Pirjo Rautiainen

POPULATION BIOLOGY OF THE *PRIMULA SIBIRICA* GROUP SPECIES INHABITING FREQUENTLY DISTURBED SEASHORE MEADOWS: IMPLICATIONS FOR MANAGEMENT
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Abstract

Many plant species inhabiting the seashore meadows of the Bothnian Bay, especially early successional ones, have become threatened. Isostatic land uplift creates virgin land for early successional species to colonise. However, at the same time it gradually elevates the habitat and eventually makes the habitat unsuitable for them. Disturbances of the waterfront may slow down succession and create new empty sites. In order to persist on the shores, pioneer species have to be able to colonise new sites by seeds, vegetative propagules or growth.

In this thesis I studied the status of an endangered early successional grass species, *A. fulva* var. *pendulina*, at the Liminka Bay. According to a matrix population model based on eight years of observations (1992–1999), the population seemed not to be in immediate danger of extinction. However, simulations based on four-year field observations (2000–2003) indicated that if the current trend continues, the species will decrease considerably in area in the next 30 years.

In the field studies no seedlings or viable seeds of *A. fulva* were found. In spite of this, high genotypic diversity was found in the *A. fulva* population, suggesting that sexual reproduction has taken place at some time during the history of the population. Analysis of the population structure revealed a low level of genotypic differentiation between subpopulations and significant substructuring within subpopulations. The overall pattern of genetic variation suggests that the population has characters of both stepping-stone and metapopulation models.

The results of the study on the ability of a seashore plant *Potentilla anserina* ssp. *egedi* to change its allocation of resources to sexual and vegetative reproduction according to competitive stress implied that the species can modify the allocation of resources to different life-history traits. For a plant living in disturbance-prone environment, it may be beneficial to be able to rapidly track the competition-free space formed by disturbances by changing its reproductive pattern.

Management studies on three endangered seashore plant species showed that deterioration of suitable habitats of *A. fulva* and *Primula nutans* var. *jokelae* could be slowed down by management, and the vegetative and/or sexual reproduction of these species was enhanced. However, in the case of *Puccinellia phryganodes*, no positive response to management was observed.

Keywords: between-genet competition, Bothnian Bay seashore meadows, genetic diversity, genetic population structure, management, population viability analysis, Primula sibirica group, threatened species, within-genet competition
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Oulu, March 2006

Pirjo Rautiainen
Abbreviations

AFLP  amplified fragment length polymorphism
TTC   2,3,5-triphenyl-2H-tetrazolium chloride
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1 Introduction

The shores of the Bothnian Bay in the northernmost part of the Baltic Sea are characterised by relatively rapid primary succession caused by isostatic land uplift (the relative land uplift is 6.87 mm/year, Johansson et al. 2004), creating virgin land for early successional species to colonise. However, the same deterministic process also elevates the habitat and together with succession by plants, eventually makes it unsuitable for early colonisers. Frequent stochastic disturbances of the waterfront by ice scouring, water level movements and floating plant debris strongly influence the vegetation dynamics of the area by destroying extant vegetation cover and concomitantly creating open space for species to colonise. In order to persist in the area, early successional species have to be able to track the new colonisable land by clonal growth or dispersal by sexual or vegetative propagules.

Many species of the Baltic shores, especially early successional ones, have become endangered (Ryttäri & Kettunen 1997), and some of them belong to the so-called *Primula sibirica* group. In addition, the seashore meadows of the Baltic are also considered threatened. According to Washitani (2001), the most important practical effort in conservation of global biodiversity is to conserve endemic species or habitats unique to a region. Endemic species usually occur in naturally small and isolated populations, and thus have an intrinsically high risk of extinction due to demographic and environmental stochasticity (Oostermeijer 2003).

Many species conservation projects have failed to achieve their aims because of insufficient data (Pavlik 1994, Heywood & Iriondo 2003). The aim of this thesis is to provide scientific information for proper management of the seashore meadows of the Bothnian Bay and the species inhabiting them.

1.1 Vegetation dynamics of seashore meadows

Vegetation dynamics can be simplified into three phases (Idestam-Almquist 2000). In the first phase free space is formed, usually by disturbance or plant senescence. In the second phase free space is colonised either by seeds or vegetative dispersal. Colonisation depends on the dispersal abilities of the species, but pure chance is also involved. In the
third phase, the persistence of the species depends on their competitive relationships, which may change as environmental conditions change, for example during succession.

Seashores are characterised by distinct zonation of plant species correlated with shore elevation (Tyler 1969, Vartiainen 1980, Bertness 1991). The zonation is formed because of different environmental conditions and competition at different elevations. Lower shores are waterlogged and disturbed by the activity of sea forces that early successional species tolerate. However, investment in traits which enable plants to tolerate stressful conditions might cause the species to be weak competitors in situations where these stress factors are unimportant (Grime 1979). The upper shore zones are thought to be shaped more by interspecific competition (Bertness 1991, Amsberry et al. 2000). Hence, plants in the prevailing upper shore are considered to be better competitors than species in the lower shores, and where their niches overlap the higher shore species eventually displace waterlogging-tolerant ones (Bertness 1991, but see also Bockelmann & Neuhaus 1999).

Isostatic land uplift on the Baltic seashores constantly creates new free land, but also causes allogenic vegetation succession where early successional species are replaced by later successional ones (Ericson & Wallentinus 1979). In addition to the land uplift, disturbances, such as ice scouring and floating plant debris, create open area by destroying existing vegetation. To persist on the Baltic shores, early successional species have to track the colonisable habitats created by land uplift or disturbances by dispersal by vegetative or sexual propagules or clonal growth. Long-term persistence requires a positive balance between new colonisations and local extinctions (e.g., Thomas 1994, Harrison and Taylor 1997, Snäll et al. 2003, Jäkäläniemi et al. 2005).

The decline of many early successional species on the Baltic shores may partially be a consequence of change and abandonment of traditional agricultural practises, such as cattle grazing and haymaking, which used to enlarge the area of low-growth meadows situated on the shoreline to upper elevations, slow down succession and maintain higher species richness by changing the competitive interactions between species (Pykälä 2000, Jutila 2001). In addition to changed agricultural practises, eutrophication of the shores may have given a competitive advantage to some invasive species, such as Phragmites australis. During the recent two decades the long-term mean sea level has been five centimetres higher on average than could be predicted on the basis of the historical linear trend (Johansson et al. 2004). This may indicate that the rising sea level is counteracting land formation by land uplift and, hence, the formation of empty space for early-successional species has slowed down.

The long-term dynamics of plant populations colonising the shores of the Bothnian Bay are governed by land uplift, but short-term dynamics are also affected by disturbances. In general, disturbances alter competitive interactions by destroying dominant species, thus enabling poorer competitors to persist in the area (e.g., Connell 1978). Seashore disturbances also affect the dispersal of species. Seeds and vegetative propagules are carried by waves, ice scouring and debris tear fragments of plants, which can act as dispersal agents. Furthermore, debris may provide a suitable germination surface for seeds and vegetative propagules, but on the other hand it can suffocate seedlings. Also, a high sea level may prevent germination of seeds.
1.2 Clonal growth and propagation vs. sexual reproduction

Many plant species living in shore and aquatic environments are clonal and rarely reproduce sexually (Cook 1987, van Groenendael et al. 1996, Silvertown and Charlesworth 2001, Santamaria 2002). Dispersal often takes place by plant fragments, which have low production costs and are able to capture nutrients while dispersed and may still disperse over considerable distances (Santamaria 2002). However, compared to seeds, clonal propagules are usually larger and more vulnerable to desiccation, and therefore have limited dispersal ability (Silander 1985).

Clonal plants multiply vegetatively by growing genetically identical daughter ramets, which may either remain integrated or become independent. The advantages of clonal growth are many. Daughter ramets may survive better than seedlings, and clonal species are not that dependent on seeds and pollination (e.g., Cain & Damman 1997). Clonal growth may be an advantageous strategy also because it enables the persistence of given genotypes in a population (Barsoum et al. 2004). The benefits of clonal integration include effective resource acquisition by plant ‘foraging’ (Slade & Hutchings 1987, Sutherland & Stillmann 1988), division of labour and resource sharing (Silvertown & Charlesworth 2001, Douhovnikoff et al. 2004). Physiological integration of ramets may give clonal plants an advantage over non-clonal species in colonising disturbed habitats, especially those characterised by high physical stress or resource deficiencies (Bertness & Ellison 1987, Shumway 1995). Long-distance clonal spreading can be an effective trait in colonisation of disturbed areas because plants can send offspring into the recently disturbed patch without going through the seed stage (Fahrig et al. 1994). The ability of many clonal plants to selectively place their clonal offspring enables them to choose the identity of their neighbours and therefore change the rate of inter- or intraspecific interactions (Lovett Doust 1981, Maddox et al. 1989).

Seeds represent a life cycle stage that combines specific adaptations for survival over unfavourable periods with a potential for new successful genetic combinations. According to Eriksson (1993, 1997), the so-called initial seedling recruitment strategy (ISR) that emphasises the importance of seed dispersal in the early stages of colonisation, as opposed to repeated seedling recruitment (RSR), where seedling recruitment occurs within patches of established genets, is a common strategy among clonal plants. However, RSR and ISR are not discrete classes, but rather end points of a hypothetical spectrum. Intraspecific competition may be responsible for the suppression of recruitment after the initial colonisation and small-scale disturbances promote within-population recruitment (Eriksson 1993).

Sexual and vegetative propagules contribute in different ways to the lifetime reproductive success of plants, as they may differ in dispersal distance, phenology and success of establishment (e.g., Winkler & Fischer 2001). As a rule, seeds disperse further than vegetative propagules and may thus aid in the colonisation of new habitats (e.g., Nishitani et al. 1999, Huber & During 2001). However, since plants may modify their growth pattern and place new ramets in favourable patches, and vegetative propagules may move considerable distances (e.g., Slade & Hutchings 1987, Sutherland & Stillman 1988, Oborny & Cain 1997), vegetative propagation may also have a dispersal function. Both seed dispersal and clonal growth can provide a mechanism for escaping from
competition (e.g., de Roij-van der Goes et al. 1995). It has been proposed that clonal reproduction would be favoured over seed production until plant density becomes so high that the survival of additional vegetatively produced individuals is unlikely (Williams 1975).

Clonal growth forms have been described using the relative terms ‘phalanx’ and ‘guerilla’ (Lovett Doust 1981). Phalanx species have highly branched clones with closely packed ramets, whereas guerilla species are less branched with loosely packed ramets. It has been suggested that the phalanx growth form increases competitive strength and the guerilla growth form allows rapid occupation of available space and increases the chance of outcrossing (Fischer et al. 2004), and that phalanx species are better competitors in more productive environments, whereas guerilla species are better competitors in poor environments (Gough et al. 2001). Some species may exhibit both of these growth forms in different habitats (Fischer & van Kleunen 2002). Sackville Hamilton and co-authors (1987) suggested that sexual reproduction is more important for species with a phalanx growth strategy, because they depend more on seeds for colonisation of new habitats.

Clonal reproduction yields genetically identical offspring, therefore highly clonal populations are expected to exhibit low genotypic diversity (Silander 1985). However, comparisons of sexual and clonal species have not revealed the expected difference in population genetic structure (e.g., Ellstrand & Roose 1987, Widén et al. 1994, McLellan et al. 1997, Auge et al. 2001). For example, Bengtsson’s model (2003) has shown that a small number of sexual individuals per generation is sufficient to make an apparently asexual population genotypically highly variable (see also Watkinson & Powell 1993).

1.3 Endangered species and their management

The number of threatened species is increasing in almost all taxonomic groups, and many habitats have also become endangered (Baillie et al. 2004). Effective conservation of species and their habitats needs to be based on a reliable diagnosis of the causes of their decline (Norris 2004). The paucity of data available for most endangered species and habitats often forces management decisions to be made with little quantitative information (Eriksson 1996, Heywood & Iriondo 2003). The first step in planning species management is to assess the biological status of the species, in other words, to determine whether the population is increasing, decreasing or stable (Schemske et al. 1994). The next step is to identify the life history stages that are most critical to population growth - aspects in the biology of the species in question that constitute the greatest vulnerability - and to analyse the biological causes of demographic variation in these stages.

There are three interacting factors or processes that might contribute to a population’s extinction (e.g., Shaffer 1981). Demographic stochasticity is most important in small populations, which are in danger of becoming extinct just because of vagaries in the age and sex structure of the population (Lande 1993, Hunter 2002). Environmental stochasticity means variation in habitat quality, such as climate, nutrients and relationships with other species, which may affect, for example, the dispersal ability of a species. Genetic stochasticity is random variation in the gene frequencies of a population. It is a huge task to study the impact of all these factors on plant populations, and there has
been a debate about the importance of taking into account the genetic status of an endangered species in conservation (Lande 1988, Holsinger & Gottlieb 1991, Falk 1992, Frankham 2005). It has been argued that if the population is large enough to survive ecological threats to population viability, it will also mitigate genetic threats (Lande 1988). However, genetic variation is necessary for evolutionary adaptation, and thus for the long-term persistence of the species, and it may also have short-term fitness consequences (Ellstrand & Elam 1993, Schemske et al. 1994, Fischer & Matthies 1998, Oostermeijer 2003). Small populations are particularly vulnerable to genetic stochasticity because of increased inbreeding, loss of genetic variation due to genetic drift and accumulation of deleterious mutations (Matthies et al. 2004). It has been shown that inbreeding can depress population fitness and increase extinction risk (Young et al. 1996, Tallmon et al. 2004). Ignoring genetic factors may lead to inappropriate management (Frankham 2005).

Estimation of the spatial dynamics of propagule movement with respect to extant landscape features may aid conservation biologists in predicting the demographic and genetic responses of a species to population subdivision (Sork et al. 1999). Quantifying the organisation of genetic variation of a rare species may help in prioritising sites and management choices that will capture and maintain that variation (Neel & Cummings 2003, Neel & Ellstrand 2003). Tero and co-authors (2003) have summarised five hypotheses of migration patterns with contrasting predictions for the population structure of a species: (i) populations or subpopulations may form a single panmictic unit or (ii) may be only fragments of a formerly continuous population (Halliburton 2004). (iii) There may also be migration only between adjacent subpopulations, i.e., the migration pattern may follow the stepping-stone model (Kimura 1953, Kimura & Weiss 1964). Populations may also represent (iv) a source-sink or (v) a classical metapopulation. These different models of population structure provide different testable hypotheses about the distribution of genetic variation within and between subpopulations. Different population structures should be taken into account when planning translocations or reintroduction of an endangered species.

1.4 *Primula sibirica* group

All the study species belong to the so-called *Primula sibirica* (*Primula nutans*) group, which is ecologically and taxonomically a heterogeneous group of plants (Ericson & Wallentinus 1979). Species of the group occur on the shores of both the White Sea and the Baltic Sea, and most of them have a more or less circumpolar distribution along the Arctic Ocean. In the Baltic, some species are restricted to the Bothnian Bay only. The disjunct pattern was first recognised by Fries (1865), who identified twelve taxa sharing the named distribution pattern. Since then plant biographers have included a variable number of species in the group. Eurola (1999) has distinguished the following six core species belonging to the group: *Carex halophila*, *C. paleacea*, *Hippuris tetraphylla*, *Potentilla anserina* ssp. *egedii*, *Puccinellia phryganodes* and *Primula nutans*. Ericson and Wallentinus (1979) have named altogether four moss and 29 vascular plant species to the group, e.g., *Arctophila fulva*, *Catabrosa aquatica*, *Salix triandra* and *Carex halophila*. 
Many of the species belonging to the group have become endangered (*Catabrosa aquatica* and *Salix triandra* are near threatened, *P. nutans* var. *jokelae*, *P. phryganodes* and *Salicornia europaea* are endangered, *A. fulva* var. *pendulina* is critically endangered and *Stellaria humifusa* is regionally extinct).

It is unclear how the species of the *P. sibirica* group have dispersed to the Baltic area. The oldest explanation is that the species dispersed to the Baltic via a water connection between the Baltic and the White Sea that existed at the end of the last ice age (Lovén 1862, Ericson & Wallentinus 1979). According to another theory the present occurrences of the species are remains of a much larger area of distribution covering the shores of all of Fennoscandia (Eurola 1999). It has also been suggested that the present distribution is a consequence of long-distance dispersal by wind or migrating birds (Havas 1961).

### 1.5 Aims of the study

To create a sound basis for the management of the threatened plant species and the area they inhabit, detailed information is needed on rates of death, birth, migration in and between populations, and on the reproduction capacity of the species. The main aim of the present study is to provide scientific information for proper management of the Bothnian Bay seashore meadows and the threatened plant species inhabiting them. First, the influence of competition on the dispersal properties of seashore plants was studied (I and V), second, the status and genetic structure of the *A. fulva* var. *pendulina* population of the Liminka Bay was assessed (II-IV), and third, the methods of managing the threatened seashore plant species were studied (V).

More specifically, the aim of the experiment with *Potentilla anserina* ssp. *egedii* (I) was to find out whether competition with conspecifics affects the allocation of resources to sexual and vegetative propagation. It was suggested that seed dispersal is adaptive as a means of escaping the competitive effect of clonal crowding in order to colonise new environments (Eriksson 1997). It was hypothesised that a lack of competition would generate allocation to rapid, short-distance spread (vegetative propagation), while the presence of competition would increase allocation to long-distance dispersal (sexual reproduction), and the allocation shift would be more pronounced where competing ramets were related.

In the second study, genetic diversity and the distribution of genetic variation between and within subpopulations of the largest extant population of *A. fulva* was analysed. Low genotypic diversity was expected in the population, as no seedlings or viable seeds have been found from the population. In addition, the following questions concerning the migration pattern between subpopulations were studied. First we examined whether the population of *A. fulva* at the Liminka Bay forms a single panmictic unit with free gene flow throughout the population. If this were the case, the subpopulations should exhibit genetic uniformity over all spatial scales. Second, are subpopulations only fragments of a formerly continuous population without any present-day migration between subpopulations. This fragmentation model predicts that subpopulations should show significant differentiation because of a lack of concurrent gene flow. Third, does the migration pattern follow the stepping-stone model, i.e., is there migration only between
adjacent subpopulations, in which case the genetic distance between subpopulations should increase with increasing geographical distance and genetic diversity should be higher near the centre of the habitat than near the ends. Fourth, do subpopulations represent a source-sink or fifth, a classical metapopulation model. Both the source-sink model and the classical metapopulation model predict that recent population bottlenecks and extinction-replacement dynamics should considerably increase linkage disequilibrium between loci within subpopulations. The source-sink model also predicts that genetic diversity will be lower in the sink subpopulations relative to the source area.

In the third study the status of the A. fulva population at the Liminka Bay was assessed and the effect of different disturbance regimes on its population growth rate was studied. Shoots of A. fulva are impossible to follow individually, and hence, a projection matrix model based on colonisation and disappearance events and patch size changes was constructed. Patches were divided into hydric and non-hydric zones according to their proximity to the seashore and the intensity of disturbance. We also studied which transitions are most critical for population growth.

In the fourth study the patch dynamics of A. fulva in terms of patch formation, growth, shrinkage and mortality under disturbances caused by ice scouring, and suffocation by plant debris and overgrowth by later successional species, were studied. The objective was to determine how local disturbance and competitive exclusion affect the patch dynamics and the persistence of the A. fulva population.

The populations of Arctophila fulva var. pendulina, Puccinellia phryganodes and Primula nutans var. jokelae have declined in number and size on the shores of the Bothnian Bay, and all the species are classified as nationally endangered. These species occur in the early stages of primary succession. They are adapted to grow in the narrow zone between the sea and taller vegetation. The fifth study examined whether displacement of the three species by later successional species could be slowed down by management. It was expected that targeted management of the species would reduce competition and enhance their sexual and/or vegetative reproduction and thus benefit their abilities to track and colonise new suitable habitats.

On the basis of the present results, practical guidelines for the management of the threatened seashore meadows of the Bothnian Bay are elaborated.
2 Material and methods

2.1 Study species

All the study species are clonal (Table 1). *Arctophila fulva* var. *pendulina* (pendant grass) is a tall grass, and because of its strong rhizomatous growth it forms dense, monospecific patches in the shallow water along the shores of seas, rivers and sometimes lakes. It flowers regularly, but no seedlings have been recorded in the study area (III). The *pendulina* variety is endemic to the Bothnian Bay and its catchment. The number of var. *pendulina* populations has declined during the last decades and the area of extant populations has diminished (Siira 1994, information from Länsstyrelsen i Norrbottens län Naturvårdsfunktionen), and the species is considered critically endangered in Finland (Rassi et al. 2001). The study population at the Liminka Bay in Finland is the largest remaining population, covering approximately 3000 m², whereas the others vary from less than a square metre to some tens of square metres in size.

*Puccinellia phryganodes* (creeping alkali grass) is a small stoloniferous grass which propagates by producing easily detaching axillary shoots. Flowers are formed, but no production of viable seeds has been reported (e.g., Sørensen 1953). The species grows in low salt marsh meadows next to the shoreline and may even dominate them if the soil is too saline for other species and/or the meadow is grazed by waterfowl (Siira & Haapala 1969, Niemelä et al., unpublished data). *P. phryganodes* has almost complete circumpolar distribution. In the Baltic its populations are restricted to the Finnish coast of the northern Bothnian Bay and their number has declined to one-third in recent decades, and therefore the species is considered endangered in Finland.

*Primula nutans* (formerly *Primula sibirica*) is a perennial, rosette-forming hemicyrptophyte herb, which reproduces by seeds and spreads by runners originating in the axils of the lower leaves and by forming new rosettes (Mäkinen & Mäkinen 1964). Flowers are insect pollinated and seeds are dispersed by water currents (Ulvinen 1997). *P. nutans* has almost a complete circumpolar distribution, and plants growing on the shores of the Bothnian Bay and the White Sea belong to the *jokelae* variety. The species grows on low-growth seashore meadows. Approximately one-fourth of the species’ known
populations have become extinct during the recent decades and the species is considered endangered in Finland.

*Potentilla anserina* ssp. *egedii* (pacific silverweed), is a perennial stoloniferous rosette-forming herb. The species aggregate *Potentilla anserina* is globally distributed but the ssp. *egedii* grows on the Arctic and cool temperate seashores of the northern hemisphere (Rousi 1965). In Finland it occurs on sandy or clay-rich seashore meadows of the Baltic Sea. The plant consists of a short vertical rhizome from which rosette leaves emerge in the beginning of the growing season in the end of May or the beginning of June. During the growing season the plant forms stolons or flowers from meristems in the axils of the rosette leaves. In autumn all aboveground parts wither and daughter ramets become independent units.

The study species *P. anserina* has a distinct guerilla growth form. *P. nutans* usually grows very densely, but is able to form stolons several centimetres long. Also *P. phryganodes* and *A. fulva* typically grow very densely, but in running water *A. fulva* may form very long floating rhizomes and on open shores *P. phryganodes* may grow long stolons.

<table>
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<th>Table 1. Characteristics and conservation status of the studied species.</th>
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### 2.2 Study areas

The study areas are located on the shores of the Bothnian Bay (Fig. 1). The Bothnian Bay is covered by ice approximately six months a year (Seinä & Peltola 1991) and ice scouring is most severe during the spring, when broken ice is carried along the shore by wind. The ice moves according to changes in the water level, and the shallow shore water may sometimes freeze to the bottom. Floating plant debris is rafted to the shores mainly at the end of spring and in the beginning of summer and becomes stranded by the declining water level. Debris can form mats more than half a metre thick and cover thousands of square metres. Although there are practically no tides in the area, the sea level may fluctuate considerably. For example, in Oulu the water level has been +183 cm
at the highest and -131 cm at the lowest, calculated from the theoretical mean water level (Finnish Institute of Marine Research 2006).

The studies of *A. fulva* var. *pendulina* (II-V) were carried out in the largest remaining population at the Liminka Bay in the estuary of the Temmesjoki River 30 km south of Oulu, Finland (Fig. 1). There are seven subpopulations at the Liminka Bay separated from each other by from 80 metres to two kilometres of open space. Five of them situate close to each other in the estuary of the Temmesjoki River. The other two subpopulations are very small and are situated approximately two kilometres south and north from the main area of the population. The subpopulations differ in their area, number of patches and amount of open space around patches.

A mowing experiment was carried out with *P. phryganodes* (V) in the Cape of Tauvo c. 50 km southwest of Oulu (Fig. 1). The area is a wide, flat, low-growth meadow that is usually relatively dry but occasionally becomes submerged by seawater. The nutrient-poor soil consists mainly of sand (Siira 1984). The sparse and low vegetation of the area is mainly dominated by *Agrostis stolonifera*, and *Juncus gerardii*. *P. phryganodes* grows in small patches 100–450 m inland from the mean water level.

Management experiments were conducted with *P. nutans* (V) in six different locations 25-50 km north of Oulu (Fig. 1). The study areas are situated in open low-growth seashore meadows, except for the area of a shrub removal experiment, which represents the upper part of the seashore meadow where *Salix phylicifolia* and *Alnus incana* tend to overshadow lower vegetation.
2.3 Role of vegetative and sexual reproduction

Approximately fifty *P. anserina* ssp. *egedii* genets were randomly chosen and collected from 14 seashore populations in August 2000 (I). The experiment was initiated in the middle of March 2001. The following treatments were applied: (i) two ramets from different genets in the same pot, (ii) two ramets from the same genet in the same pot and (iii) a single ramet in a pot. In the case of two individual plants in a treatment, one of them was randomly selected to serve as an experimental unit.

When analysing absolute plant performance ANCOVA models were applied, where total root length was included as a covariate. After ANCOVA the effects of competition and ramet relatedness was studied by calculating orthogonal contrasts. To analyse the relative allocation to sexual and vegetative reproduction, all measured biomasses were expressed relative to total biomass. After ANCOVA, comparisons between the treatment means were carried out using orthogonal contrasts.
2.4 Genetic diversity and structure of the *A. fulva* population

For study II shoot material was collected from six of the seven existing *A. fulva* subpopulations at the Liminka Bay in 2000 and 2001. Genetic diversity was quantified as the percentage of within-population polymorphic loci and Nei’s unbiased expected gene diversity. Non-random association of alleles has also been used to infer whether organisms recombine. Non-random association was analysed statistically by calculating linkage disequilibrium between loci.

The presence of genetic structure in the total population was analysed using the Bayesian method of Holsinger and colleagues (2002). The presence of genetic structure on the subpopulation and regional levels was tested by using Analysis of Molecular Variance (AMOVA) (Exoffier *et al.* 1992). In order to analyse whether or not there has been any gene flow between the subpopulations, we used the 2MOD programme (Ciofi *et al.* 1999) to estimate the relative likelihoods of drift vs. immigration models since a certain time. Assignment analysis was used to estimate the dispersal pattern between subpopulations by identifying individuals that are possible immigrants. The assumption on association between genetic and geographic distances was tested using pairwise F<sub>ST</sub> values as genetic distances between subpopulations.

2.5 Seashore disturbance and patch dynamics of *A. fulva*

In study III the area and location of all patches of *A. fulva* were assessed once a year at the end of every growing season from 1992 to 1999. The patches were classified by area as small, medium or large. The annual patch survival and transitions between size classes were used to construct Lefkovitch transition matrices (Lefkovitch 1965). The population was divided into hydric and non-hydric zones based on position relative to the shoreline. The data yielded seven inter-annual transition matrices for the population as a whole and for the hydric and non-hydric habitat zones separately. Elasticities were used to identify the transitions that are the most effective management targets. A life table response experiment was used to identify the transitions contributing most to the differences between growth rates of the two different subpopulations (Caswell 2001). Patch numbers for the whole population were simulated for 20 years.

Seed germination and seed bank studies were done to provide background information for the matrix model, i.e., are the new patches initiated from seeds. Spikelets of *A. fulva* were collected in 1998 and incubated in Petri dishes for eight weeks at 20°C and a 16:8 light:dark cycle. At the end of the experiment ungerminated seeds were dissected and their viability checked with a 1.0% solution of TTC. Seeds collected in 1999 were dissected and examined microscopically and their viability was checked using TTC. To assess the seed bank, soil samples were taken from three patches. From each patch five soil samples were collected in PVC cylinders. The samples were divided into 5 cm layers and 10 mm of soil from each layer was placed in a Petri dish. The samples were kept in a growth chamber at 20°C and a 16:8 light:dark cycle for eight weeks. After eight weeks the seedlings were identified and the samples were subjected to cold stratification. After
this the samples were again placed in the growth chamber for eight weeks and checked again for seedlings.

In study IV *A. fulva* patches were mapped and their area was measured individually in the beginning and at the end of four consecutive growing seasons from 2000 to 2003 (except the beginning of growing season 2000). The data were used to estimate the following parameters for the model: probability distributions for size transitions in winter and summer seasons, frequency of new patch formation, size of the new patches, dispersal distance and the probability of disappearance of extant patches.

A spatially explicit model was constructed to predict the development of the *A. fulva* population and to analyse the contribution of patch growth, disturbance and dispersal to the estimated risk of extinction. A modelled year was divided into summer and winter seasons that differ with respect to the growth and disturbance of patches. Most of the new *A. fulva* patches are formed in spring when existing patches are often fragmented by disturbance. The modelled new patches were assumed to be formed as fragments of the nearest existing patch, with a rate that equals the rate of new patch formation in the observation data. This was also the basis for the measurement of the dispersal distance from the edge of the nearest existing patch. The model assumes that the dispersal distance is an exponentially distributed random variable, with the mean distance corresponding to the observed one. New patches were initiated with an exponentially distributed patch size, which was based on the maximum likelihood fit with the observed area of the new patches. The dispersal of new patches was modelled as equally probable in all directions.

The winter disturbance of *A. fulva* patches was modelled in two stages. The first stage models the survival probability of a patch as a logit-linear function of patch area. In the second stage a patch that was not completely destroyed in the disturbance event will lose a uniformly distributed random proportion of its area. If a patch is 50 m$^2$ or under in size, the maximum disturbance proportion is independent of patch size. Otherwise the maximum proportion of patch area that may be lost is a decreasing share of the patch size.

Many parameter values in the model were taken from a specified random distribution and therefore all the simulations were replicated 100 times in order to get an estimate of the distribution of the response variable. Each replicate of the simulation was run for 30 time steps (years), which included both a summer and a winter season. The simulations were initiated with the observed distribution and location of patches at the end of the growing season in 2003. The effect of the model's parameters on the development of the system was analysed by plotting the rate of change in the total area of the patches (total and subpopulations separately) against different parameter values ranging around the observed ones.

### 2.6 Management of seashore plant populations

A management experiment (V) with *A. fulva* was carried out in 2000-2003. Three experimental quadrats were established beside fourteen randomly chosen *A. fulva* patches and allocated to one of three treatments: (i) control, (ii) mowing and (iii) mowing and
additional turning of soil with a shovel to a depth of 10-20 cm. Mowing was repeated annually in mid/late July and soil turning was done in the first two years.

A mowing experiment with *P. phryganodes* (V) was carried out in 2000-2003. Twelve randomly chosen *P. phryganodes* patches were subjected to mowing treatment and another twelve served as controls. In the first year mowing was done in late September and in the following years at the end of July.

Two separate management experiments were carried out with *P. nutans* (V). First, a mowing experiment was conducted in 1999-2002 in five *P. nutans* populations. Ten quadrats were established in each population and half of them were randomly allocated to a control and the other half to mowing treatment. Second, a shrub removal experiment was carried out in one population in 2001-2004. The experimental design and the number of quadrats were similar to those in the mowing experiment.

The influence of management on all three species was analysed using R statistical software (Ihaka & Gentleman 1996). In the analysis, the number of *A. fulva* and *P. phryganodes* shoots and *P. nutans* rosettes, seedlings and flowering individuals were used as response variables, as was also the cover of *P. phryganodes*. When the number of individuals in the year concerned (shoots, rosettes, flowering shoots or seedlings) was used as a response variable, a generalised linear model with a log-link was built for the data analysis, as is customary with variables following a negative binomial distribution. The impact of treatment was evaluated by the change in model deviance that follows a Chi-square distribution. Additional analysis of orthogonal contrasts with z-statistics was applied in analysis of the general effect of treatment on the number of *A. fulva* shoots and the potential difference between the two different management treatments included. The change in the cover of *P. phryganodes* following treatment was analysed by one-way ANOVA following an arcsine-root transformation of the cover estimates.
3 Results

3.1 Role of vegetative and sexual reproduction

*Potentilla anserina* ssp. *egedii* plants grown alone performed consistently better than plants that experienced either between-genet or within-genet competition (1). The negative effect of competition on clonal reproduction was pronounced, since the control genets produced nearly twice as many stolons and daughter ramets, and their runners and daughter ramets had considerably higher biomass than did the ones that faced competition. Due to reduced reproductive output in daughter ramets, both the biomass and the number of flowers also decreased in the presence of competition. The number and the biomass of the flowers of the mother ramet were independent of competition, which was the only exception to the general pattern.

Competing plants allocated slightly more of their total aboveground biomass to sexual reproduction than did plants grown alone, although the difference was not statistically significant. In turn, treatment had a significant effect on the biomass allocated to flowers by the mother ramet: plants allocated equally to the flowers of mother ramets in both competition treatments, but when grown alone they allocated significantly less to flowers than in the presence of a competitor. Treatments did not affect the proportion of biomass allocated to flowers of the daughter ramets or their flowering frequency.

Total allocation of biomass to vegetative structures did not vary significantly between the treatments. In contrast, when the biomass of mother ramets and the biomass of stolons and daughter ramets were analysed separately, a pronounced influence of competition could be seen. Plants that grew alone allocated, in relative terms, significantly less biomass to mother ramets than plants in the competition treatments. In contrast, the relative biomass allocated to vegetative reproduction (i.e., daughter ramets and stolons) was higher when the plants grew alone. There was no difference between the two competition treatments in total vegetative biomass or in biomass allocated to vegetative reproduction.
3.2 Genetic diversity and structure of the *A. fulva* population

Each examined shoot of *A. fulva* exhibited a unique AFLP pattern (II). Thus, the genotypic diversity in the sample equalled the sample size. The proportion of polymorphic loci in different subpopulations varied between 65% and 82%. The mean genetic diversity within each subpopulation, assuming Hardy-Weinberg equilibrium (\(H_e\)), was 0.267; the estimate, assuming total inbreeding (\(H_i\)), was slightly lower (0.197).

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 \(H_i\).

No significant association was detected between the estimated subpopulation area and genetic variation, suggesting that there is no equilibrium between drift and migration within subpopulations. Significant linkage disequilibrium between loci was found at each subpopulation.

In the Bayesian analysis of population structure the posterior mean estimate of FST was 0.071±0.006, and there was very strong evidence that the estimate was greater than 0, which suggests that there are significant genetic differences between the subpopulations, even though the low value of the estimate indicated that the levels of differentiation are not very high.

In AMOVA the variance components were highly significant at all hierarchical levels. Most of the variation appeared within patches. The variation between subpopulations explained only a small amount of the total variation, and the small \(\Phi_{ST}\) estimate between subpopulations also suggested a low amount of genotypic differentiation between subpopulations. However, all the pairwise exact tests between subpopulations were significant, suggesting that the genotype frequencies in different subpopulations are dissimilar. There was much more variation among patches within subpopulations, and the reasonably large estimate of differentiation between patches also suggested significant genotypic substructuring within subpopulations.

The 2MOD simulations indicated that the subpopulations of *A. fulva* are not totally isolated units, as suggested by the fragmentation model, 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 high. Assignment success was over 50% only in the most isolated northernmost subpopulation. All the mixing was not between adjacent subpopulations.

The scatterplot and the Mantel correlation analysis suggested a relatively strong positive association between pairwise \(F_{ST}/(1-F_{ST})\) values and linear geographical distance between subpopulations.
3.3 Seashore disturbance and patch dynamics of *A. fulva*

3.3.1 *Area covered by* *A. fulva* *patches*

The area covered by *A. fulva* patches at the Liminka Bay has varied considerably during the study years 1992-2003 (Fig. 2). It was lowest in 1995, when patches covered an area of c. 2100 m². After that the total area rose and peaked in 2000 at approximately 4800 m². Since then the area has declined steeply every year, reaching a cover of approximately 3000 m² in 2003.

![Graph showing area covered by A. fulva patches from 1992 to 2003.](image)

**Fig. 2.** Total area covered by *A. fulva* patches in 1992-2003.

3.3.2 *Projection matrix model*

Seeds of *A. fulva* collected in the summer of 1998 did not germinate and showed no respiration when tested with TTC, indicating that the seeds were not viable (III). Seeds collected in 1999 also showed no respiration and, under microscopic examination, only 10% contained endosperm and none contained embryos. Soil samples in the seed bank experiment did not produce any *A. fulva* seedlings, even though seedlings of several other
species living in similar conditions appeared, and we concluded that *A. fulva* neither reproduced by seeds nor had a viable seed bank.

When all *A. fulva* patches of the Liminka Bay area were pooled, the population growth rate, measured as the dominant eigenvalue $\lambda = 1.054$ of the mean transition rate matrix, suggested that the number of patches is increasing (III). The stochastic simulation with the seven transition matrices from 1992-1999 showed an increasing number of patches in the hydric zone ($\lambda = 1.14$), while the patch number decreased in the non-hydric zone ($\lambda = 0.96$).

The elasticity analysis showed the growth of the pooled population to be most sensitive to changes in the small-to-small transition, which includes both the probability of small patches staying small or small patches producing new small patches. The division of the population into hydric and non-hydric zones did not change the result much: the probability of staying in the small class still had the highest elasticity in both zones, but the probability of staying in the large size class now seems to be more important in the hydric zone than in the non-hydric zone.

According to the results of the life-table response experiment, the growth from a small to a medium-sized patch explains most of the variation in growth rates between the two zones, but the transition from a medium to a large patch and production of small patches by a medium-sized patch also contributed highly to the difference between growth rates of the two zones.

According to the stochastic simulation, the cumulative extinction probability of the whole population is low, only about 4.5 % in 150 years. When analysed separately, the hydric zone yields a zero extinction probability, whereas the non-hydric zone is expected to become extinct within 40 years. The median time to extinction in the non-hydric zone is about 25 years.

### 3.3.3 Spatially explicit model

During the summers of the study period many of the patches lost a small area, but they also had the capacity to increase their area rapidly. The change in size during winters was negative or zero, with a skew to the left, indicating disturbances. There have been few very large disturbances during the winters of the four-year observation period. On average, 13 % of the patches were not disturbed during winter. The mean probability of a patch disappearing in winter was 7.88 %. Smaller patches were more prone to disappear than larger ones. Fitting the logit-linear extinction risk model to the observation data resulted in parameter estimates of $\alpha = 1.4447$ and $\beta = 0.0916$ for the whole population. The relationship between the patch size (on a logarithmic scale) and the probability of the patch surviving a disturbance event is thus an increasing, sigmoidal function. In the patches that survived, the proportion of patch area removed in a disturbance was highly variable among small patches. The maximum disturbance proportion was lower in large patches, which was accounted for by a decrease of the disturbance maximum with increasing patch size. Between the minimum and maximum, the disturbance appeared to be uniformly distributed.
The origin of a new *A. fulva* patch was considered to be the nearest existing patch. The mean dispersal distance was 12.98 metres. The probability of a new patch being formed from an existing patch was 23.99%, and new patches were very small.

Based on the 30-year simulations, carried out using the estimated values of the model’s parameters, the total area covered by the *A. fulva* subpopulations at the Liminka Bay was predicted to decline steeply during the first five years and more gradually for the rest of the simulated time span. The number of patches declined at a fast rate for the first two years, followed by a phase of more gradual decline. In this scenario, the total area of the patches will approach zero in 15 years and the number of patches, in 30 years.

Small changes in the parameters of the patch survival function resulted in similar changes in the simulated cover of the patches at the end of the 30-year period. For example, an increase in the steepness of the survival function leads to a more rapid increase in survival probability as the patch grows larger, increasing the cover of patches at the end of the simulated period. Changing the parameter $\alpha$, which determines the size-independent part of the logit survival, shows how important for population growth it would be if the survival of the small patches could be increased. The patches that survived the winter lost a proportion of their area that followed a decreasing function of the patch size. If the slope ($\mu$) of this function could be increased, it would have a large positive effect on the population.

An increase in the dispersal distance leads to a minor reduction in the total area of patches. This negative impact is mostly due to the increased probability of dispersing to an uninhabitable space. In turn, the sensitivity of population growth to the new patch formation probability seems to be very high: for example, the observed probability is 0.24, and if it could be doubled it would increase the end area covered by the patches. A reduction of the same magnitude would naturally reduce the area of patches, but to a lesser extent compared to an increase.

Growth in the initial size of a new patch seems to have a positive effect on population growth. The larger the patches are, the less vulnerable they are to disappearing in winter.

The fate of subpopulations one and two, where the vegetation is most closed, seems to be the worst. An increase in many of the parameters described above would have a positive effect on population growth as a whole, but in the case of these two subpopulations no such positive effect can be seen, at least not in the values we have tested.

### 3.4 Effects of management on seashore plants

The number of shoots of both grass species, *A. fulva* and *P. phryganodes*, declined during the course of the experiment in the non-managed control plots and only *A. fulva* responded positively to management. The number of *A. fulva* shoots increased in mowed plots and in plots that were both mowed and had their soil turned. The analysis of contrasts revealed that the control quadrats and the treated ones differed during all the study years. More *A. fulva* shoots were constantly found in the treated quadrats than in the controls, and, moreover, of the two different treatments the combined cutting and soil turning scored a higher median number of shoots in all the years than did mere cutting.
The cover and the number of shoots of *P. phryganodes* declined in both the mowed and the control quadrats during the experiment, and there was no statistically significant difference between the control and the mowing treatments in any of the study years.

In general, mowing markedly increased the number of *P. nutans* individuals in all the demographic stages. In the first year after the start of the experiment the control and the mowed quadrats did not differ from each other, but in two years the number of sterile rosettes and flowering individuals exceeded those in the control quadrats. Seedlings were separated from the sterile rosettes only in the last study year, and then the number of seedlings was statistically significantly higher in the mowed quadrats than in the control ones.

In the shrub removal experiment the total number of *P. nutans* individuals was higher in the treated quadrats compared to the control ones already one year after the start of the experiment. This was mostly due to the increased number of sterile rosettes in the treated quadrats when compared to the control quadrats in 2002 and the following study years. Also, the number of seedlings was higher in the treatment quadrats every year after shrub removal. Removing shrubs also increased the flowering of *P. nutans* in the quadrats in the last two years, and these differences were statistically significant.
4 Discussion

4.1 Sexual and vegetative reproduction of seashore meadow plants

Plants growing on shores are often clonal (Santamaria 2002) and they may experience both interspecific and intraspecific competition and competition with their own genet. Our results of the competition experiment (I) imply that *Potentilla anserina* ssp. *egedii* can modify the allocation of resources to different life-history traits according to competitive stress. The mother ramets of *P. anserina* allocated more to flowers when experiencing competition, whereas allocation to vegetative growth was more intense when competition was absent. However, allocation patterns were independent of the relatedness of the competitors. Whether a plant was competing with ramet from the same genet or a different genet did not explain variation in either vegetative spread or sexual reproduction. It may be the case that, once the physical connection between ramets has disappeared, there are no means for a ramet to identify its relatives, although, according to Schenk and colleagues (1999), plants may have the potential to recognise their relatives. According to Schmid and Bazzaz (1990), shoots of phalanx species are more likely to interfere with members of the same genet than shoots of guerilla species, and Falik and co-authors (2003) suggest that avoidance of competitive interactions with self would be greatest among closely spaced clonal species.

In competition-free conditions *P. anserina* grows according to a guerilla strategy (sensu Lovett Doust 1981), exploiting free space by allocating more biomass to vegetative spread, and eventually in production of novel meristems. Clones did not, however, escape competitive stress by means of vegetative propagation, but instead the mother ramets increased flowering and, apparently, seed production. Such flexibility is likely to reflect a shift in the optimal allocation strategy during the life cycle of a plant, with a guerilla growth form with rapid exploitation of free space in a new patch by vegetative spread favoured. When spread becomes limited by competition, long-distance dispersal in space (seeds) or time (persistence) becomes beneficial.

Competition with other species may restrain both sexual and vegetative reproduction. When the surrounding vegetation of *Primula nutans* was mowed, both vegetative and sexual reproduction were enhanced as the number of flowering individuals, seedlings and
vegetative rosettes increased (V). The effect of shrub removal was first seen as an enhancement of vegetative reproduction and later as an increase of sexual reproduction. Sunlight filtered through green leaves may have also inhibited seed germination from the seed bank (Baskin & Baskin 1998). The seedling stage is also susceptible to light, and the poor survival of seedlings in tall and dense swards may be a consequence of low light levels (Bullock 2000). In addition, the amount of litter was clearly higher in the control quadrats at the end of the experiment. Litter may affect seed germination and seedling establishment by shading, changing the surface temperature and humidity of soil, or allelopathy (Crawley 1997, Baskin & Baskin 1998). It has been shown that many Primula species need bare ground for successful germination (Whale 1984, Endels et al. 2004), and removal of vegetation may have created proper microsites for seed germination and seedling establishment (Brys et al. 2004; Hoffmann & Isselstein 2004).

A. fulva flowers regularly, but the lack of viable seeds and a seed bank indicated sexual reproduction to be unsuccessful (III). Moreover, according to the observations, no seedlings have been found in the Liminka Bay area in many years. The dispersal of A. fulva seemed to be based on clonal growth and fragmentation of rhizomes.

However, a relatively high amount of genetic variation found in the A. fulva subpopulations suggests that sexual reproduction has taken place despite conflicting field observations (II). The existence of unique AFLP genotypes in each sample suggests that the effect of clonal growth contributes little to within-subpopulation diversity, or that the effect occurs over a smaller spatial scale than our sampling scheme.

There may be some other explanations for the high genetic variation in the population. The clones may be so long-lived that somatic mutations are accumulating in different parts of the clone, thus confusing the clonal origin of shoots. Pure clonality may also be a recent phenomenon in A. fulva. In some cases, a high level of clonal or genotypic variation has been found in clonal species with low levels of seedling recruitment (e.g., Jonsson et al. 1996, Auge et al. 2001, Lundqvist & Andersson 2001). For example, in Carex bigelowii no seedlings have been observed in natural stands, even though the species is genotypically highly variable (Jonsson et al. 1996). It might be that A. fulva may have experienced periods of successful seed recruitment in the Bothnian Bay area in the past. In a species with exceptionally long-lived genets, very rare occasions of successful recruitment could result in high local genetic variation or it may have been established initially by a diverse colonising cohort (Eriksson 1997). Successful pollination can, for example, be quite rare in the Liminka Bay population. Every summer in the four study years (2000-2003) the sea level was so high during the flowering of A. fulva that inflorescences were underwater for considerable times, and therefore pollination may have been prevented.

Bengtsson’s model (2003) has shown that a small number of sexual individuals per generation is sufficient to make an apparently asexual population highly variable. On the other hand, the observed significant linkage between loci in each subpopulation suggested non-random association of alleles, which may indicate a loss of recombination in A. fulva. Bengtsson’s model (2003) shows that because of the “memory-effect”, a population started by a small number of sexually derived propagules may retain its initial genotypic variation for a very long time, even if it later reproduces almost exclusively asexually. In so-called initial seedling recruitment (ISR) species seedlings only take part in the initial colonisation of a site, and further development of the local stand is due to
clonal propagation (Eriksson 1997). A positive correlation is found between the occurrence of the ISR and persistent below-ground clonal structures (Eriksson 1989), such as *A. fulva* rhizomes.

### 4.2 Population structure and gene flow of *A. fulva* at the Liminka Bay

Reliable estimates of population differentiation are crucial in conservation of endangered species because it is necessary to understand whether the populations or subpopulations are genetically isolated from each other, and if so, to what extent. Knowledge of population structuring provides valuable guidelines for the management of the species. There are an increasing number of studies on the structure of genetic variation within and between fragmented populations of endangered species (e.g., Colas *et al.* 1997, Gaudel *et al.* 2000, Schmidt & Jensen 2000, Neel & Ellstrand 2003), and some of them have also attempted to interpret the genetic structure in terms of metapopulation processes (e.g., Cruzan *et al.* 2001, Senneville *et al.* 2001, Tero *et al.* 2003, Hilfiker *et al.* 2004).

In the population of *A. fulva* at the Liminka Bay, significant differences in genotype and allele frequencies were found between subpopulations (II). Variation was higher among patches within subpopulations than between subpopulations, and accordingly the estimate of differentiation at the patch level was much higher than at the subpopulation level. The subpopulations of *A. fulva* were not genetically uniform over all spatial scales, and therefore, they do not form a single panmictic population. The results of 2MOD analysis indicated at least some migration between the subpopulations and, hence, they cannot be regarded as fragments of a formerly continuous population where no present-day migration between subpopulations takes place.

Genetic diversity within subpopulations appeared to be highest near the centre of the population, and there was a positive correlation between the geographical and genetic distances, as expected according to the classical stepping-stone model of gene flow (Halliburton 2004). However, the pattern of the two distances suggests that the significant correlation is only due to pairwise distances between the most isolated northernmost subpopulation and the other subpopulations, and in fact there is no association between geographic and genotypic correlations when the distance is less than 1000 m. Therefore, the results support only some predictions of the stepping-stone model where migration should happen only between adjacent subpopulations (see, e.g., Tero *et al.* 2003). Also the assignment analysis suggests that the migration events have not taken place merely between the adjacent populations. Moreover, the absence of a relationship between subpopulation area and genetic diversity suggests that there was no equilibrium between drift and migration in subpopulations as expected in this model.

The absence of a relationship between area and genetic diversity in all of our study subpopulations seems to point to metapopulation-type dynamics with recent colonisations and extinctions (Ohta 1982). However, the evidence does not seem to support the source-sink model, where permanent, high-quality source subpopulations should be net exporters and ephemeral low-quality subpopulations net importers of migrants. Although the level of genetic diversity was high in the two central subpopulations, implying a structure of a few source subpopulations with a high level and several sink subpopulations with low
levels of genetic diversity, according the assignment analysis the migration was multidirectional between central populations and the more peripheral ones. Consequently, the genetic structure of the *A. fulva* population resembled a classical metapopulation more than a source-sink population.

### 4.3 Status of the *A. fulva* population at the Liminka Bay

The long-term data on the populations of *A. fulva* at the Bothnian Bay show that from the conservation point of view the situation of the species is poor (Siira 1994, Siira 2006 unpublished). Many of the populations have become extinct. The total cover of *A. fulva* in the Liminganlahti Bay population has changed considerably during 1992-2003 (Fig. 2) (Siira 2006, unpublished). At its lowest the area has been c. 2100 m², and at its highest, c. 4800 m². In 1995-2000 the total area increased considerably. The mowing carried out in parts of the Liminka Bay in 1993-1998 may have benefited the species in some areas, such as on the north side of the Tennesjoki River (Siira 2006, unpublished). However, it has also been harmful to some *A. fulva* stands, as in the northernmost subpopulation mowing in 1996 enabled plant debris to accumulate on the *A. fulva* patches, which were destroyed in large areas.

According to the matrix population model (III) based on the seven years (1992-1999) of observations of the *A. fulva* population at the Liminka Bay, the population was not in immediate danger of extinction, although in the non-hydric zone the number of patches was decreasing. It seemed that the shoreline disturbance of the hydric zone was sufficient for maintaining a viable population. In contrast, in 2000-2003 the situation of the population was more critical. Over this four-year period there was a considerable decrease of overall cover of *A. fulva*, and the simulation indicated that if the current trend continues, the *A. fulva* population will decrease in area and number of patches considerably in the next 30 years. Winter disturbances have been very severe during the study years, and the summer growth of the patches was not able to compensate the losses. Neither was the formation of a large number of new patches, especially in the summer of 2001, able to reverse this undesirable trend. It appeared that most of the new patches were formed at small bays on the shoreline, where the prevailing wind direction moved the rhizome fragments. Unfortunately, these new patches were quite rapidly outcompeted by closing vegetation on the shoreline. At the same time competitive exclusion, ice scouring and suffocation by debris diminished the area of older patches, resulting in a decrease in the total area covered by *A. fulva*.

The results of the two models were opposite, which highlights the importance of long-term monitoring of studied species in the field of conservation biology in order to produce reliable predictions about the fate of the population. The matrix population model was constructed on the basis of eight years of observations, but the size of the patches was measured only once in the growing season, therefore the change in the patch areas during winter and summer could not be differentiated. In the matrix population model the habitat was divided into two zones to assess the effect of different disturbance regimes on population growth. The data for the spatially explicit model was collected only during four years, but twice in the growing season. Therefore, the growth of the
patches during summer and the effect of wintertime disturbance on the patch area could be assessed. The matrix model used ignores potential density dependency. However, especially in plants, spatial aggregation may create local spots with high density which are not available for colonisation. This feature is implicitly taken into account in the spatially explicit model, where the amount of suitable habitat is determined. The patches could not grow outside the suitable habitat, and new patches were prevented from growing inside the existing ones.

It is tempting to speculate that some kind of ecological threshold value has been reached where the formation of a free area is not enough for the survival of the A. fulva population at the Liminka Bay (e.g., Scheffer & Carpenter 2003, Huggett 2005, Lindenmayer & Luck 2005, Pascual & Guichard 2005). Huggett (2005) defines an ecological threshold as a point or zone of alternative states, and when it is passed a system turns to a different state. The change is caused by a small additional change in one or more factors in the environment. At the regional scale the declining trend of A. fulva in the Bothnian Bay has been going on for some decades. Many A. fulva populations have become extinct and now it seems that the largest extant population is following this trend. The land uplift on the coast of the Bothnian Bay has offered a suitable refugium for A. fulva, but it might be that the present rate of land uplift combined with the minor sea level rise (Johansson et al. 2004) that counteracts the land formation makes the competition-free zone too narrow for it to persist in the area. In addition, the eutrophication of the Baltic has benefited some species, such as Phragmites australis, thus reducing the competition-free space available for early-colonists even more and making them susceptible to extinction.

During 2000-2003 the wintertime disturbances have been severe. Many A. fulva patches disappeared completely and many lost large proportions of their area, and growth during the summer was not fast enough to compensate the losses. The summer growth has been restrained by competitive exclusion and suffocation by plant debris. If the current trend continues during the coming years, the fate of the A. fulva population at the Liminka Bay seems poor. However, our study period could naturally represent four exceptionally bad years for A. fulva. If this is the case, the simulations produced too pessimistic predictions about the fate of the study population.

4.4 Effects of management on growth and reproduction

The purpose of the experimental management, to slow down the displacement of Arctophila fulva var. pendulina, Primula nutans var. jokelae and Puccinellia phryganodes by later successional species, was fulfilled with A. fulva and P. nutans (V). A. fulva benefited from both mowing the surrounding vegetation and turning the soil. The number of shoots increased in both treatments, while it decreased almost to zero in the untreated quadrats during the experiment. Turning the soil together with mowing was more effective than mere mowing. Management was particularly effective especially against tall, tussock-forming sedges (e.g., Carex aquatilis), which formed dense stands impenetrable by other species. Mowing restrained their growth only slightly, because the sedges regenerated rapidly from the basal meristems left intact by mowing: a common
phenomenon for tiller-forming species (e.g., Stammel et al. 2003). Soil turning, however, decreased the cover of sedges effectively. It imitated the effect of ice scouring by breaking the tussocks and the roots of the sedges. It also cut the roots and the rhizomes of A. fulva, but the species was able to rapidly grow new shoots from the small rhizome fragments. This is probably an adaptation to its unpredictable environment, where ice scouring both destroys vegetation and creates open areas, and floating plant debris may bury vegetation. The advantage of such adaptation was clearly seen in the 1960s, when the mouth of the Temmesjoki River was dredged (Siira 1994): A. fulva rapidly spread to areas where the soil was disturbed. However, despite the increase in the number of shoots in both the mowing and soil turning treatments, A. fulva was not able to form as dense stands as it usually does in open space (P. Rautiainen, personal observation).

In contrast to A. fulva, P. phryganodes showed no positive response to mowing. Despite the fact that mowing decreased the cover of competing vegetation at the start of the experiment while most of the creeping shoots of P. phryganodes were left intact, the cover and the number of shoots of P. phryganodes decreased during the experiment. Interspecific competition for light and space may not be the main reason for the decline of P. phryganodes along with succession in Tauvo, as the total cover of vegetation was fairly low in the study area already in the beginning of the experiment. Relatively dry summers during the study years may have enhanced the replacement of P. phryganodes by later successional species. The nutrient-poor, dry and rather coarsely textured sandy soil of the area may not provide optimal conditions for the shallow-rooted species. Srivastava & Jefferies (1996) studied the habitat requirements of P. phryganodes with transplant experiments and concluded that high salinity, low soil moisture and low nitrogen content were associated with the decreased growth and survival of transplanted tillers of P. phryganodes (see also McLaren & Jefferies 2004).

Management resulted in an increase of both vegetative and sexual reproduction of the herb, P. nutans. Its response to mowing and shrub removal was parallel. Removal of surrounding vegetation increased the total number of P. nutans individuals and the number of sterile rosettes, seedlings and flowering individuals. The increase in the number of individuals was due to both increased vegetative and sexual reproduction. The results are similar to those obtained with Primula veris (Brys et al. 2004) and P. vulgaris (Endels et al. 2004).

4.5 Implications for management

Genetic variation within and between subpopulations of A. fulva indicated that the species has maintained a high level of genetic and genotypic diversity at the Liminka Bay (II). However, our analysis did not allow estimation of the amount of inbreeding within subpopulations. It is possible that the genetic diversity of A. fulva may be decreasing due to inbreeding, which may expose the population to extinction because of genetic degradation.

Some characteristics of the A. fulva population structure seem to follow the classical metapopulation model (e.g., Tero et al. 2003, Halliburton 2004), which suggests that the subpopulations are more or less ephemeral. Accordingly, conservation and management
efforts directed to this species should focus on conservation of the required habitat of the species instead of extant subpopulations.

Although there seems to be no specific immediate genetic threats to the survival of *A. fulva* at the Liminka Bay, other populations of *A. fulva* are much smaller and may be more vulnerable to extinction (see, e.g., Ellstrand & Elam 1993, Hunter 2002). The long-term survival of these populations may require active translocation of individuals from larger populations. In general, to implement recovery of fitness and genetic diversity, the individuals chosen for introduction to small inbred populations should be genetically differentiated from the population to which they are being introduced (e.g., Frankham *et al.* 2002) and there should be high genetic variation among the transplants (e.g., Williams 2001, Oostermeijer 2003). Given that there is no local adaptation or co-adapted gene complexes within populations, the best selection strategy for reintroductions is often to choose the most unrelated individuals from the source populations (e.g., Earnhardt *et al.* 1999). Accordingly, the genotypes of the individuals chosen for introduction, and in the case of augmentation, the genetic composition of the potential recipient population, should be studied before translocations. If this is not possible in practice, our results provide a rough guideline for selection of individuals for such translocation purposes. First, because there was no association between subpopulation area and genetic diversity, it is not necessary to choose the individuals from the largest subpopulations. Second, because we found a large amount of genotypic variation among patches within subpopulations, one should collect individuals from several patches within subpopulations in order to augment existing subpopulations to establish new ones with a high level of genetic diversity. Finally, we found isolation-by-distance between subpopulations, suggesting that collecting individuals as far apart as possible would decrease relatedness between the chosen individuals.

However, plants may also be adapted to local conditions or there may be co-adapted gene complexes within populations (Heywood & Iriondo 2003, Beebee & Rowe 2004). An input of new genes in this case may disrupt local adaptations or lead to a break-up of favourable gene combinations. Unfortunately, determination of local adaptation by the existence of co-adapted gene complexes is not easily done, in contrast to assessing differentiation using neutral genetic markers. Because we did not have this kind of information on *A. fulva*, introduction or reinforcement could be best achieved through material from the closest subpopulation.

Since it is impossible to influence the land uplift of the Bothnian Bay, the most feasible way to promote the persistence of early-successional species in the area is to try to alleviate competitive pressure by late-successional species, create open space for colonisation and improve dispersal and establishment of the early successional species. In addition to the methods used in the management experiment (V), other means of management could be employed to prevent the disappearance of the populations. Large-scale mowing of the seashore meadows and subsequent litter removal would enhance the conditions for early successional species, but especially *A. fulva* and *P. phryganodes* may also need soil turning to aid the establishment of vegetative propagules. However, soil turning is very labourious, as it should be done for many years and no heavy machinery can be used due to the soft soil, and therefore soil turning could be feasible only when small populations are at immediate risk of extinction. Grazing and concomitant trampling by cattle could provide an effective management tool for preserving and creating suitable
open habitats for the species (Siira & Pessa 1992, Pykälä 2000, Jutila 2001). Trampling
also promotes an increase in topsoil salt content (Siira 1971), thus making the
environment relatively better for salt-tolerant species, such as *P. phryganodes*. However,
grazing and mowing which could potentially slow down succession can also create other
problems, because they may considerably increase environmental stochasticity
(Oostermeijer 2003). For example, after mowing, *A. fulva* patches may be more
vulnerable to suffocation by debris because there is no taller vegetation around the
patches to prevent debris from floating and standing on them (Siira 2006, unpublished).
Even though the species is adapted to disturbances of the waterfront, it takes time to
recover from suffocation by debris.

The results of the spatially explicit model (IV) of *A. fulva* indicate that the best way to
increase the growth of the *A. fulva* population is to increase new patch formation. Also
the elasticity analysis of the matrix population model (III) showed that the population
growth rate is most affected by changes in the persistence of small patches within their
size class or the production of new small patches.

Because rhizome fragments of *A. fulva* loosened by ice usually end up in small bays
and are quite rapidly replaced by other species, transplantation of *A. fulva* and other
species to non-vegetated habitats could be worth trying (see, e.g., Colas *et al.* 1997,
Handa & Jefferies 2000, Heywood & Iriondo 2003). Rooting of rhizome fragments to
open areas is difficult because wind pushes them towards the shore. Therefore, rooting of
the transplants should be aided and they should be anchored, for example with poles.
Since a considerable portion of the transplants might disappear in the disturbances, a
large number of fragments should be used. Dispersal of the species by vegetative growth
could perhaps be aided by ploughing the soil in lower meadows wherever it is feasible.
However, most important for the long-term conservation of seashore meadows and the
species inhabiting them is to cut down the amount of leaching of nutrients from arable
soils in order to reduce the eutrophication of the Baltic Sea.
5 Conclusions

Management decisions concerning threatened plant species and habitats are often made with insufficient data. In order to manage a population correctly, we must first understand its structure and the factors affecting it. In this thesis, genetic, modelling and experimental approaches were used to obtain information for the management of threatened seashore plants and their habitat.

There are no specific immediate genetic threats to *A. fulva* var. *pendulina* in the Liminka Bay area. A relatively high amount of genetic variation was found in the population. The migration pattern and distribution of genetic diversity among the subpopulations of the area were mainly similar to the stepping-stone population structure. However, the population did not seem to be in drift-migration equilibrium, manifesting a classical metapopulation-type population structure in *A. fulva*.

The results from a matrix population model based on eight years of observations (1992-1999) of *A. fulva* at the Liminka Bay indicated that the population is not in immediate danger of extinction. However, according to a simulation model based on four-year observations (2000-2003) of the population, the situation of the population is more worrying. The simulation indicated that if the current trend continues, the population of *A. fulva* will decrease considerably in area and number of patches during the next 30 years. The results of the model indicate that the best way to increase the growth of the *A. fulva* population is to increase patch formation. The conflicting results of the matrix population model and the spatially explicit model highlight the importance of long-term monitoring of studied species in the field of conservation biology in order to produce reliable predictions about the state of the population.

The management experiment with three early-successional species, *Arctophila fulva* var. *pendulina*, *Primula nutans* var. *jokelae* and *Puccinellia phryganodes*, showed that displacement of the first two species could be slowed down by management. The results with *P. phryganodes* were the opposite. In *A. fulva* and *P. nutans* vegetative and/or sexual reproduction was enhanced when the surrounding vegetation was removed. In addition to the methods used in the experiment, also other means of management could be used. Grazing and concomitant trampling by cattle could provide an effective management tool for preserving and creating suitable habitats. Transplantations of the studied species and possibly also other threatened species, for example to non-vegetated habitats which have
recently risen from the sea, might help to lower the risk of regional extinction. However, most important for the long-term conservation of seashore meadows and the species inhabiting them is to cut down the amount of leaching of nutrients from arable soils to reduce the eutrophication of the Baltic Sea.

The results of the study on a clonal seashore plant *Potentilla anserina* ssp. *egedii* indicated that the species is capable of modifying its allocation of resources to sexual and vegetative reproduction according to competitive stress. The low-lying seashore meadows where the species grows are characterised by unpredictable but frequent disturbances. For a plant living in such environment, it may be beneficial to be able to track the competition-free space formed by disturbances by changing its reproductive pattern.
References


Lovén S (1862) Om några i Vettern och Venern funna Crustaceer. Öfversigt af Konliga vetenskasakademiens förhandlingar 18: 258-314.


**Original papers**


Original papers are not included in the electronic version of the dissertation.


439. Tero, Niina (2005) Genetic structure at different spatial scales in metapopulations of Silene tatarica

440. Säkkinen, Hannele (2005) Variation in the blood chemical constituents of reindeer. Significance of season, nutrition and other extrinsic and intrinsic factors


443. Jäkäldaniemi, Anne (2005) Adaptation, population viability and colonization-extinction dynamics of Silene tatarica in riparian habitats

444. Kivahde, Maja (2005) Effects of authentic learning and e-learning in an introductory chemistry laboratory course


446. Oksanen, Pirtti O. (2005) Development of palsa mires on the northern European continent in relation to Holocene climatic and environmental changes

447. Harjumaa, Lasse (2005) Improving the software inspection process with patterns

448. Ylianttila, Mari (2005) Structure-function studies of the peroxisomal multifunctional enzyme type 2 (MFE-2)


450. Poykko, Heikki (2005) Host range of lichenivorous moths with special reference to nutritional quality and chemical defence in lichens


452. Auola, Jyrki (2006) Environmental factors controlling the position of the actual timberline and treeline on the fells of Finnish Lapland
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POPULATION BIOLOGY OF THE PRIMULA SIBIRICA GROUP SPECIES INHABITING FREQUENTLY DISTURBED SEASHORE MEADOWS: IMPLICATIONS FOR MANAGEMENT