierarchical levels indicated highly significant differentiation between populations in all three regions, even though the majority of the total variation was found among individuals within populations (see Table 2 in paper IV). The FCA analysis, however, suggested structuring only in the White Sea region. In the Bothnian Bay and the Barents Sea regions the overall diversity was probably too low for the FCA analysis to detect structuring.

Species that grow in hydrologically connected habitats, *e.g.*, aquatic macrophytes, often have high levels of gene flow among populations and relatively low levels of inter-population differentiation (Nies & Reusch 2005, Chen *et al.* 2007). Also, Siberian primrose grows in a habitat where the seed dispersal is affected by water dynamics. There was no spatial structuring within the populations in any of the regions, and dispersal seemed to be most efficient at a local (population) scale (IV). Isolation by distance was found only in the Bothnian Bay region when the distance between populations was over 120 km. Similarly, in a related species, *Primula sieboldii* growing along streams in Japan, an efficient seed and clonal propagule dispersal during flooding has resulted in a very low level of differentiation among subpopulations within streams (Kitamoto *et al.* 2005).

The estimated mean *N_0* values were very small for all of the studied Siberian primrose populations, and no large differences between the regions were found (IV), even though the species is not considered endangered in the White Sea. The decline of Siberian primrose populations of Northern Europe has been acknowledged only quite recently, however. According to the results of Bayesian
demographic simulations, the decline started several thousands of years ago when the species colonised the areas (II). In Siberian primrose the phylogeographic history of the Northern European area has strongly influenced the amount of diversity extant in the three study regions today. During post-glacial recolonisation of the area, the species went through several bottlenecks, which has resulted in loss of genetic diversity in the populations (II).

Compared with widespread taxa, many rare and endangered species may become genetically depauperate because small populations are subject to genetic drift and inbreeding. Both of these processes can lead to loss of genetic diversity (e.g., Bauert et al. 1998). In heterostylic species imbalance of the frequency of the two flower morphs (pin versus thrum) is expected to further reinforce genetic drift effects by reducing mating opportunities especially in small, isolated populations (Allee effect) (Byers & Meagher 1992, Washitani 1996). Correlations between population size, flower morph imbalance and population persistence have been found in other primrose species, for example in Primula vulgaris (Endels et al. 2002). Biased flower ratios have led to lowered seed production and further, to lowered seed germination in several Primula species (e.g., Jacquemyn et al. 2002, Kery et al. 2003, Brys et al. 2004). These may be the future of small Siberian primrose populations, especially when populations are declining as the current trends suggest.

4.1.3 Mode of reproduction and dispersal mechanisms of Siberian primrose

Siberian primrose reproduces both by seeds and runners (Mäkinen & Mäkinen 1964), and clonal propagation has frequently been observed in the Bothnian Bay populations (Degerman-Fyrsten 2001). However, at the study scale, no signs of extensive clonal growth were found (IV). In Siberian primrose the runners usually are less than 15 cm long (Björnström T, pers. comm.). Consequently, with the 50 cm sampling intervals used in my study, the probability of finding clone mates was not very high. In order to study the clonality of Siberian primrose, a smaller scale study should be conducted with samples collected at shorter distances. For instance P. modesta, a closely related species also with a dense growth habit (shoots mostly within 5 cm of each other), had identical MLGs and its genets were of the same clone (Shimono et al. 2006).

The absence of clear genetic structure within the Siberian primrose populations could indicate that seed and pollen dispersal are not restricted within
the populations. Furthermore, the clonal propagation or heterostyly did not seem to have any effect on the local structure. Heterostyly is thought to efficiently decrease the inbreeding coefficient in large populations because it decreases inbreeding by allowing successful mating to occur only between individuals that have different flower morphs. The high $F_{IS}$ estimates observed in a few study populations suggested that heterostyly does not prevent the intramorph mating very efficiently. The high estimates can, however, be explained with several factors, including inbreeding, selfing and the Wahlund effect. In Siberian primrose, inbreeding cannot be completely ruled out since both intramorph and intermorph crossings can produce viable seeds (Degerman-Fyrsten 2001). We did not find signs of null alleles or large allele dropout in the data.

The Siberian primrose pollinating insects, Lepidoptera and Bombus, can have rather long flying distances (Mäkinen & Mäkinen 1964), but pollen dispersal is not likely the main contributor for dispersal between populations located tens of kilometres apart. The size and structuring of populations have an effect on the behaviour of pollinators. In insect pollinated species pollen is often dispersed only over short distances. For example, in a related species, *P. veris*, the distance of pollen dispersal is less than 12 m (Richards & Ibrahim 1978), and in *P. sieboldii*, it is usually less than 20 m (Kitamoto et al. 2007). The dynamics of the sea are most likely more important than pollen flow for regional dispersal between Siberian primrose populations. The seeds have no special physiological dispersal mechanism, but they are able to float (Hyvärinen M, pers. comm.), and may disperse with water currents when the shores are flooded, which occurs several times during the year. Occasional submergence of the habitat may sometimes prevent pollination (Björnström 2006), but may also contribute to seed dispersal. Movements of ice in winter can also move the seeds, as well as genets and ramets to new sites.

### 4.1.4 Post-glacial dispersal of Siberian primrose

Many arctic taxa had refugia in southern localities during the latest glaciation (reviewed in Hewitt 2000). In recent studies Beringia has also been recognised as an important area for the initial divergence and expansion, and also for continuous *in situ* survival of species such as the arctic blueberry (Alsos et al. 2005).

In the present study the inference of a possible colonisation scenario of Siberian primrose in Northern Europe was based on the distribution of microsatellite variation among the three study areas in Northern Europe. The
genetic structure of Siberian primrose at the Northern European scale was investigated in order to identify the mechanisms responsible for the presently disjunct distribution. These included (i) post-glacial colonisation from a single or several southern, eastern or northern refugia, (ii) fragmentation of a formerly broader distribution or (iii) continuous migration among the three regions (II).

No evidence supporting the theory of an Arctic Ocean origin of the Bothnian Bay populations, or indication of a southern refugia was found. Neither did we find evidence supporting the theories of (ii) a formerly broader distribution of Siberian primrose or (iii) possible long distance dispersal between the regions. Instead, the results seemed to indicate the previously hypothesized eastern origin for the populations (II).

Frequent founder effects and genetic drift during post-glacial colonisation of Northern Europe have resulted in an overall low level of genetic diversity in the studied Siberian primrose populations. The highest level of genetic diversity observed in the White Sea area compared to the Barents Sea and Bothnian Bay areas, together with the distribution of private alleles between the regions, supported the eastern refugia hypothesis. Further support was suggested by the results of the Bayesian coalescent simulations. The estimates of time span after initial population expansion in the different regions suggested that Siberian primrose colonised the White Sea area several thousand years before it reached the other two areas, which at that time were probably still covered with ice. Later, when the ice melted, the species also colonised the Barents Sea and the Bothnian Bay areas, both at roughly the same time. The populations of those most western areas represent the leading edge of colonisation from eastern refugia and have a lower level of genetic diversity than the White Sea populations because of population bottlenecks during colonisation. The observed pattern of a strong genetic structure between the different regions may well be explained by the lack of gene flow between the regions.

No evidence of dispersal of Siberian primrose between the different regions was found, and regional dispersal seemed to be efficient over distances less than 120 km (II, IV). Because dispersal seems to be efficient only over rather short distances, it seems most likely that Siberian primrose colonised the Bothnian Bay area during the Baltic Ice Lake or the Yoldian Sea period, when there were only narrow isthmuses between the Bothnian Bay and the White Sea water areas (e.g., Jantunen 2004). It most likely spread first to the White Sea area, and subsequently colonised both the Barents Sea and the Bothnian Bay regions from there. At that time the shoreline of current Bothnian Bay was located much further east. During
subsequent times the shoreline moved west as a result of land uplift, and the species followed the shoreline. This scenario would also explain the formerly known inland populations (Mäkinen & Mäkinen 1964), which persisted in suitable habitats when the shoreline receded further westwards.

Recently discovered plant macrofossils from west Beringia have indicated that during the last cold-stage, arctic species coexisted there with aquatic littoral, meadow and steppe taxa (Kienast et al. 2005). Among the species of fossils found was *Potamogeton vaginatus*, which is one of the *Primula sibirica* group species. It seems likely that the refugia of Siberian primrose were also located somewhere in Beringia, but the exact location remains to be discovered.

### 4.1.5 Taxonomy of Siberian primrose

Mäkinen & Mäkinen (1964) put forward a division of the European subspecies *finmarchica* into two varieties based on morphology: var. *finmarchica* occurring at the Arctic Ocean, and var. *jokelae* at the Bothnian Bay and the White Sea. However, our results of the genetic divergence between the three study regions indicated that the White Sea populations are as closely related to the Bothnian Bay as to the Barents Sea populations. The genetic distances between the regions, together with the results of the FCA (see Fig. 3 in paper II) and SAMOVA analysis (see Table 3 in paper II) and the neighbour joining tree (see Fig. 2 in paper II), suggested that rather than two groups, the populations form three groups that correspond to the three regions. Therefore, the strict separation of the subspecies *finmarchica* into two varieties (Mäkinen & Mäkinen 1964) does not seem necessary (II).

### 4.2 Pendant grass

#### 4.2.1 Genetic diversity of pendant grass

Because of the apparent clonal reproduction of pendant grass, a low amount of genetic diversity was expected in the Liminka Bay population. On the contrary, every sampled individual exhibited a unique AFLP genotype, and the genotypic diversity was equal to the sample size (III). The genetic diversity of the population was also relatively high. The mean Nei’s expected heterozygosity within subpopulations assuming random mating (*H_*\text{e}) and total inbreeding (*H_*\text{i})
scored 0.267 and 0.197, 653 respectively. Nybom (2004) has reviewed studies using nuclear DNA markers for assessment of among and within-population diversity in wild plants. When comparing the present results to her review, pendant grass seems to exhibit a high level of genetic diversity, considering that the grand mean of $H_{e N}$ within populations in AFLP based studies in the review was 0.23. The level of diversity in pendant grass seems to be typical for outcrossing species with 0.24 as a mean $H_{e N}$ in dominant marker based studies.

4.2.2 Clonal vs. sexual reproduction

According to field observations, the reproduction and dispersal of pendant grass in the Liminka Bay is governed mainly by clonal growth and fragmentation of rhizomes (Rautiainen et al. 2004). The capability of clonal populations to maintain their genotypic diversity is regarded as lower than sexual populations (Balloux et al. 2003) and prolonged exclusive clonal growth can eventually lead to a monoclonal population even on the basis of genetic drift (Honnay & Bossuyt 2005). Because of the apparent clonal reproduction of pendant grass, a low amount of genetic diversity was expected in the Liminka Bay population. On the contrary, every sampled individual exhibited a unique AFLP genotype, and the genotypic diversity was equal to the sample size (III). Also, compared to other species, the observed level of genetic diversity in pendant grass was high (Nybom 2004) and most similar to the estimates in outcrossing species.

Observed high genotypic and genetic diversity suggested that clonal growth contributes little to the within-subpopulation diversity, or that its effect occurs over a spatial scale less than the used sampling scheme (III). On the other hand, the observed significant linkage between loci in each subpopulation suggests a non-random association of alleles. This may indicate clonal reproduction in pendant grass (III), even though there may be other reasons for the linkage disequilibrium (Ohta 1982, McVean 2002).

To distinguish if the different pendant grass genotypes were the result of mutations or recombination, the character compatibility analysis was used (see Fig. 3 in paper V, see also Fig. 3). According to the results, both clonal and sexual reproduction were important. The results suggested that sexual recombination has substantially contributed to the observed high genotypic and genetic variation. The main mechanisms behind the spatial genetic structure seemed to be founding events with a few propagules, and clonal extension after initial foundation of subpopulations/populations. This is common to clonally reproducing species, where
new areas are often occupied by seedlings and subsequently by clonal spread (Eriksson 1993). The clonal growth assumption is consistent with the field observations of pendant grass (Rautiainen et al. 2004).

Following an initial colonisation event, there is a rapid loss of genets in populations in which there is no further seedling recruitment. Such populations can become dominated by a few large clones. Lack of recombination hinders adaptation to a changing environment and makes the population more vulnerable to a selective sweep (e.g., Silvertown & Charlesworth 2001), which effectively narrows the genotypic diversity. Despite of the evident clonal reproduction and recent decrease in the number of populations, pendant grass has maintained a high level of genetic and genotypic diversity, and a large effective neighbourhood size in the Liminka Bay population. Given the reasonably large neighbourhood size, the “memory-effect” (see Bengtsson 2003) may have retained the initial genotypic variation of the previously sexually reproducing population for a very long time, even though the species may at present reproduce mainly asexually.

On the other hand, the input of only a few seedlings each year into a population of ramets can act as a powerful mechanism for maintaining genet diversity in populations (Bengtsson 2003). This has been shown in a computer simulation of seedling recruitment in Ranunculus repens by Watkinson and Powell (1993). It is possible that in pendant grass, successful pollination and seed set does happen occasionally. The yearly fluctuation of weather conditions can have an effect on sexual propagation of pendant grass. For example, if the sea level is high during flowering and the inflorescences spend considerable time under water, pollination may be prevented (Rautiainen 2006). It is possible that in recent years the weather conditions have not been favourable for sexual reproduction. Another possible explanation for the absence of seedling observations could be that the seeds germinate predominantly within extant patches, where the seedlings cannot be distinguished from other shoots. Within the patches, the germination conditions may be more suitable for the seeds and provide a less disturbance prone environment for the seedlings to be established than in the area outside. With more densely collected shoot samples, probably genotypically identical shoots would have also been found.

Another factor that maintains genetic variation in a population is migration from other populations. There seemed to be considerable amount of dispersal from the Kainunkylä population to the Liminka Bay population, which may have been one factor maintaining variation in the Liminka Bay (Table 1).
4.2.3 Local scale population structure of pendant grass

Genotypic differentiation between the pendant grass subpopulations in the Liminka Bay was very low but significant. More variation was found among patches within subpopulations than between subpopulations, and the estimate of differentiation at the patch level was much higher than at the subpopulation level. Most of the variation was found within patches. Both the significant linkage between loci among subpopulations and the significant patch level component in the AMOVA analysis suggested hierarchical structuring within the subpopulations. Correspondingly, the spatial autocorrelation analysis revealed a microspatial genetic structure in each studied subpopulation (V).

Because the subpopulations were not genetically uniform over all spatial scales, they did not seem to form a single panmictic population. According to the 2MOD results there was at least some migration between the subpopulations. Hence, the subpopulations could not be regarded as fragments of a formerly continuous population, with no present day migration.

The highest genetic diversities within subpopulations were found near the centre of the population, and a positive correlation was found between the geographical and genetic distances, as expected in a classical stepping-stone model of gene flow (Hutchison & Templeton 1999, Wilkins & Wakeley 2002, see also Tero et al. 2003). However, the pattern of the two distances (see Fig. 2 in paper III) suggested that the significant correlation was only due to pairwise distances between the most isolated northernmost subpopulation and the other subpopulations. In fact there was no association between geographic and genetic distances, when the distance was less than 1,000 m. Therefore the results supported only some features of the stepping-stone model, where mainly adjacent subpopulations interact through migration and gene flow. Similarly, the assignment analysis (see Table 4 in paper III) suggested that migration is not restricted to just the adjacent subpopulations, but also happens between other subpopulations. In addition, the absence of an inverse relationship between the subpopulation area and genetic diversity suggested that there was no equilibrium between drift and migration in the subpopulations as expected in a stepping-stone model.

The absence of a relationship between all of the studied subpopulations indicated metapopulation type dynamics in the Liminka Bay population with recent colonisations and extinctions (Ohta 1982). The multidirectional migration pattern resembled the classical metapopulation model (e.g., Tero et al. 2003). This
is in agreement with a study of the population dynamics of pendant grass at patch level, showing concurrent extinctions and formation of new patches (Rautiainen et al. 2004). The same dynamics can be expected to work on the subpopulation level as well, causing extinctions and colonisations of subpopulations, since some of the subpopulations consist of only a few patches.

There was a spatial range of genetic similarity in each subpopulation of pendant grass (V). In general, there were quite large positive kinship coefficients at short distances and negative values at intermediate distances, which supported the concept that new patches often originate from a nearest existing patch (Rautiainen et al. 2004). However, the average values were less negative, or even positive, as distances increased to the limits of the subpopulations. This pattern did not correspond with the shape of autocorrelation correlograms produced by theoretical models of spatially restricted gene flow (e.g., Epperson 2003), suggesting that the observed spatial genetic structure in pendant grass is generated by clonal or sexual founding events.

4.2.4 Regional structure of pendant grass

Even though pendant grass seems to lack a specialised means of long-distance dispersal (Rautiainen et al. 2007a), the assignment analysis over the samples from the three populations indicated efficient dispersal of propagules between populations over long distances (Table 1). Also, the UPGMA-dendrogram, AMOVA and Mantel test suggested that Liminka Bay and Kainuunkylä were most related, and the Liminka Bay and Kautokeino most divergent. At a local scale, new pendant grass patches are established by floating rhizome fragments that are moved by water currents and winds (Rautiainen et al. 2007a). Similar mechanisms are probably also at work at a regional scale. The observed regional scale structure could in fact be a result of hydrochoric dispersal of clonal propagules or seeds.

A large amount of the samples (27%) collected from the Liminka Bay were assigned to the Kainuunkylä population (Table 1). Dispersal seemed to be unidirectional, following the flow of the Tornionjoki River downstream. Measured by waterways, these two populations are located approximately 180 km apart. Such long-distance dispersal could be enabled by propagules carried by the river, which flows southwards providing a possible migration corridor from the upper course of the river to the Bothnian Bay. Furthermore, no dispersal between the Kautokeino and the other populations was detected (Table 1). None of the
samples collected from the Kautokeino population were assigned to the other populations or vice versa. The Kautokeino population is not connected by waterways to the two other study populations (Fig. 2) because the Kautokeinoelva River runs north to the Barents Sea/Arctic Ocean. Consequently, any propagules originating from the Kautokeino population carried by the river would disperse northwards.

The UPGMA-dendrogram divided the populations into three major clusters corresponding to their geographical locations (Fig. 2, Fig. 4). Each population seemed to form its own cluster. The Kautokeino population was most distant from the two other populations. The Kainuunkylä and Liminka Bay populations were more closely related. Similarly, in AMOVA analysis most of the variation was found between groups when the Kautokeino population was placed in one group, and the Liminka Bay and Kainuunkylä populations in another group. The population level component, however, was not significant (Table 2).

In the Mantel test, correlation of genetic and geographic distances was significant in all of the populations. The correlation seemed to be linear between the Kainuunkylä and Liminka Bay populations, as expected in IBD with restricted gene flow (Fig. 5). The Kautokeino population, however, seemed to be genetically more differentiated. This can be explained by the lack of gene flow between the Kautokeino and the other two populations.

4.2.5 Taxonomy of pendant grass

Elven and Johansen (1981) reported on the studied pendant grass population in Kautokeino less than 30 years ago. At that time they speculated that it could be of a Bothnian Bay origin. However, the plants in the population are morphologically different from the plants in the Bothnian Bay (Rautiainen 2002), which does not support their view. Also according to the results of this thesis, the Kautokeino population is not likely of Bothnian Bay origin. It may be speculated that the Kautokeino population more likely originates either from populations in Russia, or from some unknown location upstream on the Kautokeinoelva River. This could not be verified in this study since the AFLP analysis of the dried samples from Russian populations could not be performed due to fragmented DNA. Furthermore, we did not get any data from the Swedish populations.
4.3 Phylogeography of the *Primula sibirica* group

Dispersal over heavy barriers is a rare stochastic process and unlikely to cause parallel distribution patterns among several ecologically divergent organisms, whereas a large scale vicariant event is likely to affect a great number of organisms simultaneously, which will show similar distribution patterns a long time after the event. Based on the results of this study, it seems that the present distribution of Siberian primrose is the result of a vicariant event taking place in Northern Europe after the last ice-age.

Long-distance dispersal in both Siberian primrose and pendant grass seems to be mediated by water. Siberian primrose seems to lack long distance dispersal between the disjunct study regions. Also, pendant grass seems to have good dispersal ability through water corridors, but no means for long distance dispersal between populations that are not connected by waterways. No conclusions of the colonisation history of pendant grass could be made on the basis of the results. However, vicariance would seem to be a more likely explanation, since dispersal over a long distance without a water connection does not seem probable.

For the study species, water is not only an element of dispersal, but at least in the Bothnian Bay region, it is also an important factor in new habitat formation by the land uplift from the sea and the disturbance dynamics of the seashore (Rautiainen *et al.* 2004). The most plausible explanation for the present distribution of the study species seems to be a vicariant event. The large *Primula sibirica* group as a whole is taxonomically and ecologically relatively heterogeneous, implying that dispersal may not be the most obvious explanation behind their distribution pattern. All of the species are capable of dispersal and multiplication by clonal fragments. Therefore, single genets may be long-lived and can cover large areas. Consequently, permanent establishment requires only rare occurrences of initial colonisation by seeds or clonal fragments. All of the species in the group grow on seashores or alluvial meadows and inhabit environments characterized by stressful conditions (saline sites, dry sands) or a high level of disturbance. This is notable because it can be an indication of the importance of water-mediated dispersal for all species in the *Primula sibirica* group.

There has been a controversy over the role of potential glacial refugia of arctic plants in Scandinavia. It has been suggested that coastal refugia (or nunataks) could explain the biodiversity hotspots of alpine species in Norway and the circumpolar distribution of plants that seem to have poor dispersal ability *(e.g.,*
Dahl 1987, 1990). However, both the polycentric biodiversity pattern and the current distributions of the suggested glacial survivors can satisfactorily be explained both by present day conditions, and by dispersal (e.g., Birks 1996, Tollefsrud et al. 1998, but see Fedorov & Stenseth 2001). Our results did not support the glacial refugia hypothesis.

4.4 The Baltic Sea

Although the geological history of the Baltic Sea is relatively short (8,000 years), the populations living in the Baltic Sea are often genetically different from Atlantic populations of the same species, probably as a result of isolation, bottlenecks and selection of adaptive traits (Johannesson & André 2006).

Genetic variation in non-neutral loci is the essential basis for population development and long-term survival (Templeton 1981, Templeton et al. 2001). Consequently, loss of genetic variation hampers the species adaptation to new selective regimes, such as climate change and environmental contamination. Genetic erosion can even lead to complete loss of a population that has evolved separately over a long period of time. As with extinct species, such an evolutionary lineage will not re-appear (Moritz 2002). Consequently, the marginal ecosystem of the Baltic Sea is vulnerable and also highly valuable, housing unique evolutionary lineages - genes, genotypes and populations that constitute an important genetic resource for management and conservation. The study species are good examples of such lineages, since Siberian primrose ssp. finmarchica var. jokelae grows only in the Bothnian Bay and the White Sea, and pendant grass var. pendulina is endemic to the Bothnian Bay.

Eutrophication of the Baltic Sea (Siira 1994), together with the recent changes in land use and the sea water level, improve the conditions for invasive species thus decreasing the open area suitable for early successional species such as Siberian primrose and pendant grass. The reduction in the amount of competition-free space available for these early colonists will make their populations susceptible to local extinction. The number and size of populations of both study species have decreased in the last few decades. As a result, the remaining populations in the Bothnian Bay are currently more fragmented and more isolated than before, and require our attention.
4.4.1 Management implications of the study species

A recent PVA analysis of Siberian primrose has suggested that the large populations in the Bothnian Bay will be able to survive (Björnström 2006). However, the observed very low level of genetic diversity in the endangered Bothnian Bay and the near threatened Barents Sea populations, together with the small $N_e$ estimates in all the study regions, does not predict long term survival for the species in these areas without management (II, IV). At least some small populations are most likely headed for local extinction, and the declining trend will probably accelerate in the future.

Based on the results of this thesis, it seems that natural ‘genetic rescue’ (e.g., Ingvarsson 2001) of Siberian primrose through long distance dispersal is not likely, and therefore active management efforts are required for long term survival of the species (II, IV). Because of the probable Allee effect in small populations (Byers & Meagher 1992, Washitani 1996), conservation efforts should be focused on large populations with relatively even flower type ratios. Restocking or augmentation from other populations could help the survival of the genetically depauperated populations in the Bothnian Bay area.

The results indicate no immediate genetic threat to pendant grass in the Liminka Bay area (III), but a recent parameter sensitivity analysis of the species has predicted that the Liminka Bay population will considerably decrease in both the area and the number of patches in the next 30 years (Rautiainen et al. 2007a). Even the patches that are long-lived and large at present will eventually disappear. In the long-term, the probability of formation of new patches is the most important factor for the persistence of the pendant grass population in the Liminka Bay. For example changes in dispersal distance are less important (Rautiainen et al. 2007a). This is in agreement with our results, which suggested that the pendant grass subpopulations at the Liminka Bay are more or less ephemeral (III). Consequently, management efforts should be targeted to conservation of the required habitat rather than of extant subpopulations.

In order to improve long-term persistence of both Siberian primrose and pendant grass in the Bothnian Bay, the successional vegetation changes should be slowed down, and dispersal and colonisation success of these early colonisers should be improved by continuous management of the populations. Rautiainen et al. (2007b) studied the effect of management on these species and concluded that deterioration of suitable habitats can be markedly slowed down by management. Combined cutting of higher vegetation and soil turning treatment had the
strongest positive effect on pendant grass, significantly enhancing its vegetative reproduction and increasing its potential to colonise new habitats. Mowing and shrub removal had a positive effect on Siberian primrose, increasing the number of sterile rosettes, flowering individuals and seedlings.

The study species seemed to benefit from management, but another species of the *Primula sibirica* group, *Puccinellia phryganodes*, did not respond as well to management (Rautiainen *et al*. 2007b). Management may temporarily increase the vigour of the present populations of these early successional species and slow down the rate of succession. Local management efforts can not, however, turn around the effects of global warming on the environment and the species, such as the growing frequency of the occasional spring floods caused by southwestern storms (Björnström 2006).

Transfer of individuals within or between populations has often proved to be efficient in reducing inbreeding depression and enhancing the vitality of populations (Frankham *et al*. 2002). In addition to management of the existing populations of the study species, long term survival of the smaller populations may require active translocations of individuals to supplement or reinforce them. Translocations could also be used for reintroductions or attempts to establish the species in formerly inhabited sites. However, plants may be adapted to local conditions, or there may be co-adapted gene complexes within populations. Input of new genes in this case might disrupt local adaptations or lead to break-up of favourable gene combinations (see *e.g.*, Vergeer *et al*. 2004). Consequently, transplanting requires that the level of genetic differentiation of the donor and recipient populations is studied. Unfortunately, determination of local adaptations or existence of co-adapted gene complexes is not easily done. Because this kind of information on the study species is unavailable at present, and correlations between genetic and geographic distances were found, introduction or reinforcement could be best achieved through material from the closest subpopulation or population to avoid disruption of local adaptation or co-adapted gene complexes (Vergeer *et al*. 2004). Furthermore, sampling should be done in as many patches as possible to maximize the level of genetic diversity.

### 4.5 Concluding remarks and future considerations

In this thesis, I examined essential aspects on species conservation, genetic diversity and population structure in two endangered seashore plant species,
Siberian primrose and pendant grass. Both species are included in a taxonomically and ecologically divergent *Primula sibirica* group.

The studies of this thesis gave new information on the diversity and population structure of the endangered study species, and new markers that will be useful in future genetic studies of primrose species. The results highlight the need for further investigation of the study species, as well as studies on other *Primula sibirica* group species.

Both Siberian primrose and pendant grass are declining in the Bothnian Bay, and on the basis of these new results, and earlier studies on the population viability and life-history traits of the species, it seems that at a long time scale, both of the species will continue to decline without efficient management efforts. Most critical for the persistence of these species will be conservation of the habitats suitable for them. Translocations could also be considered in order to enhance the diversity of the existing populations and to establish new populations.

By examining the present day structure of Siberian primrose, it was possible to make inferences on the colonisation history of the species in the Northern European area. The current distribution of Siberian primrose seemed to be a result of a vicariant process that took place after the last ice-age when the species colonised the Northern European area. Due to changes in the sea water level and land uplift after the retreat of the Eurasian ice sheet, the Baltic Sea basin has alternatively been in connection with the Arctic Sea, or with the North Sea through Southern Sweden, or the Danish straits. The isostatic adjustment after the ice age has uncovered large areas of land from the Baltic Sea basin that previously were under water. These geological changes have resulted in the current disjunct distribution of the species. Siberian primrose spread first to the White Sea area, probably from the east, and subsequently colonised the Bothnian Bay and the Barents Sea in the west. The areas became isolated when the water connection disappeared as a result of land uplift. For pendant grass, similar processes seem probable, because the Kautokeino population appears to be very distinct from the Bothnian Bay and Tornionjoki populations. However, more extensive analysis could not be done with the sample set used for the present study. More samples from Northern European populations, and also from Russian populations, would be valuable for making inferences about pendant grass. More species should be studied to apply the comparative phylogeography approach and get a broader picture of the history of the *Primula sibirica* species group and to get more support for the results presented here. Studies of old herbarium specimens could also give valuable information on the diversity of the populations in the past.
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