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**REPRODUCTION ECOLOGY AND GENETIC
DIVERSITY OF DECLINING SEDGE (*Carex*) SPECIES**

**VÄHENEVA ARVUKUSEGA TARNALIHKIDE (*Carex*)
PALJUNEMISÖKOLOOGIA JA GENEETILINE
MITMEKESISUS**

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to by their Roman numerals in the text. The papers are reproduced by kind permission of the publishers.

- I Kull, Thea and Kull, Tiiu. 2006. Habitat loss and reproduction biology as related to decline in rare *Carex* species. *Ekologia* (Bratislava) 25: 280–288.
- II Kull, Thea, Kull, Tiiu and Sammul, Marek. Reduced light availability and increased competition diminish the reproductive success of wet forest sedge *Carex loliacea* in deteriorated habitats. *Plant Species Biology*. Accepted.
- III Kull, Thea and Oja, Tatjana. 2007. Low allozyme variation in *Carex loliacea* (Cyperaceae), a declining woodland sedge. *Annales Botanici Fennici* 44: 267–275.
- IV Kull, Thea and Oja, Tatjana. 2010. Allozyme diversity and geographic variation among populations of the locally endangered species *Carex magellanica* subsp. *irrigua* (Cyperaceae). *Folia Geobotanica* 45: 323–336.

The contributions of the authors to the papers were as follows:

Paper	Original idea	Study design	Data collection	Manuscript preparation
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ABBREVIATIONS

- TAA Herbarium of Vascular Plants and Bryophytes in the Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences
- TU Herbarium of Vascular Plants in the University of Tartu, Museum of Natural History

1. INTRODUCTION

The variety of ecosystems, species, populations, and genetic diversity within species guarantees biological diversity. Due to direct and indirect consequences of human activity a large number of species have gone extinction already and many others are reduced in their population size. A negative trend in biological diversity also characterizes the relatively well preserved Estonian flora since between 1970–2005 about 40 vascular plant species from a total of over 1440 have lost more than half of their localities in Estonia (Kukk and Kull 2005). Several species of the genus *Carex* are among them. A similar decrease in *Carex* species has been observed by De Bruijn (1980) in the Netherlands, where a great number of *Carex* species have become extremely rare since 1950, mostly because of habitat destruction. In Estonia, the genus *Carex* is represented by 70 species (Kukk 1999) out of the 2000 species occurring worldwide (Bernard 1990) and nine of them are under nature protection (Leht 2010).

This thesis concentrates on five of the most declining *Carex* species in Estonia which, although more widespread before 1970s, have been less recorded since. These species grow in wet habitats including sphagnum swamps (*C. chordorrhiza*, *C. pauciflora*), swamp forests (*C. disperma*, *C. loliacea* and *C. magellanica* subsp. *irrigua*), quagmires and lakeshores (*C. magellanica* subsp. *irrigua*). Population size, seedling survival and degree of habitat specialisation play an important role in species' persistence. The first aim of the thesis was to document the current distribution of these declining sedge species in Estonia and reveal the causes of their apparent reduction. A more detailed study was carried out on *C. loliacea*, the species of old wet forests and an indicator species of minerotropic swamp forests. Throughout its distribution range, this species is declining due to cutting and drainage of forests (Gustafsson 1994, Schweitzer and Polakowski 1994, Garve and Giffe 1997, Oldham 1999, Pawlikowski 2001, Hallanora *et al.* 2002, Korpela 2004, Macdonald 2005). In Estonia, the species has survived only in one fourth of its former localities. In order to elaborate a scientifically based conservation plan for the prevention of a further decline in this species a study of its reproduction biology was undertaken. Seed germination phase is of great importance for species persistence and later fitness. Germination and seed dormancy of several *Carex* species have been studied by many authors (Schütz 1997,

2000; Vellend 2000; Schütz and Rave 2003; Esmaeili *et al.* 2009), but data about the reproduction ecology of *C. loliacea* still remain scarce. In this thesis the germination rate of *C. loliacea* seeds under different light conditions and the survival rate of seedlings of *C. loliacea* were studied.

The level of genetic diversity is related to the size and fragmentation of a population and may reflect the rarity of species. Endangered and rare species with fragmented and small or declining population sizes are expected to have low genetic diversity (Oostermeijer *et al.* 2003, Leimu and Mutikainen 2005). In this thesis genetic diversity of two declining *Carex* species was investigated by electrophoretic separation of enzymes. Allozyme variation within and among populations of *C. magellanica* subsp. *irrigua* and *C. loliacea* in Estonia, Fennoscandia, South Central Alaska and Poland was investigated in order to find any possible correlation to species decline and to assess geographical variation in population genetic structure. In addition to *C. loliacea*, *C. magellanica* subsp. *irrigua* was chosen for genetic study as it is a rare and declining taxon in the Baltic countries and Central Europe, belonging to the Red Data Book List of many countries, but rather common throughout North Europe (Mossberg *et al.* 1992, Hämet-Ahti *et al.* 1998) and North America (USDA, NRCS 2008).

2. REVIEW OF THE LITERATURE

2.1. Habitat conditions

Modern human activities such as building, mining, agriculture etc. changes the abundance of species that are more sensitive to growing conditions. Habitat fragmentation is one of the major threats to species diversity and to the genetic and demographic structure of fragmented populations (Honnay *et al.* 2005). Species from stable and ecologically complex environments like old forests are especially threatened (De Bruijn 1980) since woodland destruction is one of the major conservation problems nowadays. Many woodland herbs have attracted attention because landscape alteration and habitat destruction caused many species to become threatened, rare or even extinct (Prieditis 1999, Whigham 2004). In order to fully understand the causes of population degradation and plan appropriate protective measures, it is essential to know if the main cause of population decline lies in species biology and ecology (e.g. Cranstone and Valentine 1983, Foster 1999, Rovira *et al.* 2004, Gough 2006,) or suboptimal habitat conditions. The combination and accumulation of these effects could also reduce genetic diversity of small and isolated populations (Vergeer *et al.* 2003, Hooftman *et al.* 2004, Vandepitte *et al.* 2007) and push small populations into an extinction vortex (Tanaka 2000). Light is an essential environmental component for successful growth of plant species. Levine and Feller (2004) measured six environmental variables (pH, N, P, soil moisture, light, and plant density) and only light (positively) and plant density (negatively) contributed significantly to the variation in potential sexual reproduction. Similar results regarding the importance of light were reported by Mayberry and Elle (2009), who found that reproductive plants were more likely to be located in canopy gaps than vegetative plants. The total allocation of flowering also increased significantly with canopy openness.

2.2. Reproduction

The rate of vegetative and generative reproduction is of great importance for the long-term survival of species. Vegetative reproduction secures the survival of the population in a stable condition. Sexual reproduction has an advantage in fast changing conditions (Cook 1985), where seed establishment, germination of seeds and survival of seedlings are

of crucial importance. Even though sedges flower and fruit quite successfully, sexual reproduction is inhibited and seedling recruitment is very low (Liu *et al.* 2009). Sedges are wind pollinated, most of them are self-compatible and frequently autogamous (Whitkus 1988). The most probable seed dispersal is with water (Leck and Schütz 2005). Dispersal by ants, adhesion to animals or internal dispersal by animals may also occur (Catling *et al.* 1990). Various environmental conditions (light, temperature, competition, predators) are responsible for germination of seeds and seedling survival (Greiling and Kichanen 2002, Isselstein *et al.* 2002, Kotowski and van Diggelen 2004, Ketterling *et al.* 2006). Most sedge germinate better in light than in shade and at relatively high temperatures (Schütz 1999, Vellend *et al.* 2000, Schütz 2002, Brändel and Schütz 2005). However, a light requirement can be lost or reduced in the course of after-ripening (Schütz and Rave 1999), and high temperature amplitudes can replace a light requirement in seeds of some sedge species (Schütz 2000). Temperature fluctuations stimulate seed germination in *Carex* species and are especially necessary for wetland sedge species.

Experiments show that sensitivity of seeds to the red/far-red ratio is common in *Carex* species. Germination under a dense canopy is strongly suppressed (Schütz 2000, Araki 2008). Sedges are typical spring germinators using the available light before canopy closure. *Carex* seeds have a strict or conditional primary dormancy that prevents the germination in unsuitable conditions. Seeds of sedges are able to form a seed bank and enter secondary dormancy at increasing environmental temperatures. The dormancy of many species in temperate regions, including sedges, can be broken by cold-wet stratification (Ketterling and Galatowitsch 2007). For example, cold-wet stratification was beneficial for germination at 10/22 °C in 28 of 32 temperate *Carex* species (Schütz and Rave 1999), but mature seeds of *C. divisa* did not germinate 2 and 6 weeks after collection, indicating innate dormancy (Esmaeili *et al.* 2009). Species of open habitats require higher temperature for germination than forest species (Schütz 1997). Forest sedges have greater capacity to respond to low temperature and to temperature fluctuations (Schütz and Rave 1999). *Carex* seeds are long-lived and buried seeds can remain viable in the soil from 6 up to 130 years (Schmid 1986, Schütz 2000).

2.3. Impact of neighbouring plants on seedlings survival

The influence of plants on each other is usually thought to be negative, and competition is often assumed to be the most important negative interaction between them. Still, shelter by neighbouring plants may be essential for the establishment of some species (Ryser 1993). During the different stages of life neighbouring plants and competition between them act differently on plants. Shading from neighbours may improve water conditions for seedlings under the canopy of adults. But as the seedling roots grow into the rooting zones of neighbours', competition for water and nutrients will also increase. So, from facilitative or neutral effects on early growth, neighbours became competitive for adult growth and reproduction, with a maximum influence on reproductive plants (Violle *et al.* 2006). Howard and Goldberg (2001) studied old-field perennials and found little to no overall effect of neighbours on germination and seedling survival, but a strong effect of neighbours on seedling growth and on adult survival and growth. A number of studies have demonstrated the negative impact of competition from neighbouring plants on *Carex* seedlings (e.g. Cranstone and Valentine 1983, Schmid 1986). A study on the effects of competition and different water levels on *C. flava* indicated that while *C. flava* suffered considerably from competition, different water level did not influence the shoot and seed mass (Suter 2009). Thus, *C. flava* also may occur under slightly dryer conditions, but is absent in wetlands, where biomass production is high and light becomes a limiting factor. During the first phases of vascular plant life bryophytes may also significantly reduce germination and seedling survival of plants (Jeschke and Kiehl 2008).

2.4. Genetic diversity

Many aspects of species biology such as mating patterns and reproductive systems can be determined using genetic analyses including inbreeding and outbreeding rates in plant species. Small populations of naturally outbreeding species usually suffer from loss of genetic diversity. In a random mating population inbreeding causes loss of allelic diversity and polymorphism. Conversely, a plant population that reproduces by selfing may have a high inbreeding coefficient in each individual, a very low heterozygosity, but high overall genetic diversity as alleles are distributed among, rather than within individuals. A lot of studies have investigated

rare vs. common species and the effect of population fragmentation on the genetic diversity (Cole 2003, Fu and Dane 2003, Honnay *et al.* 2005, Leimu and Mutikainen 2005). Small and fragmented plant populations often suffer from reduction in genetic variation (Durka 1999, Paschke *et al.* 2002, Oostermeijer *et al.* 2003, Mateu-Andres 2004, Šingliarova *et al.* 2008, Chung 2009) that is expected to be associated with low fitness. But, recent studies have revealed many exceptional cases of high genetic variation in threatened or geographically restricted species (Neel and Ellstrand 2001, Eckert *et al.* 2008).

Genetic diversity of numerous *Carex* species has been investigated according to their growth form, rarity, habitat or phylogeny (Table 1). Both cross- and self-pollination has been observed among *Carex* species (Bruederle and Fairbrothers 1986, Waterway 1990, Ford *et al.* 1991, Whitkus 1992, Alekseev 1996, Huh *et al.* 2000, Ford *et al.* 2009). According to the growth form *Carex* species are divided into two groups. The first group has a caespitose growth habit where a lot of flowers of the same genet are close to each other and the chance of selfing is high (Ford *et al.* 1991). The species of the second group are rhizomatous and spikes are widely separated from each other. In this case the nearest neighbour may not represent the same individual (genet) and the chance of outcrossing is increased (Ford *et al.* 1998b). The two species investigated genetically in this thesis (*C. loliacea* and *C. magellanica* subsp. *irrigua*) are loosely caespitose and belong rather to the first group. Inflorescence morphology and growth form facilitate selfing in many caespitose *Carex* species that have multiple pistillate and staminate spikes on each stem or spikes with two sexes. Consequently, self-compatible species are expected to have lower allele frequencies because of frequent inbreeding, which leads to loss of alleles and increased populations' differentiation (Derieg *et al.* 2008). Thus, clonal architecture plays an important role in shaping mating patterns and population genetic structure in *Carex*, whereas the monoecy and protogyny in many *Carex* species may provide only limited opportunities for outcrossing (Friedman and Barret 2009). Sedges, like most Cyperaceae are wind-pollinating species but those growing in forest understory suffer from low wind speed which contributes the low level of outcrossing. Many forest plant species lack specific adaptation for long-distance seed dispersal, and their seed production is usually low. Species with low seed recruitment and long generation time lose genetic diversity mainly through sudden reductions in populations' size due to habitat loss and fragmentation. Low levels of polymorphism may also

result from genetic drift in small populations after genetic bottlenecks (Waterway 1990).

Table 1. Overview of mating mode, endemism, expected heterozygosity (H_c), growth form, and habitat of different *Carex* species.

Taxon	Mating mode	Endemic	H_c	Growth form	Habitat	Source
<i>C. breviculmis</i>	outcrossing	no	0.174	rhizomatous	forest	Huh <i>et al.</i> 2000
<i>C. humilis</i>	outcrossing	no	0.274	rhizomatous	forest	Huh 2001
<i>C. latebracteata</i>	outcrossing	yes	0.153	caespitose	forest	Ford <i>et al.</i> 2009
<i>C. mendocinensis</i>	outcrossing	yes	0.097	caespitose	forest	Waterway 1990
<i>C. cryptolepis</i>	selfing	yes	0.006	caespitose	open site	Derieg <i>et al.</i> 2008
<i>C. digitata</i>	selfing	no	0.106	caespitose	forest	Tyler 2002
<i>C. lutea</i>	selfing	yes	0.049	caespitose	open site	Derieg <i>et al.</i> 2008
<i>C. misera</i>	selfing	yes	0.04	caespitose	cliff	Schell and Waterway 1992
<i>C. pachystachya</i> complex	selfing	no	0.054	rhizomatous	mountain	Whitkus 1992

The genetic variation of a species may differ across its geographical area. Tyler (2002) for example investigated genetic diversity of *Carex digitata* throughout its native European range and found that the highest allelic richness was in Fennoscandia, i.e. in areas geographically central in the distribution range of the species. Even though cluster analysis on the level of populations did not recover any geographical structure, several alleles were predominately found in Fennoscandia and regional allelic richness was the highest there. The lowest within-region allelic richness was found in populations and regions close to the distributional edges of the species.

Genetic richness varies widely across different plant species: the number of polymorphic alleles is reportedly 18.3% for self-pollinators and 51% for outcrossers (Gottlieb 1981). The genetic richness and diversity among *Carex* species also has a wide variation. For example *C. gynodynamis* has an extremely low level of allozyme variation. All individuals studied were

monomorphic for the same allozymes for 16 of the 17 isozymes assayed (Waterway 1990). In *C. pachystachya* complex extremely low levels of polymorphism were reported with more than half the individuals being monomorphic and no heterozygotes were found in any of the populations (Waterway 1990).

Allozymes are reliable and useful markers for studying population genetic structure (Prentice *et al.* 2006, Oja and Paal 2007, Hedren 2008, Pedersen and Hedrén 2010) and they are also useful in evaluating the mating systems and evolutionary processes of taxa. A recent comparative study (Conte *et al.* 2008) shows that despite lower polymorphism of allozymes, all estimated values of genetic parameters showed a high congruence between SSR and allozymes.

3. AIMS OF THE STUDY

The decline of biodiversity is a global issue and for conservative purposes it is important to study the biology of declining species. Of equal importance is knowledge of the ability of species to persist in changing habitats, their reproductive ability as well as the genetic structure of these species. The general goal of this study was to address these issues using the example of five declining sedge species in Estonia.

First, we aimed to describe the contemporary distribution of five of the most declining *Carex* species according to the database of the Atlas of Estonian Flora (Kukk and Kull 2005). The distribution of many *Carex* species in Estonia is poorly investigated and available data are not sufficient. We hypothesized that the current distribution of *Carex* species might be different to that shown in the database.

Second, we estimated the germination rate of *C. loliacea* seeds in different light conditions. Environmental conditions change greatly during forest management operations and light is of crucial importance for plant growing. We assumed that changes in light conditions influence the germination success of declining *Carex* species.

Third, we investigated the competitive ability of seedlings of *C. loliacea* in order to evaluate the sensitivity of the species to habitat deterioration during the early stages of its life cycle. Cutting and drainage cause variation in the forest herb layer, usually favouring the growth of taller herbs. We hypothesised, that this high vegetation suppresses seedlings of small sedges.

Fourth, we assessed genetic diversity of *C. magellanica* subsp. *irrigua* and *C. loliacea* among and between populations and their genetic variation among geographic regions. Rare and declining species are expected to have low genetic diversity. We assumed that if the degree of rarity in different geographic district varies the genetic diversity should also differ.

4. MATERIAL AND METHODS

4.1. Study species

In this thesis five *Carex* species were studied: *Carex chordorrhiza* L. f. (I), *C. disperma* Dewey (I), *C. loliacea* L. (I, II, III), *Carex magellanica* Lam. subsp. *irrigua* (I, IV) and *C. pauciflora* Lightf. (I). On the basis of the database of Atlas of Estonian Flora (Kukk and Kull 2005), these species have declined in Estonia by a factor of four or more since the 1970s.

Carex chordorrhiza L. f. belongs to the subgenus *Vignea* (Beauv. ex Lestib.) Peterm. and is a circumpolar species. It is widely distributed in Eurasia and North America and was formerly more common in Central Europe (Hultén and Fries 1986). *Carex chordorrhiza* is a rhizomatous sedge. The flowers are borne in 3 to 8 small aggregate spikes at stem tips. One culm may produce 3–20 seeds (Rabotnov 1980). It occurs primarily in fens and *Sphagnum* bogs.

Carex disperma Dewey belongs to the subgenus *Vignea* (Beauv. ex Lestib.) Peterm. It is a boreal species belonging to the circumpolar plants (Hultén and Fries 1986). The species forms loose tufts with slender rhizomes. Stems are 10–60 cm long, very slender and weak. Fruit production is very low, 2–6 seeds per stem (Rabotnov 1980). *Carex disperma* grows in moist forest understory.

Carex loliacea belongs to the subgenus *Vignea* (Beauv. ex Lestib.) Peterm. It is a boreal-montane species, belonging to circumpolar plants but with wide gaps in distribution in the North Atlantic and Bering Sea areas (Hultén and Fries 1986). It forms sparse tufts and has gynecandrous spikes (a spike with upper flowers pistillate and lower staminate) and one culm may produce up to 25 seeds. It grows in mixed-spruce swampy forests.

Carex magellanica Lam. subsp. *irrigua* (Wahlenb.) Hiit (synonyms: *C. irrigua* (Wahlenb.) Sm. ex Hoppe, *C. paupercula* Michx.) is a boreal-montane taxon occurring in the northern hemisphere (Hultén and Fries 1986). The species belongs to the subgenus *Carex*. It forms small clumps usually with 3–6 shoots each shoot having 1–4 female spikes with an average seed production of 15 to 29 seeds per spike. The species grows in peaty soils of *Sphagnum* bogs, minerotrophic woodland fens, wet meadows, and in quagmires of lakeshores.

Carex pauciflora Lightf. belongs by Egorova (1999) to subgenus *Psyllophora* (Degl.) Heuff. It is a boreal-montane species and belongs to the circumpolar plants (Hultén and Fries 1986). It is a rhizomatous sedge with a single spike consisting of 1 to 4 staminate flowers and 1 to 7 pistillate flowers. The mean seed set for one culm is 5 seeds. This species is found in *Sphagnum* bogs, usually on open mats, but also in partial conifer shade.

Overview of measured parameters of five study species is given in Table 2.

Table 2. Measured parameters of five study species.

	<i>C. chordorrhiza</i>	<i>C. disperma</i>	<i>C. loliacea</i>	<i>C. magellanica</i> subsp. <i>irrigua</i>	<i>C. pauciflora</i>
Habitat loss	x	x	x	x	x
Habitat conditions			x		
Vegetative growth	x		x	x	x
Germination	x	x	x	x	x
Influence of light on germination			x		
Competition			x		
Genetic diversity			x	x	

4.2. Habitat study and reproduction biology (I, II)

The study of habitat loss (I) was based on the database of the Atlas of Estonian Flora (Kukk and Kull 2005) and on herbarium records in TAA and TU. In total 81 localities where *C. chordorrhiza*, *C. disperma*, *C. loliacea*, *C. magellanica* subsp. *irrigua* and *C. pauciflora* have been recorded since 1921 were revisited in 2001–2002. In most locations revisited the most recent data originate from before 1971.

The study of habitat conditions of *C. loliacea* (II) was carried out in 10 populations in Estonia, 11 in Finland and four in Sweden where on 50x50 m squares the number of clumps was counted to estimate the abundance of *C. loliacea*. On the same plots the type of habitat and impact of drainage (heavy, moderate, light, absent) were estimated. Within the

plots 1m² quadrates were used to estimate the cover of vegetation and moss layer, number of flowering culms of *C. loliacea*, and the content of available P, K, total N and organic matter in the soil. Light conditions were characterized using the hemispherical photography technique from a digital photo taken above the plot.

To study the clonal growth, the rhizome length of ramet and number of branches on one rhizome were measured from excavated rhizome systems of *C. loliacea*, *C. chordorrhiza*, *C. pauciflora* and *C. magellanica* subsp. *irrigua*. *Carex disperma* was not excavated due to the limited number of plants. Sexual reproduction was studied in 5 species using a comparative germination experiment in a natural environment and a garden. To estimate germination ability in natural conditions, thin nylon bags (5×5 cm) with 10 seeds in each were planted under a 1 cm moss layer. In the garden the seeds were sown in boxes which were placed in half shade and watered regularly (I).

To estimate the effect of light on germination, a laboratory germination experiment with seeds of *C. loliacea* was carried out (II). Seeds were collected in Estonia, Finland and Poland. Three treatments each with a different light environment were designed: “neutral shade” (14 h photoperiod at seed level of about 21 $\mu\text{mol m}^{-2}\text{s}^{-1}$, R/FR ratio 1), “green shade” (15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at seed level, R/FR ratio 0.25) and dark. Germination was carried out in the laboratory with a fluctuating temperature of 17/28 °C after a two month period of cold-wet stratification. Germination was recorded at 2–3 day intervals. Radicle emergence was the criterion of germination. After two months seeds that had not germinated were put back into the refrigerator at 4 °C for one month to break any possible dormancy. Thereafter seeds were returned into the germination environment, and seeds from the dark treatment were now placed in “neutral shade” conditions. After two weeks when maximum germination had passed, seeds still not germinated were placed once again for one month into a refrigerator and subsequently germinated and counted.

For statistical analysis a logistic regression method with SAS GENMOD procedure was applied. The model was over-dispersed and so the scale parameter was estimated by the square roots of Pearson’s Chi-Square divided by the degrees of freedom. Spearman Correlation Coefficients were used for analysing rhizome length. In addition, statistical analysis using the program STATISTICA 8 was carried out. General Regression

Model with two-directional stepwise selection procedure was used to test for the effect of habitat conditions on abundance of *C. loliacea*. ANOVA with subsequent Tukey's post-hoc test was applied to investigate the germination of *C. loliacea* seeds in different light treatments.

4.3. Impact of neighbouring plants on seedlings survival (II)

Carex loliacea is already extinct or very rare in many parts of its distribution area. In Estonia it is also declining but there are still sufficient populations to collect seed material for germination and competition experiments. The survival ability of *C. loliacea* seedlings in closed vegetation was tested by means of a removal experiment. Experimental plots were established at three sites in areas where the species was also naturally present: Vägari (VG) – drained forest, no flooding; Laeva (LV) – old wet forest (Fennoscandian herb-rich forest), extensive flooding in spring and Järvelja (JS) – old wet forest with no drainage and minimum human impact. The Järvelja site is largely covered with *Sphagnum* sp. mosses and may flood in wet years. In all sites 12 permanent plots (25×25 cm) were established. Half the plots were treated with herbicide (ROUND UP) for eliminating the aboveground competition from natural vegetation. On the other half of the plots (plots with competition) the vegetation remained intact. Five seedlings were planted in each plot. The survival and growth of seedlings was observed four years.

Generalized Linear Model (GLZ) model with binomial error distribution and logit-link function in the program STATISTICA 8 was used to test the influence of competition removal treatment on survival of *C. loliacea* seedlings.

4.4. Genetic diversity (III, IV)

Two species with different inflorescence type and various rarity levels were chosen for the genetic study. *Carex loliacea* is declining over its distribution area, while *C. magellanica* subsp. *irrigua* is still quite common in the northern part of its distribution range. *Carex loliacea* has male and female flowers in one spike but *C. magellanica* subsp. *irrigua* in different spikes. For isozyme analyses, seeds of *C. loliacea* and *C. magellanica* subsp. *irrigua* were collected from Estonia, Finland, Sweden, South-

Central Alaska, Norway (*C. magellanica* subsp. *irrigua* only), and Poland (*C. loliacea* only) (Figure 1). At each site seeds were collected from at least five mother plants located at least 2 metres apart from each other.

Ten (*C. magellanica* subsp. *irrigua*) to eleven (*C. loliacea*) enzymes were examined: malate dehydrogenase (MDH), shikimate dehydrogenase (SKD), aspartate aminotransferase (AAT), superoxide dismutase (SOD), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucoisomerase (PGI), peroxidase (PRX), phosphoglucomutase (PGM), alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP) and esterase (EST). Tissue extracts were prepared from leaves of seedlings. The extracts were subjected to electrophoresis in vertical polyacrylamide gel slabs. After electrophoresis, the gels were stained for isozymes by applying standard histochemical methods (Wendel and Weeden 1989). The isozyme results are described at the level of electrophoretic isozyme phenotypes that are interpreted as corresponding to respective genotypes according to Wendel and Weeden (1989). All population genetic statistics was described using conventional designation of alleles.

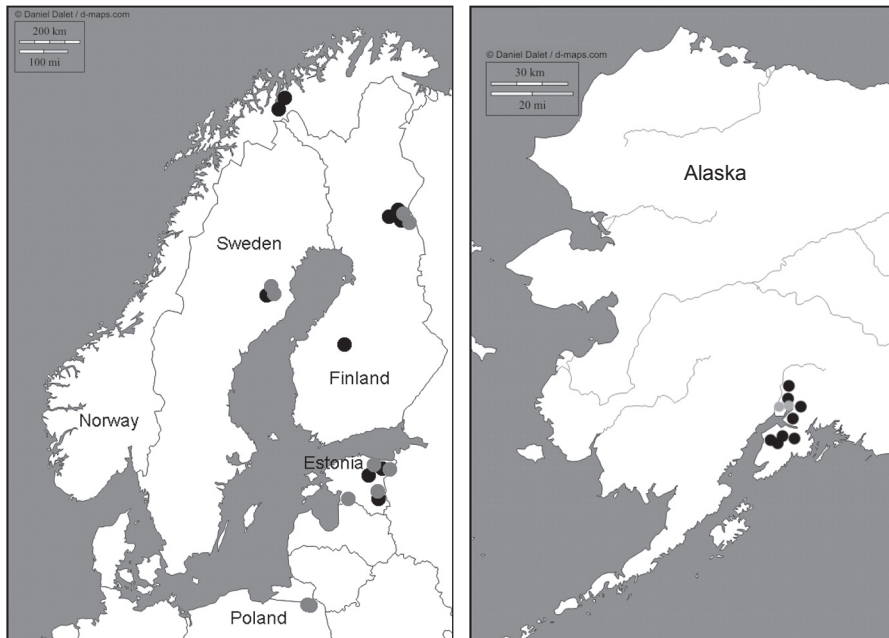


Figure 1. Study sites for allozyme analyses of *C. loliacea* (grey dots) and *C. magellanica* subsp. *irrigua* (black dots) in Europe and in Alaska.

To characterize the genetic diversity quantitatively, the following parameters were calculated for each local population studied: the number of alleles per locus (A), the percentage of polymorphic loci (P), Wright's fixation index (F), the observed (H_o) and expected (H_e) heterozygosity. In addition to these characteristics for *C. magellanica* subsp. *irrigua* effective number of alleles (A_e) and inbreeding coefficient (F_{is}) were calculated. Program POPGENE (version 1.31) (Yeh *et al.* 1999) was used to calculate parameters of genetic diversity for *C. magellanica* subsp. *irrigua*. Allele frequencies were calculated for each population and were used to calculate Nei's genetic distance (Nei 1978) between populations. These distances were subjected to UPGMA cluster analysis to study patterns of between-population variation. UPGMA were performed using program TFPGA (version 1.3) (Miller 1997).

5. RESULTS

5.1. Habitat study

As a result of revisiting former habitats, it appeared that some species have persisted in their old localities better than indicated in the database of the Atlas of Estonian Flora (Kukk and Kull 2005). *Carex chordorrhiza* and *C. loliacea* were found in most of the places previously recorded, while *C. chordorrhiza* was even found in 15 new sites. *C. pauciflora* was found in 58% of revisited places and 10 new localities were identified for this species. However, the abundance of *C. magellanica* subsp. *irrigua* and *C. disperma* were found to have decreased: they were found in less than half of the revisited sites. No new localities were found for *C. loliacea* and *C. disperma* (Table 3).

Table 3. Data of the occurrence of the studied *Carex* species in atlas grid cells in different time periods (I).

Species	Found in grid cells of the distribution database of the Atlas		2001–2002			
	1921–1970	1971–2000	revisited	found	%	new
<i>C. loliacea</i>	63	20	22	20	91	0
<i>C. chordorrhiza</i>	76	26	19	15	79	14
<i>C. pauciflora</i>	81	30	12	7	58	10
<i>C. disperma</i>	63	9	15	5	33	0
<i>C. magellanica</i> subsp. <i>irrigua</i>	65	21	13	3	23	1

The detailed study of 25 populations of *C. loliacea* showed that the species grows in rich paludified forests (16 sites), in minerotrophic swamp forests (7 sites) and in drained peatland forests (2 sites). The variation of light conditions between sites was low (16–25% openness) and the content of N, P and K in soil was widely variable between sites with values 0.08–2.8% for N, 4–135 mg/kg for P and 12–878 mg/kg for K. Using a General Regression Model with stepwise selection of factors no significant effect of these habitat characteristics on the abundance of *C. loliacea* in any models was found. Neither did the habitat characteristics reveal any significant correlation to each other.

5.2. Reproduction

5.2.1. Vegetative reproduction

The study of the rhizome systems showed that the mobility (rhizome length) was highest for *C. chordorrhiza* (mean 12.9 cm). The other species studied had remarkably shorter rhizomes (mean 2.0 cm – 4.1 cm). *Carex chordorrhiza* and *C. loliacea* had the highest variation coefficient for rhizome length and for number of rhizome branches, while mean number of rhizome branches did not differ much among the species (Table 4).

Table 4. Clonal growth parameters of the four studied species (I).

Species	Rhizome length (cm)					Rhizome branches				
	N	mean	min	max	Var. coef (%)	N	mean	min	max	Var. coef (%)
<i>C. chordorrhiza</i>	90	12.9	0.1	90	126	96	1.1	0	13	185
<i>C. pauciflora</i>	180	4.1	0.3	35	96	204	1.1	0	4	87
<i>C. magellanica</i> subsp. <i>irrigua</i>	89	2.2	0.3	7.5	68	94	1.3	0	4	75
<i>C. loliacea</i>	31	2.0	0.1	6.5	102	35	1.4	0	5	116

**Carex disperma* was not measured because of the too small number of plants.

5.2.2. Generative reproduction

The comparative germination experiment revealed differences within and between species in germination rates in natural conditions and in the garden (Figure 2).

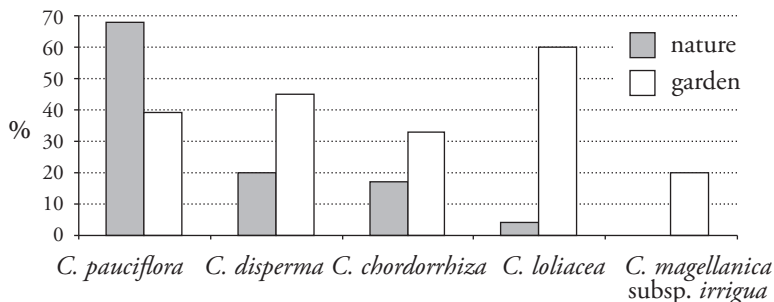


Figure 2. Germination rate of *Carex* species in natural conditions and in the garden experiment.

The laboratory experiment in different light conditions indicated that the germination of *C. loliacea* seeds is highly dependent on light conditions ($p = 0.0001$) (Figure 3).

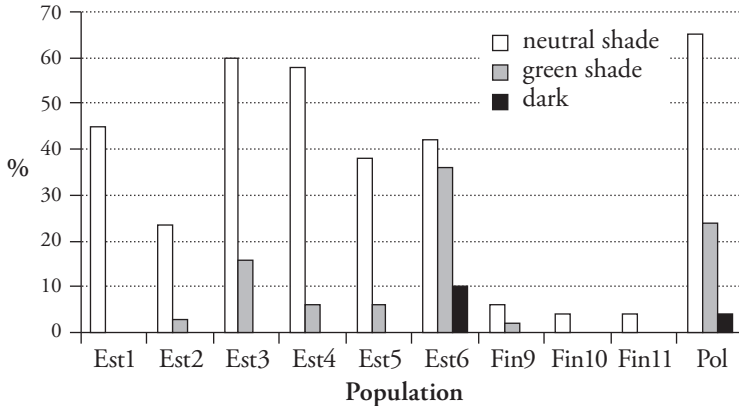


Figure 3. Germination of *C. loliacea* seeds from different populations following the first stratification in three different light treatments. Est – Estonia, Fin – Finland, Pol – Poland.

The germination started on seventh day and the maximum germination occurred during the following four days. The rather high germination temperature induced secondary dormancy in seeds that did not germinate during the first two weeks. After the second and third stratification the germination rate increased again, so that after the third stratification

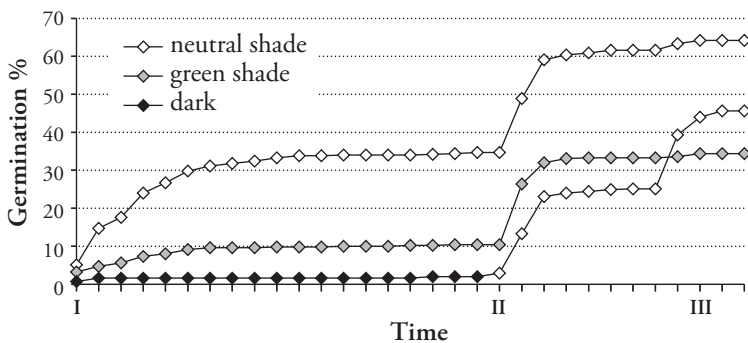


Figure 4. Germination (in days) of *C. loliacea* seeds in three different light conditions after three stratification periods (I, II, III). After the second stratification period seeds of dark treatment were moved into conditions of the ‘neutral shade’ treatment (II).

in ‘neutral shade’ 64% of seeds and in ‘green shade’ 34% of seeds had germinated. Seeds from the dark treatment were moved into the ‘neutral shade’ treatment after the second stratification and the final germination in this treatment was 46% (Figure 4).

5.3. Impact of neighbouring plants on seedlings survival

The competition experiment showed that during the four years of the experiment survival of seedlings of *C. lolicea* was significantly higher ($p = 0.0002$) on plots without competitors than on plots with intact vegetation (Figure 5). This result did not apply to the Laeva site where no effect of treatment was detected. An important abiotic factor in the survival of seedlings on this site was flooding, because in spring there were extensive overflows in some study plots and seedlings there suffered considerably. Additionally, the negative impact of *Populus tremula* leaf litter was observed at the Laeva site. Small seedlings suffered from low light under fallen leaves and died.

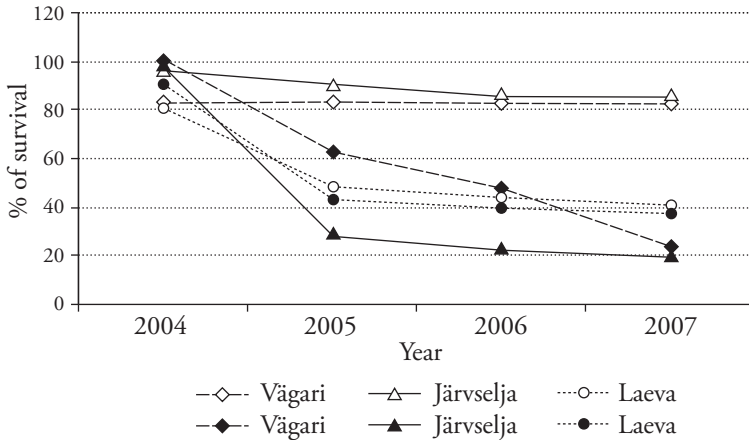


Figure 5. Survival of *C. lolicea* seedlings in three different sites during four years in the competition experiment. Filled signs – with competitors, open signs – without competitors (**II**).

5.4. Genetic diversity

The two species differed considerably in the percent of polymorphic isozyme loci. *C. magellanica* subsp. *irrigua* showed much higher genetic diversity than *C. loliacea*: the percent of polymorphic loci for *C. magellanica* subsp. *irrigua* was 55.6, while for *C. loliacea* the same number was only 5.6. The mean number of alleles per polymorphic locus was 1.05 for *C. loliacea* and 2.2 for *C. magellanica* subsp. *irrigua*. In *C. loliacea*, only one (MDH-A) of the 18 isozymes was polymorphic, whereas *C. magellanica* subsp. *irrigua* showed higher polymorphism with five polymorphic isozymes out of nine. Over the latter taxon, three alleles (*Pgi-b*, *Prx-b*, *Aat-b*) had a frequency of over 0.7 and were dominant in most populations. One allele (*Prx-a*) had a frequency of less than 0.1 and was present in only one Norwegian population (N1). One Estonian population (E2) was monomorphic at all loci. Level of heterozygosity was low in both species. *Carex loliacea* had very low heterozygosity in only the three largest Estonian populations. Two populations had four heterozygous individuals (MDH-A1/2), one population was polymorphic for allozymes MDH-A1 and MDH-A2, but no heterozygotes were detected. *Carex magellanica* subsp. *irrigua* had heterozygosity only in Fennoscandian populations, while Estonian and Alaskan populations were totally homozygous. The deficiency of heterozygotes in both species compared with the panmictic Hardy-Weinberg equilibrium indicates the prevailing self-fertilization in these species. Quantitative characteristics of genetic diversity for the two species studied are given in Table 5.

Table 5. Mean values of allozyme diversity for *C. magellanica* subsp. *irrigua* and *C. loliacea*. P – % of polymorphic loci, H_o – observed and H_e – expected heterozygosity, F_{is} – inbreeding coefficient, t – outcrossing rate, F_{st} – fixation index (IV).

Taxon	P	H_o	H_e	F_{is}	t	F_{st}
<i>C. magellanica</i> subsp. <i>irrigua</i>	55.6	0.004	0.073	0.949	0.008	0.666
<i>C. loliacea</i>	5.6	0.0006	0.027	0.930	0.011	0.887

5.4.1. Genetic variation and differentiation among geographic regions

Carex loliacea and *C. magellanica* subsp. *irrigua* showed similar distribution of allozyme genetic variation between different geographical districts. The UPGMA dendrogram of the Nei's genetic distances shows that for both species Alaskan and Fennoscandian populations were more similar to each other and Estonian population formed a distinct group (Figure 6, Figure 7). The unexpectedly high Nei's genetic distance value 0.9 in *C. loliacea* reflects differentiation of the two geographic population groups by two MDH-A allozymes A1 and A2.

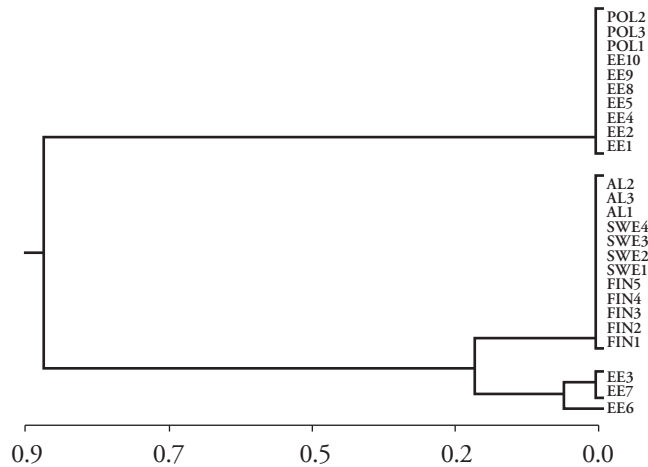


Figure 6. UPGMA dendrogram of the Nei's genetic distances among *C. loliacea* populations (III).

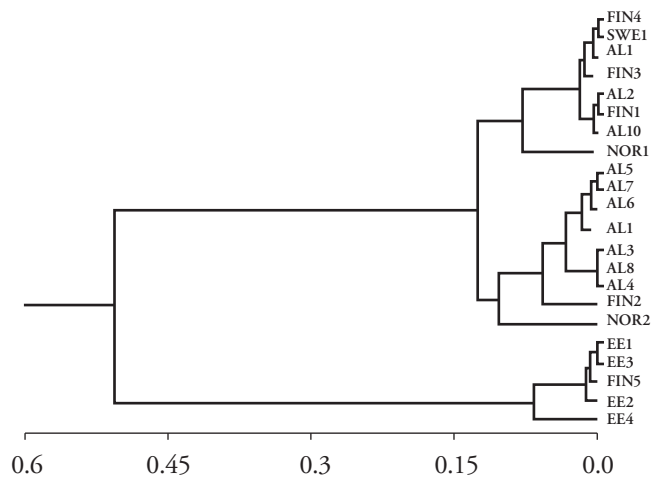


Figure 7. UPGMA dendrogram of the Nei's genetic distances among *C. magellanica* subsp. *irrigua* populations (IV).

6. DISCUSSION

6.1. Habitat study

The use of intensive methods of forest management in the 20th century has led to the large-scale drainage and logging of Estonian forests. Despite the fact that the total area of forested land has increased, the area under swamp forests decreased from 492,000 ha in the 1960s (Laasimer 1965) to 182,000 ha by the end of the 20th century (Viilma *et al.* 2001). *Carex loliacea* and *C. disperma* were often found in fresh wet clear-cut areas indicating their habitats are not so much threatened by the forest cutting but are rather affected by the groundwater level. Although *C. loliacea* was found in almost all localities revisited, the populations were as a rule quite small and they could be relicts of former larger populations. *Carex disperma* was found in only 33% of the old localities and the populations were small as well. That no new localities for forest species *C. loliacea* and *C. disperma* were found may indicate that while the species are able to persist in unsuitable conditions they may not be able to spread. The largest and most viable populations of these species were found in Järvelja primeval forest, where the influence of human activity is minimal.

The detailed study of *C. loliacea* populations (II) identified a variety of wet forest habitats for this species, mostly rich paludified forests and minerotrophic swamp forests. Despite studying many different habitat characteristics, no significant relationship between habitat factors and abundance of *C. loliacea* was found. Such a result may indicate that the species does not grow in unsuitable conditions. Another explanation may be that at least the persistence of long-lived mature plants is not so strongly affected within the range of suitable abiotic environmental conditions. As changes in the community take place slowly, it enables mature plants to survive for some period even in heavily altered conditions. This may be the reason why we can even occasionally find *C. loliacea* in severely drained areas.

It is essential to take into account that species requiring similar habitats may decline at different rates owing to various biological reasons. The loss of swamp forests, especially through drainage, has affected *C. disperma* more strongly than *C. loliacea*, although they grow in similar habitats and

are close even morphologically. *Carex magellanica* subsp. *irrigua* mainly grows in minerotrophic swamp forests and since these forest types are now often drained the species is suffering from a lack of suitable habitats. The area of Sphagnum bogs, the most common habitat of *C. chordorrhiza* and *C. pauciflora*, has decreased slightly less than the area of wet forests, and these species have actually survived better than indicated in the Atlas of Estonian Flora. Habitat loss is one of the main factors that threatens rare species, however, species biology – particularly reproductive biology – plays a crucial role.

6.2. Reproduction

Comparing the persistence of the species in their former habitats with data on their reproductive success indicates that germination ability as well as variation of rhizome parameters are important for maintaining the species in their localities. Vegetative mobility of a species allows it to relocate within a locality and seek out better conditions for its daughter ramets (Hutchings and Bradbury 1986). The variability of vegetative parameters enables higher flexibility regarding environmental conditions. This conclusion is supported by the occurrence of *C. chordorrhiza*, *C. pauciflora* and *C. loliacea*. These species had high and variable rhizome length and also the highest percentage of re-discovered sites (Table 3), indicating that these species can persist at suitable habitats for several decades. The variability of branching was the highest in *C. chordorrhiza* and in *C. loliacea*, which shows the plasticity of these species as well as their higher survival rate. These species had also best persistence rates at their old sites.

The ratio between sexual and clonal reproduction varies between species and depends on habitat conditions. It has been shown that a trade-off exists between the success of sexual reproduction and vegetative mobility (Silvertown and Doust 1993) whereby predominantly sexually reproducing species often form shorter rhizome increments. Species with high seed production and which are growing in appropriate conditions for seed germination have higher success of generative reproduction and the ability to find new localities (Eckert 2002). According to the literature (Novikov 1980, Novikov and Abramova 1980a) *C. chordorrhiza* reproduces mainly vegetatively, whereas *C. pauciflora* and *C. loliacea* sexually. Novikov and Abramova (1980b) reported that seeds of *C. chordorrhiza*

had not germinated in their laboratory experiment. In the present study, the seeds of this species germinated equally in the garden experiment and in natural conditions, but not at very high rate. We can conclude that *C. chordorrhiza* is able to reproduce vegetatively at existing sites and spread generatively into new ones. The seeds of *C. pauciflora* had highest total germination among species studied, as was the case also in many new sites. Contrary to the hypothesis that species which reproduce mainly by seeds have short rhizomes, the rhizomes of *C. pauciflora* were up to 35 cm long. Among the species studied, only *C. chordorrhiza* had longer rhizomes. Seeds of *C. loliacea* and *C. disperma* germinated well in garden experiment, but remarkably less in the natural environment, showing their susceptibility to environmental conditions. In the garden the pots were watered and the conditions were more stable. These two species have very small seeds (2–3 mm) and smaller seeds should be transported and reach new sites easier, however, neither of these two species were found in any new locality. The seeds of *C. magellanica* subsp. *irrigua* did not germinate at all in the natural environment, and the species was found at only a few old and one new site. Both factors, poor germination and loss of habitats, probably play a role here.

Unlike the germination rate in the natural environment experiment, the germination of *C. loliacea* seeds in the laboratory was rather high, but depended significantly on the light conditions. This result is in agreement with many other studies that have demonstrated the importance of light on seed germination across a range of species (Hilton 1984, Schütz and Rave 1999, Isselstein *et al.* 2002, Baskin *et al.* 2004). It has been shown that small-seeded species require more light for germination, irrespective of adult plant light requirements (Jensen 2004, Jankowska-Błaszczuk and Daws 2007). Thus, as *C. loliacea* has rather small seeds, changes in habitats leading to an increase in canopy density may reduce the success of germination. The successful time for germination of *C. loliacea* seeds in the forest environment is restricted to the short period in spring prior to leaf canopy closure. Our experiment demonstrated that under the canopy at low R/FR ratio ('green shade' treatment) germination is significantly reduced. Observations in studied habitats also confirm the requirement of light of *C. loliacea* as the species is often found growing in gaps of forest canopy caused by tree fall. The results from the comparison of three stratification periods in the present study also support the view that *C. loliacea* is a strict spring germinator. The first stratification period released seeds from primary dormancy. For some seeds a high

germination temperature induces secondary dormancy. A further stratification cycle released some of these seeds from the secondary dormancy. Such behaviour enables *C. loliacea* to develop a seed bank in the soil and ensure long-term persistence of a population.

6.3. Impact of neighbouring plants on seedlings survival

The germination and competition studies show that the impact of neighbouring plants do not only reduce the germination rate, but also decrease the survival rate of seedlings of *C. loliacea*. However, Ryser (1993) and Schmid (1986) found that competition may not be the only explanation for the depressed seedling growth. Physical hazards (like frost, drought) and pathogens may reduce seedling establishment even more than competition from neighbouring plants, and the presence of neighbouring plants may even protect seedlings from extreme abiotic conditions. Still, the data from our removal experiment clearly show that survival, growth and flowering are reduced in strong competition conditions. Only in the Laeva site was the competitive effect absent. This might, however, be explained by the flooding experienced at the site every spring and from plots without other plants some seedlings might be simply flushed off. In Vägari and Järvelja sites important competitors were bryophytes. In Järvelja, a very dense *Sphagnum* sp. layer covered the study plots resulting in the death of *C. loliacea* seedlings. In Vägari, *Hylocomium splendens* was the most prevalent moss on study plots.

Draining of habitats of *C. loliacea* is harmful to survival of species as it is followed by increase in vegetation density and competition intensity. Similar effects could be observed in forest clear-cut areas where *C. loliacea* initially took advantage of improved light availability, germinated well and spread widely, but became severely suppressed by the shrub cover in later stages of succession. Very often whole populations of *C. loliacea* disappeared under the dense cover of shrubs and broadleaved plants at forest clear-cuts (personal observations).

Ehrlen *et al.* (2006) showed that distribution of forest herbs is mainly limited by the availability of seeds. The results of the present study show that the reason for decline in *C. loliacea* populations is not the lack of seeds. Most tussocks flowered successfully. However, *C. loliacea* suffers from conditions unfavourable for germination (lack of light) and for

seedling survival (competition by neighbours). Thus, the changing quality of habitats and negative effects of drainage and cutting of trees may explain the decrease in distribution of *C. loliacea*. Therefore, the emphasis should be laid on the preservation of habitats of *C. loliacea* and on the conservation of natural environmental conditions.

6.4. Genetic diversity

Carex loliacea with its small and fragmented habitats had extremely low genetic diversity within and among populations. Fourteen populations of *C. loliacea* out of the 17 studied, i.e. 82% of the sites, were monomorphic at all isozyme loci and the percentage of polymorphic loci was only 5.6. Similarly very low genetic variation has been found in some other *Carex* species. Waterway (1990), for example, reported extremely low polymorphism and no heterozygotes in any of the populations for *C. gynodynamis*, an endemic to the California Floristic province. Whitkus (1992) found a low degree of genetic variation in the *C. pachystachya* complex where the mean number of alleles per polymorphic locus was 1.2 and only 20% of the loci were polymorphic. Schell and Waterway (1992) found low levels of isozyme heterozygosity and polymorphism in populations of the rare and endemic sedge *Carex misera* with 30% of loci polymorphic and in average 1.5 alleles per polymorphic locus at the species level. These parameters of the *C. pachystachya* complex and of endemic *C. misera* show still higher allozyme variation than in *C. loliacea* which has 1.05 alleles per polymorphic locus and only 5.6% of loci polymorphic, despite that *C. loliacea* is not an endemic but has wide circumpolar distribution. While *C. loliacea* had only few heterozygous individuals in the largest Estonian populations, *C. magellanica* subsp. *irrigua* possessed slightly more heterozygotes in Fennoscandian populations where the total genetic variability was higher than in Estonian and Alaskan populations.

Many studies have indicated that caespitose species have a lower level of intrapopulation genetic variation than rhizomatous species, e. g. Jonsen (1998). *Carex magellanica* subsp. *irrigua* and *C. loliacea* are caespitose species and have no special features for long-distance seed dispersal. Restricted seed dispersal results in small spatial clumps of genetically closely related tussocks due to the lack of nonrelative mating partners in the neighbourhood.

Genetic diversity within and between populations is greatly influenced by the mating system of species (Hamrick and Godt 1996). Selfing species usually have a higher genetic variation between populations while in outbreeding species variation within populations predominates. Mean genetic differentiation F_{st} for *C. loliacea* was 0.887 and 0.666 for *C. magellanica* subsp. *irrigua*, indicating that the level of differentiation between populations is very high and that only 11.3% and 33.4% of the diversity, respectively, lies within populations. In this study, populations of *C. magellanica* subsp. *irrigua* from the central abundant distribution region in Fennoscandia had even higher levels of F_{st} than populations in peripheral areas for this species in Alaska and Estonia, although higher differentiation was expected in small and isolated Estonian populations. The high level of differentiation between populations may be caused by the low gene flow over long distances of the species studied. Wind pollination of small herbs is hindered in forest understory and closed vegetation. As *C. magellanica* subsp. *irrigua* is a long-lived perennial, a possible explanation for lower value of F_{st} in Estonian populations may be the so called delayed loss of genetic diversity, i.e. after reduction of habitat size the genetic differentiation in isolated small populations stays for some time at the same level after fragmentation (Leblos *et al.* 2006).

Carex magellanica subsp. *irrigua* and *C. loliacea* are both declining taxa in Estonia, but their current level of genetic diversity is different. However, the mean genetic diversity of *C. magellanica* subsp. *irrigua* ($H_c = 0.073$) and *C. loliacea* ($H_c = 0.027$) is still considerably lower than a mean value for rare species ($H_c = 0.142$), reported in the review of Cole (2003). One reason for the low genetic diversity in both species could be their fragmented distribution pattern. Differences in inflorescence type also could contribute to the different level of allozyme diversity in these two sedge species. *Carex loliacea* has male and female flowers on the same spike, while *C. magellanica* subsp. *irrigua* has male and female flowers on different spikes. The close proximity of male and female flowers may favour geitonogamy and cause lower genetic diversity in *C. loliacea*. However, the observed high mean inbreeding coefficient F_{is} values (Table 5) indicate that both species are predominantly inbreeding.

6.4.1. Genetic variation and differentiation among geographic regions

Based on Nei's genetic distances, *C. loliacea* and *C. magellanica* subsp. *irrigua* showed similar genetic differentiation among the geographic regions studied. In both species, Estonian populations form a group distinct from Fennoscandian and Alaskan populations (Figure 6, Figure 7). *Carex loliacea* revealed a distinct geographic differentiation between Polish and Fennoscandian populations with alternate allozymes at the polymorphic MDH-A isozyme. However, no differentiation was found between Swedish and Finnish populations (Figure 5). In the Estonian populations both allozyme variants, MDH-A1 and MDH-A2, were represented. This pattern of intraspecific geographic differentiation in *C. loliacea* may reflect the impact of the last glaciation in Northern Europe. We may suppose that Poland was a refugium during the last glaciation from where *C. loliacea* migrated northwards, while Northern Poland is today the southernmost area in Europe where the species still exists. The migration route to Northern Europe could have been from central regions of Russia or Alaska that remained unglaciated during the last Ice Age (Velichko *et al.* 1997). Thus, *C. loliacea* could have migrated to Estonia from two different sources: from the south (Poland) and from the east (Russia), but to Fennoscandia most probably from Russia because allozyme MDH-A1 characteristic of Poland populations was not found in the Fennoscandian populations. Further investigations of local populations in different geographical regions, especially from Russia, would be necessary to elucidate the postglacial phylogeographic history of *C. loliacea*.

The populations of *C. loliacea* were small throughout its distribution area, but according to the population size and fragmentation level we can divide the populations of *C. magellanica* subsp. *irrigua* into two groups. Estonian populations form the southern peripheral group, where populations of *C. magellanica* subsp. *irrigua* as a rule are small and fragmented and the species is declining. In northern areas, in Alaska and Fennoscandia, the species is still rather common with large populations (personal observations). UPGMA dendrogram supports the differentiation between these two groups at the regional level: southern (Estonian and the southernmost Finnish population) and northern (Fennoscandian and Alaskan populations) (Figure 7).

Based on the distribution map of Hulten and Fries (1986), the geographic distribution range of *C. magellanica* subsp. *irrigua* could be distinguished into two regions: North American and Eurasian. Fennoscandia is the central distribution area of *C. magellanica* subsp. *irrigua* in the Eurasian region with large populations and higher genetic diversity. The Estonian populations have a marginal position in the Eurasian range of the species and suffer from habitat fragmentation and population decline, and probably therefore have reduced allozyme polymorphism and heterozygosity. In Central Europe the species occurs rarely and grows only in mountains (Dite and Pukajova 2003). The absence of heterozygotes in Alaskan populations is not so easy to explain. *Carex magellanica* subsp. *irrigua* seems to be common there with numerous large populations (personal observations) and one would expect high polymorphism and heterozygosity. One explanation could be that low genetic diversity is related to the edge position of the North American range. Hulten and Fries (1986) also indicate that the occurrence of *C. magellanica* subsp. *irrigua* in Alaska and in most part of North America consists of numerous, but rather isolated populations that has potentially restricted gene flow and decreased the level of genetic polymorphism.

6.5. Future projects

The results of the current thesis show that the distribution of some *Carex* species, e. g. *C. chordorrhiza* and *C. pauciflora*, has declined less than it is shown in the database of the Atlas of Estonian Flora. Thus, it would be also reasonable to examine the old localities of other declining *Carex* species to collect data on their present distribution in Estonia, habitat condition, genetic diversity and reproductive performance in relation to population sizes and geographical isolation.

Carex limosa is a morphologically close species to *C. magellanica* subsp. *irrigua*, however, more common in Estonia. Comparative genetic and reproduction study on these two species could be informative and might give us some indication on why *C. magellanica* subsp. *irrigua* is rarer than *C. limosa*.

Another species pair that would be interesting to compare in more detail is *C. loliacea* and *C. disperma*. These species grow in similar habitats and are morphologically similar. Analogous experiments on germination

and competition performed with *C. loliacea* could be undertaken for *C. disperma*. Both species are declining, but although the current study preliminarily showed that *C. disperma* has a greater extinction risk, the reasons for the differences remain to be elucidated. Genetic diversity in the remnant Estonian populations in relation to the habitat type, population size and isolation remains to be studied. As in the beginning of the study there was too little material available, *C. disperma* was excluded from the detailed study. More information about the localities has been collected during this work that makes it possible to investigate this species more thoroughly.

7. CONCLUSIONS

This thesis showed the differences in persistence of the five most declining *Carex* species in Estonia. *Carex disperma* and *C. magellanica* subsp. *irrigua* were growing in only one third of revisited sites, while *C. chordorrhiza*, *C. pauciflora* and *C. loliacea* were found in most of the pre1970's sites. Thus, fortunately the distribution situation is not as critical for some of the species studied as indicated in the database of the Atlas of Estonian Flora. Finding that in most revisited sites the majority of populations of *C. loliacea* were small however might indicate that they were remnants of earlier large populations. During fieldwork *C. chordorrhiza* and *C. pauciflora* were found in many new previously unrecorded localities, but for the wet forest species *C. loliacea* and *C. disperma*, no new localities were found. Species that were better preserved in their old localities (*C. chordorrhiza*, *C. pauciflora* and *C. loliacea*) had higher variation of vegetative reproduction (rhizome length and branching) indicating more extensive plasticity in response to changing environmental conditions.

Laboratory germination experiments with differing light conditions demonstrated that under a canopy at a low R/FR ratio, germination of *C. loliacea* seeds is significantly reduced indicating first, *C. loliacea* is a strict spring germinator and second, the species is unable to reproduce under dense shrub and herb layer. The study also showed that seeds of *C. loliacea* are able to develop a seed bank in the soil which may help species persistence when habitat environmental conditions become unfavourable.

The data from the removal experiment clearly showed that survival, growth and flowering of *C. loliacea* are reduced by neighbouring plants. It indicates the species is not able to compete with dense vegetation overgrowing its habitat after disturbance.

The results of the present study also show that a lack of seeds is not a reason for decline in *C. loliacea* populations since most tussocks flowered and set seeds successfully. *C. loliacea* however suffers from unfavourable conditions for germination (lack of light) and seedling survival (competition by neighbours). Thus, the changing quality of habitats and negative effects of draining and cutting of trees may explain the decrease in distribution of *C. loliacea*.

Carex magellanica subsp. *irrigua* and *C. loliacea* both showed low genetic diversity at the allozyme level and indicated the presence of self-pollination. However, *C. magellanica* subsp. *irrigua* had slightly higher genetic diversity than *C. loliacea*. The different inflorescence type could cause the different levels of allozyme diversity in these two sedge species. *Carex loliacea* has male and female flowers on the same spike, while *C. magellanica* subsp. *irrigua* has male and female flowers on different spikes. Level of heterozygosity was low in both species. In the 17 populations of *C. loliacea* investigated, few heterozygous individuals were found among the seed progeny in only three largest Estonian populations. *Carex magellanica* subsp. *irrigua* had heterozygous individuals only in Fennoscandian populations, Estonian and Alaskan populations were totally homozygous.

Carex loliacea and *C. magellanica* subsp. *irrigua* showed similar genetic variation between different geographical districts. According to allozyme differentiation, the Alaskan and Fennoscandian populations were more similar to each other while the Estonian population formed a distinct group in *C. magellanica* subsp. *irrigua* and in the case of *C. loliacea*, Estonian and Polish populations formed a united group.

Results from this study confirm the general view that genetic diversity and population size are often positively correlated, and that small populations frequently have lower genetic diversity than large populations. The populations of *C. loliacea* are often small and widely separated. This causes restricted gene flow among them and may also lead to greater self-pollination or cross-pollination between nearby close genetic relatives within populations thereby decreasing genetic variation and increasing the risk of extinction. As *C. loliacea* is in decline throughout its distribution area, it is necessary to pay more attention to the preservation of habitats of *C. loliacea* and to conservation of natural environmental conditions in Estonia to avoid the species becoming very rare or even extinct. There is also a need to pay close attention to the conservation of *C. magellanica* subsp. *irrigua* in Estonia, which is affected by anthropogenic disturbance. Each population of this species probably represents a set of different autogamous genetic lineages and for conservation it is important these are preserved. Lowered genetic diversity may reduce plant fitness and potential for adaptation to changing environmental conditions and stochasticity. And, rather than focusing on conservation of these species, a more effective way perhaps, is to pay further attention to preserve habitats, which goes hand-in-hand with conservation of these species.

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SUMMARY IN ESTONIAN

VÄHENEVA ARVUKUSEGA TARNALIIKIDE (*Carex*) PALJUNEMISÖKOLOOGIA JA GENEETILINE MITMEKESISUS

Tänapäeva arenevas ning üha enam inimõju all olevas maailmas toimuvad muutused mõjutavad kõigi Maal elavate organismide elu. Mõnele liikidele võivad toimuvad muutused olla isegi soodsad, kuid üha enam kaotavad paljud liigid sobivaid kasvukohti, hääbuvad või surevad välja. Selleks, et säilitada olemasolevat liigirikkust, on oluline tunda potentsiaalsete hävimisohus olevate liikide bioloogiat. Eesti Taimede Levikuatlase (Kukk ja Kull 2005) andmetel on viimase 35 aasta jooksul ligi 40 Eestis kasvavat taimeliiki kaotanud kuni kolm neljandikku oma kasvukohtadest. Käesoleva doktoritöö raames uuriti viie Eestis kõige enam leiukohti kaotanud tarnaliigi levikut tänapäeval. Detailsemalt uuriti lodutarna (*C. loliacea*) seemnete idanemisvõimet erinevates valgustingimustes ning lodutarna ja sagristarna (*C. magellanica* subsp. *irrigua*) geneetilist varieeruvust nii populatsioonide siseselt, populatsioonide vaheliselt kui ka erinevates geograafilistes piirkondades.

Eesti Taimede Levikuatlase andmetel on ajavahemikul 1970–2005 kõige enam leiukohtade arv vähenenud viiel järgneval liigil – alsstarn (*C. chordorrhiza*), õrn tarn (*C. disperma*), sagristarn (*C. magellanica* subsp. *irrigua*), lodutarn (*C. loliacea*) ja õievähene tarn (*C. pauciflora*). Nende liikide peamiselt ajavahemikust 1920–1970 pärit leiukohtade taaskülastamisel selgus, et kõige paremini on oma varasemates leiukohtades säilinud alsstarn ja lodutarn. Rohkem kui pooltest varasematest leiukohtadest õnnestus leida ka õievähene tarn. Õrn tarn ja sagristarn olid säilinud varasematest leiukohtadest vähem kui pooltes. Uuritavate liikide klonaalsete tunnuste mõõtmise tulemusena selgus, et alsstarnal ja õievähesel tarnal oli risoomi liikuvus ja risoomi tunnuste plastilisus kõige suurem, mis on tõenäoliselt aidanud neil teiste liikidega võrreldes oma olemasolevates leiukohtades paremini ellu jääda. Ka lodutarnal, mis on pigem mätasja kasvuvormiga, oli suur klonaalsete tunnuste plastilisus ning see asjaolu on võimaldanud liigil olemasolevates kasvukohtades mõnevõrra paremini säilida. Samas olid säilinud lodutarna populatsioonid reeglina väikesearvulised. Võib eeldada, et need on jäänukpopulatsioonid kunagistest suurtest populatsioonidest.

Võrdlev idandamiskatse looduslikes tingimustes ja aias näitas, et tarna-seemnete idanemine on keskkonnatingimuste suhtes väga tundlik. Aias, kus keskkonna tingimused olid stabiilsemad, oli kõikidel liikidel (v.a. õievähene tarn) idanevus parem. Laboratooriumis erinevates valgustingimustes läbi viidud idandamiskatse tõestas, et lodutarna seemnete idanevus varjutingimustes on pärsitud ning edukaks idanemiseks vajab see liik häid valgustingimusi. Seega – kui näiteks kasvukohas toimuvad muutused, mis viivad rohustu tihenemiseni, on sellistes tingimustes lodutarna seemnete idanemine raskendatud. Valguse vajadust kinnitavad ka vaatlused kasvukohtades, kus lodutarna taimed kasvavad metsas reeglina hõredama võraga kohtades või üksikute puude ümberkukkumise tagajärjel tekkinud valguslaikudes.

Konkurentsikatse lodutarna idanditega näitas, et liik pole konkurentsivõimeline teiste liikidega ning isegi kui seemnetel õnnestub idaneda, siis ümbritsevas tihedas taimestikust ei suuda lodutarna seemikud ellu jääda. Vanu kasvukohti taaskülastades selgus, et lodutarna taimed tunnevad ennast sageli hästi lageraie aladel, kus esimestel raiejärgsetel aastatel on rikkalikult valgust ja niiskust ning puuduvad konkurendid. Raiesmike hilisemal rohustumisel ja võsastumisel kaovad lodutarna jaoks soodsad tingimused ning liik kaob nendest kasvukohtadest. Tänu liigi pikaealisusele võivad lodutarna täiskasvanud isendid püsida olemasolevas kasvukohas mõnda aega ka pärast kasvukoha tingimuste halvenemist, kuid seemned pole võimelised seal enam idanema ega seemikud tihedas konkurentsis ellu jääma.

Lodutarna ja sagristarna geneetilist mitmekesisust ja varieeruvust uuriti Eesti, Soome, Rootsi, Norra, Poola ja Alaska populatsioonides isoensüümanalüüside abil. Lodutarn, mis on vähenev või haruldane kogu oma levikuareali piires, näitas äärmiselt madalat geneetilist mitmekesisust ($H_c = 0,027$) kogu liigi piires. Isoensüümtunnuste alusel täheldati mõningast geograafilist erinevust populatsioonide vahel, mis võib kajastada liigi jääja järgseid levimissuundi. Nei (1978) geneetiliste distantside põhjal moodustasid omavahel sarnase grupi Alaska ja Fennoskandia populatsioonid ning Eesti ja Poola populatsioonid.

Sagristarn on Eestis vähenev ning Kesk-Euroopas haruldane või hävinud liik, samas on ta tavaline areala põhjaosas – Skandinaavias ja Alaskal. Sagristarna liigisisene geneetiline mitmekesisus ($H_c = 0,073$) oli küll suurem kui lodutarnal, kuid siiski väiksem keskmisest haruldaste liikide

näitajast ($H_c = 0,142$) (Cole 2003). Geneetiline mitmekesisus oli kõige madalam Eesti populatsioonides; siin olid ka kõige väiksemad populatsioonid. Sagristarna liigisisese allosüümse varieeruvuse geograafiline jaotus oli sarnane lodutarnale – ka sagristarna puhul moodustasid Fennoskandia ja Alaska populatsioonid Eesti populatsioonidest eraldiseisva grupi. Lodutarna ja sagristarna geneetilise mitmekesisuse uurimine kinnitas üldist seisukohta, et väikesed ja fragmenteerunud populatsioonid on geneetiliselt vaesestunud. Seetõttu on vähenevate liikide puhul oluline hoida säilinud suuremaid populatsioone, et tagada liigi geneetiline mitmekesisus ning seeläbi ka võime paremini kohaneda muutuvate elupaigatingimustega.

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I

PUBLICATIONS

HABITAT LOSS AND REPRODUCTION BIOLOGY AS RELATED TO DECLINE IN RARE *Carex* SPECIES

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Abstract

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Five *Carex* species were studied that have declined in Estonia by a factor of four or more since the 1970s. These species grow in open sphagnum swamps (*Carex chordorrhiza*, *C. pauciflora*) and in swamp forests (*C. disperma*, *C. loliacea* and *C. irrigua*). Old localities dated since 1921 on the basis of the database of the Atlas of Estonian Vascular Plants, and herbarium records were revisited in 2001–2002. For each species reproductive traits (rhizome increment, seed production, germination rate) were measured. The rate of refinding varied, being higher for *Carex loliacea*, *C. chordorrhiza*, and *C. pauciflora*, and lower for *C. disperma* and *C. irrigua*. The analysis confirms a hypothesis that the change in the distribution is correlated to differences in vegetative and sexual reproduction traits of the species. Higher vegetative plasticity, more extensive clonal growth, and higher rate of seed germination may in some extent buffer the influence of habitat destruction.

Key words: *Carex chordorrhiza*, *Carex disperma*, *Carex irrigua*, *Carex loliacea*, *Carex pauciflora*, clonal growth, species decline, plasticity, reproductive traits

Introduction

Survival and expansion of populations depend upon the preservation of habitats and on the reproductive capacity of species. Loss of biodiversity, including local decrease in plant species and an increasing number of rare species, has become an important issue during recent decades. There are two principal types of rarity which both need attention: (1) formerly common species that have recently reduced to small population size, and (2) historically rare species (Brigham, 2003). The loss of habitats through intensive land use has caused the diminishing or even extinction of more sensitive plant species (Garve, Kiffe, 1997; Lienert et al., 2002). Negative trends in biological diversity are characteristic even for the relatively well preserved Estonian flora.

Trends and changes in species frequency can be recorded using grid-square databases of flora mapping (e.g., Hodgson, 1986; Kull et al., 2002). Comparison of data from before and after 1970s in the Atlas of Estonian Vascular Plants (Kull et al., 2002) revealed that several species had disappeared from the localities where they occurred before the 1970s. During the last 30 years 40 vascular plant species (from 1441) have lost three quarters of their localities in Estonia (area cca 45 000 km²). Among them are several species of the genus *Carex*. In Estonia the genus *Carex* is represented by 70 species (Kukk, 1999) out of the 2000 species occurring worldwide (Bernard, 1990). Ten of them belong to the Estonian Red Book (Lilleleht, 1998). The genus has very broad ecological amplitude, growing in dry as well as in wet habitats. In this study five *Carex* species are studied that were more widespread before the 1970s, but are less recorded afterwards. The occurrence of these species has fallen by a factor of four or more. These species grow in sphagnum swamps (*Carex chordorrhiza*, *C. pauciflora*) and in wet woods like swamp forests (*C. disperma*, *C. loliacea* and *C. irrigua*). While wet forests are among the most rapidly degrading vegetation types in most of Europe, many of such areas have still largely survived in the eastern and north-eastern regions (Preditis, 1999). De Bruijn (1980) has observed similar decrease of many *Carex* species in the Netherlands, where since 1950 a great number of *Carex* species had become extremely rare, mostly because of habitat destruction.

The rate of vegetative and sexual reproduction is of great importance for the long-term survival of species. Vegetative reproduction secures the survival of the population in stable conditions. Sexual reproduction has an advantage in fast changing conditions (Cook, 1985). Even though sedges flower and fruit quite successfully, sexual reproduction is not so common and vegetative reproduction is predominant in the genus (Jonsson et al., 1996).

The aim of this study was to clarify the causes of the apparent reduction of five sedge species in Estonia. For this purpose, the old localities of these species were revisited and the reproductive traits which could be responsible for the poor spreading of these species were identified.

Material and methods

The study was based on the database of the Atlas of Estonian Vascular Plants and on herbarium records. The compilation of the database of the Atlas of Estonian Vascular Plants started in the early 1970s. Data collection, both in the field and from herbaria, was done by a number of persons; reliable data from literature and from different projects were also included. The Central European grid system (6°N x 10°E) is applied. The size of the grid-squares in Estonia is about 100 km² (11.1 x 9.45 km) and the database includes the lists of the flora for 494 grid-squares.

The herbarium records used in the present study to find the old localities of the studied species are preserved at the Institute of Agricultural and Environmental Sciences in Tartu and at the University of Tartu. It was impossible to use all material because many site descriptions were too inaccurate to permit revisits. Old localities recorded for the checked herbarium specimens were dated from the period between 1921 and 2000 (77% of the sites noted for the study species were recorded before 1971); altogether 81 sites were revisited in 2001–2002 (Table 1). The recorded sites were based on flora grid-squares.

Table 1. Data of the occurrence of the studied *Carex* species in atlas grid cells in different time periods.

Species	Found in grid cells of the distribution database of the Atlas					
	1921–1970	1971–2000	2001–2002			
			revisited	refound	[%]	new
<i>Carex chordorrhiza</i>	76	26	19	15	79	14
<i>Carex pauciflora</i>	81	30	12	7	58	10
<i>Carex irrigua</i>	65	21	13	3	23	1
<i>Carex disperma</i>	63	9	15	5	33	0
<i>Carex loliacea</i>	63	20	22	20	91	0

Species

Five *Carex* species were studied. From these, *Carex chordorrhiza* L.f., *C. pauciflora* Lightf., and *C. irrigua* (Wahlenb.) Sm. ex Hoppe are boreal-montane circumpolar species; *C. disperma* Dewey and *C. loliacea* L. are boreal circumpolar species (Hultén, Fries, 1986). These species are growing in sphagnum bogs, quagmires and swamp forests.

Vegetative and sexual reproduction

The parameters of clonal growth (vegetative reproduction) of the species were measured from the excavated rhizome systems. Due to the rarity of species, the number of excavated plants had to be small. The rhizome length of a ramet, number of branches on one rhizome and order of the branch were measured on fragments of 5 clones of *C. chordorrhiza*, 20 clones of *C. pauciflora*, on 6 clones of *C. irrigua*, and on 2 clones of *C. loliacea*. The rhizome length was measured from the branching point up to the base of the shoot. The order of the branch was counted starting from the oldest branch. *C. disperma* was not dug out owing to the scarcity of the plants. Due to the rarity of species the number of destructive measurements was kept close to critical minimum. Also, the availability of local seeds was low.

Sexual reproduction was studied using germination experiments both in natural conditions and in the garden. In the first case thin nylon (5x5 cm) bags with 10 seeds in each were planted under a 1 cm moss layer in Pupastvere mire in September 2001. *C. pauciflora*, *C. irrigua*, and *C. disperma* were studied using 5 bags, *C. loliacea* 8 bags, and *C. chordorrhiza* 11 bags. Germination was checked three times in summer 2002. In the garden experiment in September 2001, the seeds were sown in boxes which were placed in a half shade, and were watered to avoid drying through.

Data on seed size and the number of seeds per shoot, typical of the species, were taken from floras and key-books (Eichwald, 1966; Hämet-Ahti et al., 1998; Krall et al., 1999).

For statistical analysis a logistic regression method with SAS GENMOD procedure was applied. The model was overdispersed and so the scale parameter was estimated by the square roots of Pearson's Chi-Square divided by the degrees of freedom. Spearman Correlation Coefficients were used for analysing rhizome length and order of branches.

Results

Vegetative reproduction

The largest mean rhizome length (12.9 cm) was recorded for *C. chordorrhiza* (Table 2). *C. chordorrhiza* and *C. loliacea* had the highest variation coefficient for rhizome length (126% and 102%, respectively) and for number of rhizome branches (185% and 116%, respectively).

T a b l e 3. Germination rate in natural conditions and in a pot experiment (in the brackets the number of seeds used).

Species	Germination in natural conditions [%]	Germination in pot experiment [%]
<i>Carex chordorrhiza</i>	17 (110)	33 (21)
<i>Carex pauciflora</i>	68 (50)	39 (18)
<i>Carex irrigua</i>	0 (50)	20 (20)
<i>Carex disperma</i>	20 (50)	45 (20)
<i>Carex loliacea</i>	4 (80)	60 (20)

T a b l e 4. Two pairs of characters that had significant effect on refinding of old sites.

Characters	p
% of seeds germinated in garden	< 0.0001
Var. coef. of vegetative mobility	0.0017
% of seeds germinated in garden	< 0.0001
Var. coef. of branching	< 0.0001

T a b l e 5. The relationship between species characters and existence of new growing sites (significant values in bold).

Characters	p
% of seeds germinated in natural conditions	0.3739
% of seeds germinated in garden	0.5994
Number of seeds per shoot	0.2002
Seed size in mm*	0.3631
Vegetative mobility	0.1091
Var. coef.	0.1557
Branching	< 0.0001
Var. coef.	0.3141
Order	0.0031
Var. coef.	0.0948

much by the forest cutting but are rather affected by the groundwater level. In intensively drained localities these species failed. *C. pauciflora* and *C. chordorrhiza* were found in the less destroyed sphagnum bogs. Habitats of *C. irrigua* varied from the sphagnum swamp to the transitional mire and the swamp forest.

Neither of characters analysed (% of seeds germinated in natural conditions, % of seeds germinated in garden, number of seeds per shoot, seed size in mm, vegetative mobility and its var. coef., branching and its var. coef., order and its var. coef.) had significant effect on refinding the old sites. However, when we put into the model characters with smaller p-value in pairs, two of them showed significance (Table 4).

For new sites factors giving significant effect were branching of a rhizome and order of rhizome, both negative (Table 5).

Discussion

Vegetative reproduction

Analysis of the rhizome systems showed that the variation of the mobility of plants was highest for *C. chordorrhiza*, slightly lower for *C. loliacea* and *C. pauciflora* and lowest for *C. irrigua* (Table 2) (rhizomes of *C. disperma* were not available for the study). Mobility of a species allows it to relocate in the locality in search for better conditions for its daughter ramets (Hutchings, Bradbury, 1986). That could be one reason why the first two mentioned species were more often refound in old localities. For all species, there occurred negative correlation between the length of the rhizome and the age of the branch (order of the branch). It means that every next branch was shorter than the previous one, being also less branched. This may be caused by two factors: (1) either longer branches have longer life-span while shorter branches die before they can branch (2) or as senescence of the clone is progressing, every next branch is shorter until the clone dies after some time.

The higher is the variability of the rhizome parameters, the more flexible is the response of the species to environmental conditions. This idea is supported by the occurrence of *C. chordorrhiza*, *C. pauciflora* and *C. loliacea* (Table 1) for which the percentage of refound sites was highest, indicating that the plants can persist at their sites for several decades. The more branches a plant rhizome forms, the better survival and vegetative reproduction are guaranteed (Jónsdóttir, Watson, 1997). Among studied species the highest mean number of branches was for *C. loliacea* and *C. irrigua* (1.4 and 1.34, respectively). The high number of branches in *C. irrigua* did not guarantee its persistence at the old sites nor the spread to new localities. However, *C. loliacea* was found at most old sites (91%). The variability of branching was the highest in *C. chordorrhiza* and in *C. loliacea*, which shows the plasticity of these species as well as their higher survival rate. These species were also persistent at their old sites.

Comparing parameters like rhizome length and number of branches among different species with their refound percentages we can note, that the lower are the plasticity (variation coefficient) of mobility and branching intensity, the higher is the decrease in a species. Species with the higher plasticity of the rhizome parameters are more persistent in their localities.

Generative reproduction

Some species are more dependent on vegetative reproduction, while others depend more on sexual reproduction. There exists a trade-off between the success of sexual reproduction and vegetative mobility (Silvertown, Doust, 1993). Species with vigorous sexual reproduction often form shorter rhizome increments. The more a species produces seeds and the better they germinate, the higher is the success of generative reproduction and the ability to find new localities (Eckert, 2002). According to the literature *C. chordorrhiza* reproduces mainly vegetatively, whereas *C. pauciflora* and *C. loliacea* rely more on seed production (Novikov,

1980; Novikov, Abramova, 1980; Novikov, Abramova, 1980b). However, during this study no seedlings of the studied *Carex* species were found in natural habitats. Comparing the size and the number of seeds in *C. pauciflora* and *C. loliacea*, it could be hypothesized that *C. loliacea*, which has more and smaller seeds, has a wider dispersal capacity and spreads more easily into new localities than *C. pauciflora*. However, this was not the case in our study. The seeds of *C. pauciflora* germinated on average better than the seeds of *C. loliacea* and the species was discovered at many new sites, while *C. loliacea* was found in any new locality.

Contrary to the hypothesis that the species which reproduce mainly by seed production have short rhizomes, the rhizomes of *C. pauciflora* were up to 35 cm long. Among studied species only *C. chordorrhiza* had still longer rhizomes.

The size of *C. irrigua* and *C. chordorrhiza* seeds was about the same (3–4 mm), but their germination success and ability to spread seeds to distant sites were different. The seeds of *C. irrigua* did not germinate at all in the nature experiment and the species was refound at only a few old and one new site. Both factors, poor germination and loss of habitats, probably play a role here. Novikov, Abramova (1980b) report that seeds of *C. chordorrhiza* did not germinate in a laboratory experiment. In the present study, the seeds of this species did germinate as well in the garden experiment as in natural conditions (but not in very high rate). It can be stated that *C. chordorrhiza* is able to reproduce vegetatively at existing sites and spread generatively into new ones. In Scotland recent studies have shown that the species may have highly specific habitat requirements that include moderately reducing hydrosol conditions and near-constant shallow inundation, both producing intermediate levels of stress (Kennedy, Murphy, 2003).

The seed size of *C. loliacea* and *C. disperma* was the smallest (2–3 mm) among the studied species. Smaller seeds should be better transported and reach new sites. Yet not a single new site was discovered for these species. *C. loliacea* was found at most of old sites (91%), while *C. disperma* was encountered at only 33% of the checked old localities. Such a difference is interesting as these species grow in similar habitats and are even morphologically close. It may be suggested that the reason for not finding them in possible new localities was low germination rate (*C. loliacea*), small seed number (*C. disperma*) and loss of the swamp forest area.

Analysis of the refinding of the studied species and the main characters of their reproductive success (both generative and vegetative) showed that variation or plasticity of mobility and branching as well as germination ability are important for species in keeping to their localities. The reason why the germination experiment in natural conditions did not reveal any significant effect may be that the summer of 2002 was very dry and the seeds, although buried in a bank of a ditch, had dried through. For getting more valuable data it is necessary to test seed germination under more different conditions as Schütz, Rave (2003) stressed. Rarity of species and scarcity of their seeds may complicate these attempts.

For conservation purposes, it is essential to take into account that species requiring similar habitats may decline at different rates, owing to various biological reasons. The loss of swamp forests, especially their draining, has affected *C. disperma* rather than *C. loliacea*.

Persistence and spreading

The rate of refinding the studied species in their old localities varied from species to species. *C. disperma* and *C. irrigua* were seldom recorded from revisited sites (33% and 23%, respectively) while *C. chordorrhiza*, *C. pauciflora* and *C. loliacea* were still growing at most of the old sites (Table 1). All these species require wet habitats for growth. *C. chordorrhiza* and *C. pauciflora* grow rather in open sphagnum swamps than in swamp forests and paludified forests as do the other studied species. The use of intensive methods in forestry have led to large-scale drainage and logging in Estonian forests in the 20th century. However, the total area of forested land has increased, while the area under swamp forests has decreased from 492 000 ha in the 1960s (Laasimer, 1965) to 182 000 ha by the end of the 20th century (Viilma et al., 2001). *C. loliacea* and *C. disperma* were often encountered in wet clear-cut areas, which means that their habitats are not threatened so much by the forest cutting but are rather affected by the groundwater level. In the intensively drained localities these species failed. For a short period tree-cutting may even improve the light and moisture conditions for *C. loliacea* and *C. disperma* as long as the clear-cut area will grow over. However, *C. loliacea* was found in almost all revisited localities. The populations were as a rule quite small, consisting of 1 to 10 tussocks. They could be relicts from larger populations existing formerly. The only large and viable population was found in Järvselja primeval forest, where the influence of human activity is minimal.

The area of bogs and transitional mires, most common habitats of *C. chordorrhiza* and *C. pauciflora*, has decreased slightly less than the area of wet forests. Habitat loss is one of the main factor that threatens rare species, but species biology – particularly reproductive biology – should also be taken into account.

Translated by the authors

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Kull T., Kull T.: Úbytok biotopov a reprodukčná biológia týkajúce sa zriedkavých druhov *Carex*.

Skúmali sme päť druhov *Carex*, ktorých počet od sedemdesiatych rokov minulého storočia v Estónsku klesol štvoralebo viacnásobne. Tieto druhy voľne rastú v otvorených rašeliníkových močariskách (*Carex chordorrhiza*, *C. pauciflora*) a močaristých lesoch (*C. disperma*, *C. loliacea* a *C. irrigua*). Na základe databázy Atlasu cievnatých rastlín Estónska sa staré stanovišťa datujú od r. 1921 a herbárne záznamy boli znova použité v rokoch 2001–2002. U každého druhu sme zmerali reprodukčné znaky (rhizomný prírastok, produkciu semien, rýchlosť klíčenia). Miera opätovného výskytu bola rozdielna – vyššia u *Carex loliacea*, *C. chordorrhiza* a *C. pauciflora* a nižšia u *C. irrigua*. Anlyza potvrdila hypotézu, že zmeny v distribúcii korelujú s rozdielmi v znakoch vegetatívnej a sexuálnej reprodukcie druhov. Vyššia vegetatívna plasticita, extenzívnejší klonálny rast a vyššia miera klíčenia semien do istej miery môžu tlmíť vplyv na deštrukciu biotopu.



Reduced light availability and increased competition diminish the reproductive success of wet forest sedge *Carex loliacea* L.

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ABSTRACT

Wet forest ecosystems in temperate region have been heavily drained and logged, often with significant negative consequences for biodiversity in these habitats. Our research focuses on population maintenance mechanisms of a declining wet forest sedge *Carex loliacea*. We studied germination under different light regimes, and seedling survival under different vegetation density using an *in situ* removal experiment. For successful germination, seeds of *C. loliacea* need light, while germination in reduced light conditions is depressed. The seeds of *C. loliacea* are able to accumulate a seed bank and exhibit seasonal dormancy cycles.

Survival of seedlings strongly depends on competition with other plant species. Our results imply that changes in habitat conditions (draining, forest cutting) affect the successful generative reproduction of *C. loliacea* primarily via the change in light conditions, which is a strong factor both at the stage of germination and seedling growth. However, adult plants are able to persist in much broader range of habitat conditions without detectable vitality loss.

KEYWORDS *Carex loliacea*, competition, flooding, germination, light condition

Running title: reproductive success of *Carex loliacea*

INTRODUCTION

Fragmentation and decline of natural old forests causes loss of forest biodiversity (Prieditis 1999, Aune *et al.* 2005, Löhmus *et al.* 2005, Whitman & Hagan 2007) and many woodland herbs have become threatened or rare (Whigham 2004). There is a considerable difference between various forest types in the decline of the associated flora (Sammul *et al.* 2008) and species of disturbance sensitive and ecologically complex environments like old forests are especially threatened (De Bruijn 1980). However, in order to fully understand causes of population degradation in declining habitats and to plan protective measures, it is essential to know at what stages of the life cycle a species is most threatened and what is the major limiting factor. Is it a reduced germination (Gough 2006), increased competitive effect of neighbouring species (Cranstone & Valentine 1983) or suboptimal habitat conditions? Various environmental conditions (Isselstein *et al.* 2002, Kotowski & van Diggelen 2004) and neighbour effects (Ryser 1993) may influence germination of seeds and survival of seedlings. It has been shown that forest canopy and understory vegetation can diminish light availability and cause a negative effect on establishment of seedlings of several forest species (Fowler 1988, Kobayashi & Kamitani 2000).

Data from the Atlas of the Estonian Flora (Kukk & Kull 2005) show that the distribution area of many *Carex* species has severely declined during the last decades. About 40% of all *Carex* species in Estonia have lost at least one third of their localities and most of them are sedges of wet habitats. As habitat specialists, sedges can provide an indication of the initial stages of larger processes and warn us of potential further degradation of species diversity. *Carex loliacea* L. is closely associated with old wet forests and it belongs to the indicator species of minerotrophic swamp forests. Throughout its distribution range, it is declining due to cutting and drainage of forests (Gustafsson 1994, Schweitzer & Polakowski 1994, Garve & Giffe 1997, Oldham 1999, Pawlikowski 2001, Hallanora *et al.* 2002, Korpela 2004, Macdonald 2005). During the last 15 years the area of alder fens in Estonia has declined 50 percent (Lõhmus 2004) and the area of all suitable habitats for *C. loliacea* has decreased about 30 percent (Ilomets 2005). However, the species has survived only in one quarter of the former localities (Kull & Kull 2006) indicating that the decline of *C. loliacea* is caused by more than habitat destruction alone. An isozyme study of *C. loliacea* also indicates that the species, being self-pollinated (Kull & Oja 2007) should not be greatly affected by habitat fragmentation.

Carex loliacea a long-lived perennial with its short slender rhizomes and loosely tufted shoots does not spread significantly by clonal growth (Kull & Kull 2006). Although fruit production is relatively high, seedlings recruitment is seldom observed and it has been shown that in many degraded habitats species persistence is largely due to its high longevity (Kull & Kull 2006). Germination and seed dormancy of several *Carex* species have been studied by several authors (Schütz 1997a, b 1998, 2000, Schütz and Rave 2003; Esmaili *et al.* 2009, Liu *et al.* 2009). However, reproduction ecology of *C. loliacea* has been largely unreported. In this study we estimate the germination rate and competitive ability of seedlings of *C. loliacea* in order to evaluate the sensitivity of early stages of life of this plant to habitat deterioration.

Thus, in order to understand the mechanism of *C. loliacea* population dynamics, we test whether (1) germination of *C. loliacea* seeds is light dependant; (2) competition by neighbouring plants influences the survival of seedlings; and (3) habitat conditions such as soil content and canopy openness influence the abundance of *C. loliacea*?

MATERIAL AND METHODS

Habitat study

Habitats of *C. loliacea* were described in 10 populations from Estonia, 11 from Finland and four from Sweden. In each site a 50×50 m square was selected from the most typical part of the habitat. In this square the number of clumps was counted to estimate population density of *C. loliacea*. On the same plot the type of habitat (rich paludified forest, minerotrophic swamp forest or drained peatland) was identified, and the impact of draining (heavy, moderate, light, absent) was estimated. Within the plot one 1m² square was used to estimate the cover of vegetation and moss layer, and number of flowering culms of *C. loliacea*. From the same plot a soil sample was taken and the content of available P and K, and total N, and organic matter in the soil were measured. Soil analyses were conducted in the Laboratory of Plant Biochemistry in the Estonian University of Life Sciences. Light conditions were characterized using the hemispherical photography technique (Anderson 1964) from a digital photo taken above the plot. From each photo an openness value was calculated using WinSCANOPY software (Regent Instruments, Canada).

Seed germination experiment

From 6 populations in Estonia, 11 populations in Finland and one population in Poland seeds of *C. loliacea* were collected for the germination experiment. Due to extremely late summer in 2003, the quantity of unripe seeds collected from Finnish populations was very high and only three populations from Finland could be used in the experiment. Fruits, consisting of nutlet and perigynium (hereafter referred to as seeds), of at least 5 separate clumps (individual genets) of *C. loliacea* were collected in July and August, 2003 and the germination experiment took place the following year. Following visual inspection damaged and unfilled seeds were discarded. The collected seeds were dried and held at approximately 20 °C in the laboratory for 4 months.

Seeds were cold and wet stratified before germination. For wet stratification, seeds were placed in Petri dishes on a 0.7 cm thick wet sand layer covered with filter paper, and covered with another layer of filter paper with 0.3 cm wet sand on it. The Petri dishes were kept refrigerated at 4 °C in darkness for 2 months. For germination, the upper filter paper and sand layer was removed. A fluctuating temperature of 17/28 °C was used to imitate the natural daily temperature variation.

To estimate the effect of light availability on germination, three treatments each with a different light environment were designed. In 'neutral shade' treatment the Petri dishes were covered with grey linen fabric to simulate open habitat conditions. In this treatment seeds were incubated in light (14 h photoperiod at seed level of about 21 $\mu\text{mol m}^{-2}\text{s}^{-1}$, R:FR ratio 1, within the 400-700 nm range). In 'green shade' treatment the Petri dishes were covered with green cotton fabric to simulate conditions occurring under a vegetation canopy (14 h photoperiod of 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at seed level, R:FR ratio 0.25, within the 400-700 nm range). In dark treatment the Petri dishes were wrapped in a double layer of aluminium foil to receive no light. In each treatment there were 50 seeds per Petri dish from every population. Due to scarcity of material, the Polish and two of the Estonian populations had less seeds (20-40) per dish. Altogether 1350 seeds of *C. loliacea* were used in the germination experiment.

Germination was recorded at 2-3 day intervals. Radicle emergence was the criterion of germination. Seed germination in the dark treatment was estimated under dim green light.

After two months seeds that had not germinated were put back into the refrigerator at 4 °C for one month to break the probable dormancy. Thereafter seeds were replaced into the germination environment and seeds from dark treatment were now treated like 'neutral shade'. After two weeks when maximum germination was passed, seeds still not germinating were placed once again for one month into refrigerator and subsequently germinated and counted as described above.

Competition experiment

The competitive impact of neighbouring plants on seedling establishment was tested by means of a removal experiment. The experiment started in May, 2004 and ended in August, 2007. Experimental plots were established at three sites with different moisture conditions and in areas where the species was naturally present: Vägari (VG) - well drained forest, no flooding; Laeva (LV) - old wet forest (Fennoscandian herb-rich forest), extensive flooding in spring; Järvelja (JS) - old moist forest with no drainage and minimum human impact. Järvelja site is largely covered with *Sphagnum* sp. mosses and may flood in wet years. In all sites 12 permanent plots (25x25 cm) were established. Two weeks prior to planting half of the plots (6) in all sites were treated with ROUND UP (glyphosate) herbicide for eliminating the aboveground competition of natural vegetation. Borders of these plots (plots without competition) were cut up to 20 cm depth with a spade to avoid root invasion into

the plots. On the other half of the plots (plots with competition) the vegetation remained intact. Five approximately one month old seedlings with three leaves of *C. loliacea* were planted into each plot.

For the following four years the sites were visited in August and the survival of *C. loliacea* plants, the number of vegetative and generative shoots per clump and the height of shoots were recorded. Seedlings and stolons of other species growing in plots without competition were removed by hand.

To estimate the possible effect of light availability on plant growth hemispherical photographs were taken from above each plot and openness value was calculated as described above.

Data analysis

Statistical analysis was carried out in the program STATISTICA ver 8. General Regression Model with two-directional stepwise selection procedure was used to test for the effect of habitat conditions on abundance of *C. loliacea*.

ANOVA with subsequent Tukey's post-hoc test was applied to investigate the germination of *C. loliacea* seeds in different light treatments.

Using Generalized Linear Model (GLZ) model with binomial error distribution and logit-link function we tested for the influence of competition removal treatment on survival of *C. loliacea* seedlings.

RESULTS

Habitat study

C. loliacea grows in rich paludified forests (16 sites), in minerotrophic swamp forests (7 sites) and in drained peatland forests (2 sites). The variation of light conditions between sites was low having 25% of mean canopy openness in Estonian, 23% in Finnish and 16% in Swedish populations. The content of N, P and K in soil was broadly variable between sites with values 0.08-2.8% for N, 4-135 mg/kg for P and 12-878 mg/kg for K. The species grows in acidic conditions having variable pH values (3.2 - 6.4) in these habitats (Table 2). General Regression Model with stepwise selection of factors proved that habitat characteristics did not have significant effect on abundance of *C. loliacea* in any models. Neither did the habitat factors possess any significant correlation to each other (Table 4).

Seed germination in different light conditions

Two-way factorial ANOVA shows significant difference of germination percentage of seeds between different light treatments ($p = 0.0001$) and countries (geographical ranges) ($p = 0.0002$). Interaction effect between light and country was insignificant. The total germination success was always higher in 'neutral shade' treatment than in treatment types 'green shade' or in 'dark' (Table 3).

Germination started on the seventh day, maximum germination occurred during the following four days and overall, germination lasted about 30 days. After that only a few additional seedlings emerged. The highest germination rate after the first stratification was in the 'neutral shade' treatment where 34% of seeds germinated. In the 'green shade' treatment 10% of seeds germinated and in the 'dark' treatment only 1.6% of seeds germinated (Table 3, Figure 1). The Tukey's HSD Test showed that germination in the 'neutral shade' treatment was significantly higher than germination in the 'green shade' and in the 'dark' treatment.

Comparing the germination of seeds from different populations across all treatments

after the first stratification period we found that seeds from the Polish population had the best germination (31.2%) followed by the two largest Estonian populations with 25.3% and 29.3% of germination (Table 3). In the 'neutral shade' treatment the maximum germination from all studied populations following the first stratification was 65% in the Polish population. Seven populations exhibited germination over 10%. In 'green shade', only three populations reached more than 10% germination and three populations showed no germination. In darkness the germination of seeds was marginal so that only very few seeds from two populations germinated. A possible reason for the low germination of seeds from Finland could have been the large number of unripe seeds in these Finnish populations.

To release primary dormancy, seeds were cold stratified before the germination experiment. The rather high germination temperature induced a secondary dormancy in seeds that had not germinated during the first two weeks. After the second and third stratification the germination rate increased again, so that after the third stratification in 'neutral shade' 64% of seeds and in 'green shade' 34% of seeds had germinated. Seeds from the dark treatment were moved into the 'neutral shade' treatment after the second stratification and the final germination in this treatment was 46% (Figure 1).

Competition experiment

The significant interaction between site and competition treatment ($p = 0.0002$) shows the competition effect in Järvselja and Vägari, while in Laeva, there was low survival of seedlings also in no-competition treatment (Figure 2). The survival of seedlings after the four years of this experiment was significantly higher on plots without competitors than on plots with intact vegetation (Table 5). This result did not apply to the Laeva site where no effect of treatment could be detected. An important abiotic factor in the survival of seedlings on this site was flooding. In spring there were extensive overflows in some study plots and seedlings suffered considerably. Additionally the negative impact of *Populus tremula* leaf litter was observed at the Laeva site. Small *C. loliacea* seedlings suffered from low light under fallen leaves and died.

All measured parameters of plants (height, number of shoots and number of flowers) indicated to preferable conditions on plots without competitors. The height of plants was significantly ($p = 0.01$) higher on plots with removed vegetation. Similarly the number of shoots ($p = 0.0004$) and the number of flowers ($p = 0.0005$) were significantly higher on plots without competitors (Table 5). We could find no effect of light availability (canopy openness) on plants growth on study plots.

DISCUSSION

In northern Europe *C. loliacea* appears to inhabit a variety of wet or moist forest habitats, mostly rich paludified forests and minerotrophic swamp forests. However, habitats in different regions as Estonia, Finland and Sweden did not vary significantly. Studying many different habitat characteristics we could not find any significant relationship between habitat factors and abundance of *C. loliacea*. Such a result is surprising but may follow from the fact that the species just does not grow in unsuitable conditions. More precisely, this indicates that the abundance is not so strictly dependent on habitat conditions, if analysing the sites where the species is present. One explanation may be that at least the persistence of long-lived mature plants is not so strongly affected within the range of suitable abiotic environmental conditions. As the changes in the community take place slowly it enables the mature plants to survive for some period even in heavily altered conditions. That is the reason why we can sometimes find *C. loliacea* even in severely drained areas.

Indeed, in habitats where natural conditions for *C. loliacea* are changed (for example

changes in light or water regime) the adult individuals may endure while the generative reproduction of this species is seriously hindered. This means primarily decreased survival during early life stages. Our study shows that germination of *C. loliacea* strongly depends on the availability of light. The results are comparable with many other studies that have demonstrated the importance of light on seed germination (Hilton 1984, Schütz & Rave 1999, Isselstein *et al.* 2002, Baskin *et al.* 2004). Hence, light could be primary controller of growth and reproduction of woodland herbs (Kotowski & van Diggelen, 2004; Whigham 2004, Jankowska-Blaszczuk & Daws 2007, Mayberry & Elle 2009). *C. loliacea* has rather small seeds (on average about 0.8 mg, personal obs.) and it has been shown that small-seeded species require more light for germination, irrespective of adult plant light requirements (Jensen 2004, Jankowska-Blaszczuk & Daws 2007). Thus, changes in habitats of *C. loliacea* leading to an increase in canopy density may reduce the success of germination.

Moreover, the successful time for germination of *C. loliacea* seeds in the forest environment is restricted to the short period in spring prior to leaf canopy closure. Our experiment demonstrated that under the canopy at low R:FR ratio ('green shade' treatment) germination is significantly reduced. Schütz (1997a) showed similar results for some other forest sedges in his study. Observations in studied habitats assure the requirement of light of *C. loliacea* as the species is often found growing in gaps of forest canopy caused by tree fall.

The results from comparison of three stratification periods in the present study also confirm that *C. loliacea* is a strict spring germinator. The first stratification period released seeds from primary dormancy. For some seeds a high germination temperature induces secondary dormancy. A further stratification cycle released some of these seeds from this secondary dormancy (Figure 1). Even though such behaviour does not guarantee a 100% germination rate, the presence of secondary dormancy enables *C. loliacea* to develop a seed bank in the soil thus providing an additional option for ensuring long-term presence of a population.

The largest populations (500-1000 individuals, one from Poland and two from Estonia) showed higher germination rate than smaller (under 500 individuals) (Table 3). In the recent isozyme study these two Estonian populations belonged to the few polymorphic populations (Kull & Oja 2007). It shows that larger populations with higher genetic diversity have also advantages in reproduction. For effective protection of this declining species it is important to identify and preserve all large populations since their vitality is higher.

Our study shows that neighbouring plants do not just reduce the germination rate, but also decrease the survival rate of seedlings of *C. loliacea*. However, Ryser (1993) and Schmid (1986) found that competition may not be the only explanation for depressed seedling growth. Physical hazards (like frost, drought) and pathogens may reduce seedling establishment even more than competition by neighbouring plants and it is probable that in some cases the presence of neighbouring plants even protects seedlings from extreme abiotic conditions. Still, the data from our removal experiment clearly show that survival, growth and flowering are reduced by neighbouring vegetation. Only in Laeva site the competitive effect was absent. This might however be explained by the flooding experienced at the site every spring and from plots without competitors some seedlings were simply flushed off. At the same time survived individuals there were largest and flowered copiously that verifies the requirement of wet habitat for successful development for *C. loliacea*. Natural habitats of *C. loliacea* frequently are disturbance prone due to flooding. Flooding may erode the forest floor and remove some vegetation, creating gaps for seed germination but similarly the individuals of *C. loliacea* might be the objects of erosion (personal obs.). In Vägari and in Järvselja important competitors were bryophytes. According to Jeschke and Kiehl (2008), the role of bryophytes during the first phases of vascular plant life is of great importance reducing significantly germination and seedling survival. In Järvselja very dense *Sphagnum*

sp. layer covered the study plots resulting in the death of *C. loliacea* seedlings. In Vägari the moss most prevalent on study plots was *Hylocomnium splendens*.

The changes in the water regime, usually due to draining, in habitats of *C. loliacea* may be harmful to species survival through bringing about an increase in vegetation density and competition intensity. Similar effects could be observed in forest clear-cut areas where *C. loliacea* initially takes advantage of improved light availability, germinates well and spreads widely, only to become severely suppressed by shrub cover in later stages of succession. Very often whole populations of *C. loliacea* disappear under the dense cover of shrubs at forest clear-cuts (personal obs.). Mayberry & Elle (2009) found in their study that *Actaea elata* a moist forest understory species similarly to *C. loliacea* prefers canopy gaps as habitats and may have a short-term flourishing in young clear-cut areas.

Ehrlen *et al.* (2006) showed that distribution of forest herbs is mainly limited by the availability of seeds. The results of the present study show that the reason for decline in *C. loliacea* populations is not the lack of seeds. In appropriate conditions the species is able to form seeds already in the second year after germination (personal obs.). Long-lived tussocks of *C. loliacea* flower abundantly and the germination rate could be enough for population maintenance but the species suffers from unfavourable conditions for germination (lack of light) and seedling survival (competition by neighbours). Thus, the changing quality of habitats and negative effects of draining and cutting of trees explain the decrease in generative reproduction of *C. loliacea*. Therefore, the emphasis should be put on the preservation of habitats of *C. loliacea* and on conservation of natural environmental conditions. Further research on longevity of the seed bank of the species would enable to consider the probability of re-emergence in restored habitats.

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Table 1. Geographic coordinates, habitat type, draining, vegetation cover (%), moss layer (%), canopy openness (%) and the approximate abundance of *C. loliacea* study sites in Finland (Fin), Estonia (Est), and Sweden (Swe). PF – rich paludified forest, SF – minerotrophic swamp forest, DP – drained peatland. * – not measured parameters.

Site	Latitude (N)	Longitude (E)	Habitat type	Draining	Canopy openness	Abundance (approx.)	No of culms	Veget. cover	Moss layer
Fin1	66°22'53"	29°18'49"	PF	absent	23.1	1000	*	50	60
Fin2	66°22'58"	29°18'48"	PF	absent	28.0	50	*	20	7
Fin3	66°22'43"	29°19'85"	SF	absent	30.2	100	*	18	55
Fin4	66°21'96"	29°18'99"	PF	light	17.5	10	*	48	90
Fin5	66°20'48"	29°19'79"	SF	absent	14.0	10	41	40	60
Fin6	66°15'67"	29°24'86"	PF	absent	25.4	100	24	20	97
Fin7	66°15'28"	29°25'72"	SF	absent	31.6	100	10	38	95
Fin8	66°26'03"	29°09'37"	PF	absent	19.0	10	13	40	80
Fin9	66°14'38"	29°14'42"	PF	absent	20.1	500	21	27	98
Fin10	66°21'49"	29°20'20"	PF	absent	32.8	100	32	20	97
Fin11	66°21'64"	29°20'57"	PF	absent	15.6	100	12	35	90
Est1	58°02'15"	25°43'45"	DP	moderate	22.9	10	7	50	70
Est2	57°56'79"	25°37'12"	SF	light	17.9	10	3	20	30
Est3	58°16'70"	27°19'30"	SF	absent	15.0	1000	23	30	90
Est4	58°38'54"	24°52'14"	PF	moderate	14.9	100	31	60	30
Est5	58°00'55"	24°36'49"	PF	moderate	31.8	100	120	70	30
Est6	59°09'82"	27°47'99"	SF	moderate	62.9	500	30	50	10
Est7	59°11'32"	27°30'13"	PF	absent	29.8	1	4	50	60
Est8	59°07'12"	27°27'24"	SF	absent	16.8	50	30	40	60
Est9	58°42'33"	26°14'50"	DP	heavy	13.7	50	5	25	97
Est10	58°28'86"	26°28'08"	PF	light	27.3	50	16	30	20
Swe1	64°05'52"	19°05'87"	PF	absent	17.0	500	50	20	95
Swe2	64°00'77"	19°35'63"	PF	absent	19.2	50	30	35	80
Swe3	64°16'58"	19°39'12"	PF	light	12.2	100	30	10	1
Swe4	64°16'13"	19°41'07"	PF	light	18.8	100	8	45	50

Table 2. Mean values of soil parameters in Finland, Estonia and Sweden with standard errors, and minimum and maximum of the values.

	Finland	Estonia	Sweden
No of populations	11	10	4
	Mean (SE)	Mean (SE)	Mean (SE)
	min-max	min-max	min-max
pH (KCl)	5.4 (0.19)	4.7 (0.26)	4.2 (0.25)
	4.0-6.4	3.2-6.1	3.5-4.7
N %	1.95 (0.35)	1.45 (0.32)	1.55 (0.54)
	0.18-2.59	0.23-2.86	0.08-2.52
P (mg/Kg)	80 (17.76)	33 (6.49)	45 (30.68)
	4-158	14-76	9-137
K (mg/Kg)	455 (141.88)	298 (92.93)	339 (105.04)
	12-1723	81-1074	25-452
Organic matter %	64 (6.88)	46 (9.99)	52 (18.11)
	7-80	7-78	2-81

Table 3. Germination of *C. loliacea* seeds in populations from Estonia (Est), Finland (Fin) and Poland (Pol) following the first stratification in three different light treatments. N – number of seeds in each treatment, N_{total} – number of seeds in all treatments, % – percentage of germination.

Population	N	Neutral shade	Green shade	Dark	Total	
		%	%	%	N _{total}	%
Est1	20	45	0	0	60	15
Est2	34	23.6	2.9	0	102	8.8
Est3	50	60	16	0	150	25.3
Est4	50	58	6	0	150	14.7
Est5	50	38	6	0	150	14
Est6	50	42	36	10	150	29.3
Fin9	50	6	2	0	150	2.7
Fin10	50	4	0	0	150	1.3
Fin11	50	4	0	0	150	1.3
Pol	46	65.2	23.9	4	138	31.2

Table 4. Correlations between environmental parameters of the different study sites.

	abun- dance	habitat type	drain- ing	total cover	moss cover	flowering culms	open- ness	pH KCl	N %	P mg/kg	K mg/kg	org. mat- ter %
abundance	1.00	-0.15	-0.13	-0.13	0.14	0.42	0.11	0.21	0.03	0.06	0.08	0.12
habitat type		1.00	0.23	-0.01	0.04	-0.31	-0.11	0.03	0.23	0.14	-0.15	0.18
draining			1.00	0.33	-0.39	-0.12	-0.11	-0.14	-0.23	-0.37	-0.47	-0.43
total cover				1.00	-0.18	0.02	0.13	0.14	-0.47	-0.28	-0.36	-0.50
moss cover					1.00	-0.09	-0.08	-0.08	0.06	-0.05	0.11	0.25
flowering culms						1.00	0.00	0.02	-0.25	-0.07	-0.10	-0.18
openness							1.00	0.27	-0.25	-0.16	-0.22	-0.22
pH KCl								1.00	-0.01	0.17	-0.15	-0.22
N %									1.00	0.58	0.33	0.66
P mg/kg										1.00	0.41	0.69
K mg/kg											1.00	0.54
org. matter %												1.00

Table 5. Average values of plant parameters and survival of *C. loliacea* seedlings in three study sites under competition (yes) and without it (no). Significant difference in between treatments in bold.

Site	Vägari	Laeva	Järvelja	p-values
Competition	yes / no	yes / no	yes / no	
No of shoots per clump	3.7 / 13.7	5.7 / 24	1.2 / 7.4	0,0004
Shoot height (cm)	13 / 17.6	16.3 / 27.3	8.7 / 18.8	0,014
No of flowers per clump	0 / 1.04	0.09 / 4.09	0 / 0.9	0,0005
Survival (%)	23 / 83	37 / 37	20 / 87	<0.0001

Figure 1. Germination (in days) of *C. loliacea* seeds in three different light conditions after three stratification periods (I, II, III). After the second stratification period seeds of dark treatment were moved into ‘neutral shade’ treatment.

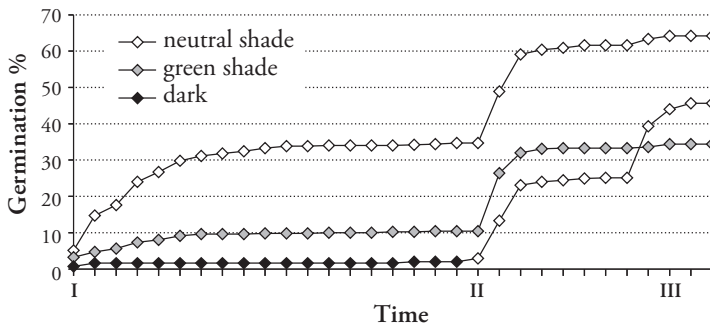
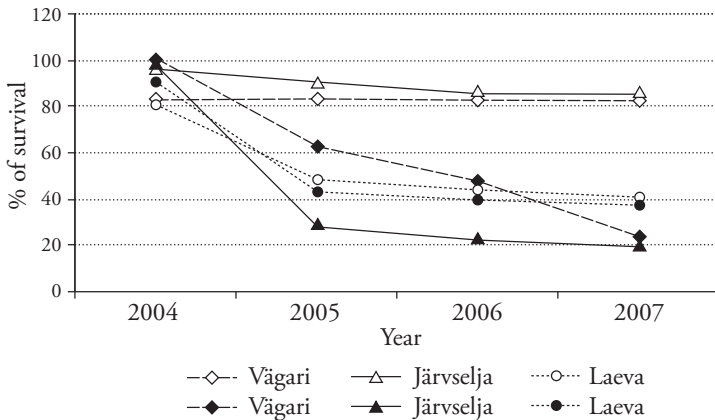


Figure 2. Survival of seedlings in three different sites during four years in the competition experiment. Filled signs – with competitors, open signs – without competitors.





Low allozyme variation in *Carex loliacea* (Cyperaceae), a declining woodland sedge

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The genetic diversity within and among 17 populations of *Carex loliacea* from Estonia, Finland, Sweden, Poland and southcentral Alaska was evaluated by isozyme analysis. An extremely low allozyme variation was found. Fourteen populations were monomorphic at all 18 isozymes. Only three Estonian populations studied showed limited variation of one isozyme, malate dehydrogenase MDH-A, with two allozymes. Almost all allozyme variation was observed as homozygous variants. Of 400 individuals analyzed, only six revealed heterozygous morphs. The high level of inbreeding ($F = 0.91$) clearly shows that *C. loliacea* is a predominantly selfing species. The variation of MDH-A showed some geographic distribution. Finnish, Swedish and Alaskan populations form one group, and Polish and Estonian populations form another. The pattern of intraspecific geographic differentiation may reflect the postglacial recolonization routes.

Key words: allozymes, *Carex loliacea*, decreasing distribution, geographical variation

Introduction

Carex loliacea is a declining species in Estonia and declining, rare or extinct in many other regions in Europe (Hegi 1939, Schweitzer & Polakowski 1994, Garve & Kiffe 1997, Pawlikowski 2001, Hallanaro *et al.* 2002). However, there is a severe lack of knowledge on a population genetic level. This information would be important since within-population genetic variation is needed to adapt to constantly changing and varying environments. Low levels of genetic variation are therefore mostly associated with

high extinction risks (Lande 1988, Young 1996). The principal reason for the decline of *C. loliacea* is presumed to be intensive land draining causing destruction and loss of habitat. The species is associated with rich wet sites, such as swamp forests, spruce mires and paludified forests. Because *C. loliacea* is sensitive to draining, it prefers relatively pristine stands, especially along forest streams or in depressions. It also favours clear-cut areas exploiting the abundant light and moisture.

Carex loliacea belongs to the subgenus *Vignea* (Egorova 1999). It is a boreal-montane

species, with a circumpolar distribution but evidently having wide gaps in the North Atlantic and Bering Sea areas (Hultén & Fries 1986). It is a morphologically uniform herbaceous perennial with short, slender rhizomes, forming sparse tufts and with gynecandrous spikes (Hegi 1939, Eichwald 1966, Hämet-Ahti *et al.* 1998, Krall *et al.* 1999). The fruit production is relatively high. According to Novikov (1980), an average of 40 generative culms and 300–400 seeds can be found on one square meter. However, seedling recruitment is rare for undisturbed vegetation (personal observations). The seeds are dispersed by water and birds (Novikov 1980).

According to the inflorescence type, *Carex* species are generally assumed to be wind-pollinated (Hesse 1980, Tarasevich 1992, Alekseev 1996, Egorova 1999). As wind-pollinated plants, sedges are supposed to be mostly outcrossed. Isoenzyme studies have shown outcrossing for many sedges and selfing for only some species (Ford *et al.* 1991, Whitkus 1992, Hedrén & Prentice 1996). Alekseev (1996) also noticed that both self- and cross-pollination exist within the genus. There are no data on the pollination mode of *C. loliacea*, and no studies of its breeding system have been carried out.

In Estonia, *C. loliacea* is frequent in the northeastern and southeastern parts; in other regions it occurs sporadically at most (Kuusk *et al.* 2003). In Finland, the species is common throughout the country (Hämet-Ahti *et al.* 1998). In Sweden, it is mostly confined to the northern and central parts (Mossberg *et al.* 1992). In Poland, the species is quite rare, restricted to the northern part of the country, which represents the southern margin of its distribution range in Europe (Pawlikowski 2001). In Alaska it grows sporadically in the southeastern three-quarters of the mainland (Tande & Lipkin 2003).

Isoenzyme data had been used successfully to study the genetic diversity in different *Carex* species (Ford *et al.* 1991, Whitkus 1992, Hedrén 1996, Hedrén & Prentice 1996, Ford *et al.* 1998, Jonsson 1998, Stenström *et al.* 2001, Tyler 2002a, 2002b, Tyler *et al.* 2002, Hedrén 2003, Tyler 2003), but there the genetic diversity and geographical structure of *C. loliacea* were not studied. The aims of this work were (1) to investigate the extent of allozyme variation in

C. loliacea, (2) to determine the mating system of the species, (3) to describe the distribution of genetic diversity of *C. loliacea* within and among populations in different regions, and (4) to discuss whether the decline of the species could be associated with its mating system.

Material and methods

Seed collection

Seeds of *C. loliacea* were collected in the summers of 2003–2005 from six populations in Estonia, two in Finland, three in Sweden, three in Poland and three in south-central Alaska (Table 1). The seed collection sites in Estonia were distributed throughout the country. The major habitats of the Estonian populations were swamp forests and paludified forests with *Picea abies* as the dominant tree species, accompanied by *Betula pubescens*, *Alnus glutinosa*, *Pinus sylvestris* and others. The Polish collection sites are situated in the northeastern part of the country. The Polish habitats were *Picea–Alnus* (*Picea–Betula–Alnus*) forests on the margin of raised bogs or fens. In Finland, the collection sites were in the northeastern part of the country, in the Kuusamo commune. The Finnish habitats were usually spruce mires on slopes and along streams. The Swedish collection sites were situated in the Vindeln commune in the central part of the country, and the habitats were similar to those in Finland. In south-central part of Alaska Peninsula *C. loliacea* was found along streams and by wet pathways, accompanied by *Picea mariana* and *P. glauca*.

Most populations of *C. loliacea* studied were small, usually occupying only few square meters. Only the EE1, EE3 and EE4 sites in Estonia and POL3 in Poland were larger, covering each up to 0.5–1.5 km². In Finland and Sweden, the species is rather common but the population size is usually small.

Each seed accession collected consisted of a bulk seed sample from an individual population. Seeds were collected from at least five separate tussocks in each population, except from the Alaskan populations, which consisted of two tussocks each. This study analysed 15–25 seed

progeny of a few mother plants from each population. Seeds collected from mother plants in the wild were formed through pollination from plants comprising a population, and the Wright's statistics allows to quantify the outcrossing rate in each population that is polymorphic for allozymes.

The collected seeds were stored in paper bags in the laboratory at approximately 20 °C for four months. For stratification, seeds were kept wet in darkness in a refrigerator at 4 °C for two months. Germination was carried out in the laboratory at fluctuating day/night temperature of 28/17 °C. Specimens are deposited in the herbarium of the Institute of Agricultural and Environmental Sciences (TAA, Tartu, Estonia).

Isozyme analysis

Eleven enzymes were examined: malate dehydrogenase (MDH, EC 1.1.1.37), shikimate dehydrogenase (SKD, EC 1.1.1.25), aspartate aminotransferase (AAT, EC 2.6.1.1), superoxide dismutase (SOD, EC 1.15.1.1), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglucoisomerase (PGI, EC 5.3.1.9), peroxidase (PRX, EC 1.11.1.7), phosphoglucomutase (PGM, EC 2.7.5.1), alcohol dehydrogenase (ADH, EC 1.1.1.1), leucine aminopeptidase (LAP, EC 3.4.11.1) and esterase (EST, EC 3.1.1.2).

Enzyme extracts were prepared from seedling leaves (about 1–2 months old) by grinding in 0.05 M Tris(hydroxymethyl)aminomethane (Tris)–0.01 M EDTA buffer containing 5 mM cysteine. After adding 20–50 mg of sucrose–Sephadex G200 mixture (4:1) to increase their viscosity, the extracts were subjected to electrophoresis in vertical polyacrylamide gel slabs (120 × 70 × 2 mm). The following four gel-buffer systems modified from Jaaska (1997) and Oja (1999) were applied for different enzymes to attain better band resolution:

Gel 1: 10% acrylamide, 0.2% N,N'-methylene-bis-acrylamide (Bis), 0.25 M Tris, 0.1 M HCl; applied for EST and SOD.

Gel 2: 10% acrylamide, 0.2% Bis, 0.15 M Tris, 0.1 M HCl; applied for LAP, ADH, AAT, SKD, PGI, PGM and 6PGD.

Table 1. Collection sites of seeds of *Carex loliacea* with geographical coordinates. *N* = number of individuals analyzed, *N_{ii}* = number of maternal individuals analysed.

Country	Code	Collection site	Lat. N	Long. E	N	N _{ii}
Poland	POL1	Romincka Forest, close to village Czarnowo Srednie	54°20'00"	22°27'29"	17	5
	POL2	Romincka Forest, "Mechacz Wrieki" nature reserve	54°20'12"	22°26'14"	25	10
	POL3	Romincka Forest, "Zytkiemska Struga" nature reserve	54°21'05"	22°37'29"	25	10
Finland	FIN1	SSO from Oulanka Biol. Station, near to Ylimäinen Hiidenlampi	66°21'48"	29°20'10"	25	5
	FIN2	Kuusamo, Juuma	66°20'48"	29°19'79"	25	5
Sweden	SWE1	Vindeln, Stora Sandsjö	64°05'52"	19°05'87"	25	10
	SWE2	Vindeln, Lappkulliden	64°16'57"	19°39'12"	25	10
	SWE3	Vindeln, Seisberg	64°00'76"	19°35'62"	25	5
Estonia	EE1	Tartumaa, Järvselja	58°16'69"	27°19'30"	25	10
	EE2	Pärnumaa, Urissaare	58°00'54"	24°36'48"	18	5
	EE3	Ida-Virumaa, near to the Poruni river	59°09'81"	27°47'98"	25	10
	EE4	Ida-Virumaa, Mustajõe	59°17'08"	27°53'61"	25	5
	EE5	Ida-Virumaa, Aadamäe	59°04'95"	26°48'93"	25	5
	EE6	Lääne-Virumaa, Suigu	59°09'03"	26°49'25"	25	5
Alaska	AL1	Alaska, 20 km N of Anchorage, near Mirror Lake	61°25'78"	149°24'61"	15	2
	AL2	Alaska, northeast of Anchorage, road to Eklutna Lake,	61°24'90"	149°10'89"	25	2
	AL3	Alaska, Wasilla, Big Lake	61°31'60"	149°51'75"	25	2

Gel 3: 7.5% acrylamide, 0.2% Bis, 0.4 M Tris, 0.1 M HCl; applied for MDH and PRX.

N,N,N',N'-Tetramethylethylenediamine (0.05 ml %) and ammonium persulfate (1 mg %) were added to the gel mixtures to initiate and catalyze their photopolymerization between two daylight fluorescence bulbs for 1 h. The upper cathode was 80 mM glycine with 10 mM Tris. The lower anode buffer was always 0.1 M Tris-acetate with the initial pH about 8.9, and it was used repeatedly as long as the pH remained > 7. Ice-refrigerated electrophoresis was carried out by applying a pulsed current at 15 mA and 20–30 V cm⁻¹ for about 2–2.5 h until the bromphenol blue marker dye reached the gel end. After electrophoresis, the gels were stained for isozymes by applying standard histochemical methods (Wendel & Weeden 1989).

The isozyme results are described at the level of isozyme phenotypes that correspond to respective genotypes. Isozymes encoded by separate loci are designated by capital letters followed by a number reflecting allozymes in the order of decreasing mobility. The allozyme numeration is unified for *C. loliacea* and its related species under study. Heterozygous phenotypes are denoted by a slash separating numbered allozymes, e.g. MDH-A1/2. Genetic interpretation of zymograms is based on the available information on the subunit structure and principles described by Wendel and Weeden (1989). In total 400 individuals of *C. loliacea* from 17 populations were used in the analyses.

Data analysis

To characterise the genetic diversity quantitatively, the following parameters were calculated: the number of alleles per locus (A), the percentage of polymorphic loci (P), Wright's fixation index (F), the observed (H_o) and expected heterozygosity (H_e). The analysis of progeny lines in studies of mating systems in plants is a widely used and accepted approach that is theoretically founded in works of Brown and Allard (1970) and Clegg (1980). We used the Wright's inbreeding coefficient F (Wright 1965) computed from polymorphic allozymes in the progeny grown

from seeds collected in the wild to estimate the extent of selfing in natural populations. An isozyme was considered polymorphic when two or more allozymes were detected, regardless of their frequency. The number of polymorphic populations of *C. loliacea* that could be used for calculations was limited. Most populations proved to be totally genetically homogenous.

Results

The isozyme variation observed among and within populations of *C. loliacea* was very low. Eleven enzymes with 18 isozymes and 19 allozymes were interpreted. Only one (MDH-A) of the 18 isozymes was polymorphic. Except for MDH-A all isozymes displayed complete homozygosity. Isozyme MDH-A showed variation with two distinctly separated allozymes A1 and A2. Isoenzymes of SKD, AAT, PGI, PGM and 6PGD were totally monomorphic in all populations, with homozygous SKD-A3, AAT-C1, PGI-A2, PGM-A1 and 6PGD-A1, respectively. Peroxidase showed three homozygous isozymes, PRX-A1, PRX-D1 and PRX-F1, in all populations. LAP revealed two homozygous isozymes, LAP-A3 and LAP-B1. EST showed two interpretable monomorphic isozymes, EST-A2 and EST-B2. SOD displayed four homozygous isozymes. Isozyme SOD-A was much faster than the remaining three isozymes, SOD-B, SOD-C and SOD-D. ADH showed a clear invariant band for isozyme ADH-A. Additional zones of activity were observed for ADH and MDH, but these were not considered because we were unable to measure them adequately for all individuals.

The mean number of alleles per polymorphic locus was 1.05 and percentage of polymorphic loci was 5.56. Mean observed and expected heterozygosity and Wright's fixation index values in polymorphic populations are given in Table 2. In all polymorphic populations the expected heterozygosity based upon Hardy-Weinberg expectations was much higher than the observed heterozygosity (Table 2), demonstrating the deficiency of heterozygotes and indicating dominance of self-fertilization in these populations. All except the three largest Estonian populations (EE1, EE3 and EE4) were monomorphic at all isozymes.

Population EE3 revealed heterozygous MDH-A1/2 in three individuals out of 25 resulting in a fixation index $F = 0.73$. Population EE4 revealed only one individual with heterozygous MDH-A1/2 ($F = 0.92$). Population EE1 was polymorphic for the same two MDH allozymes, but no heterozygotes were detected, indicating no outcrossing in this population. The distribution of the two allozymes showed some geographic pattern. MDH-A1 was the only variant in the Polish populations and a prevalent morph in Estonian populations. The Finnish, Swedish and Alaskan populations were monomorphic for alternate allozyme A2 (Table 3 and Fig. 1). However, the Alaskan population AL3 showed two individuals heterozygous for A1/2, but no homozygous variant A1, indicating a possible outcrossing with gene flow from neighbouring population with homozygous allozyme A1 not sampled. The geographic distribution of the allozymes is shown in Fig. 1.

Discussion

Levels and patterns of genetic variation in populations of plant species depend on the mating system, population size, seed dispersal etc. The great decrease in population sizes of wild plant species due to ongoing habitat fragmentation leads to smaller and more isolated populations, whose reduced genetic variation makes them more vulnerable (Oostermeijer *et al.* 2003, Godt *et al.* 2005). *Carex loliacea* has small and fragmented habitats and the isoenzyme analysis indicates that the species has extremely low genetic diversity within and among populations. Fourteen populations out of the 17 studied, i.e. 82% of the sites, were monomorphic at all 18 isozymes. Only the three largest Estonian



Fig. 1. Sampling areas of *Carex loliacea* and geographical distribution of MDH enzyme phenotypes: MDH-A1 = grey dot, MDH-A2 = black dots, both MDH-A1 and MDH-A2 = black square.

populations (EE1, EE3 and EE4) displayed low polymorphism of one isozyme, MDH-A, with two allozymes.

Table 2. Genetic variability at the MDH-A isozyme in polymorphic populations EE1, EE3, EE4 of *Carex loliacea*: number of individuals analyzed (N), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F).

Population	N	H_o	H_e	F
EE1	25	0	0.40	1
EE3	25	0.12	0.45	0.73
EE4	25	0.04	0.50	0.92

Table 3. Allele frequencies for MDH-A allozymes in the *Carex loliacea* studied. 18 loci coding for SKD, AAT, PGI, PGM, 6PGD, PRX, LAP, ADH, SOD and EST were totally invariable. The site codes are the same as those in Table 1.

Allele	POL1	POL2	POL3	EE1	EE2	EE3	EE4	EE5	EE6
<i>Mdh</i> -A1	1.000	1.000	1.000	0.720	1.000	0.340	0.540	1.000	1.000
<i>Mdh</i> -A2	0.000	0.000	0.000	0.280	0.000	0.660	0.460	0.000	0.000
	FIN1	FIN2	SWE1	SWE2	SWE3	AL1	AL2	AL3	
<i>Mdh</i> -A1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	
<i>Mdh</i> -A2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.960	

Few authors have found similarly very low allozyme variation in *Carex* species. Waterway (1990) reported extremely low polymorphism and no heterozygotes in any of the populations of *C. gynodynama*, an endemic in the California Floristic province. All individuals sampled of that species were monomorphic for the same allozymes for 16 out of 17 isozymes studied. Only one isozyme, GPI-1, showed any variation. Waterway (1990) supposed that the low level of polymorphism in *C. gynodynama* might be caused by genetic bottlenecks. A low degree of genetic variation was also found in the *C. pachystachya* complex (Whitkus 1992), in which the mean number of alleles per polymorphic locus was 1.2 and only 20% of all loci were polymorphic. Schell and Waterway (1992) found low levels of isozyme heterozygosity and polymorphism both within and between populations of the rare and endemic sedge *C. misera*. They found an average of 1.5 alleles per polymorphic locus and 30% of loci polymorphic for this species. Still, these parameters of the *C. pachystachya* complex and of *C. misera* show a higher allozyme variation than in *C. loliacea*, which has 1.05 alleles per polymorphic locus and only 5.5% of loci polymorphic, despite the fact that *C. loliacea* is not an endemic but has a wide circumpolar distribution. Hamrick and Godt (1996) showed that the extent and distribution of genetic diversity within and among populations are greatly influenced by the mating system. The three Estonian polymorphic populations of *C. loliacea* possessed *F* values ranging from 0.73 to 1, indicating that selfing is highly prevalent within the populations. The capability of *C. loliacea* populations consisting of a single tussock to produce seeds able to germinate (pers. obs.) argues in favour of self-fertilization. In addition, the close proximity of pistillate and staminate flowers and the loosely caespitose growth habit promote selfing in such sedges and may therefore result in a low level of heterozygosity (Ford *et al.* 1998). Ford *et al.* (1998) classified *Carex* species into two groups. Group 1 consists of species with hermaphroditic spikes and caespitose growth. The likelihood of selfing is increased in this group. The species have low intrapopulation variation but high interpopulation and inter-specific variation. Group 2 is characterised by

prevalent rhizomatous growth and widely spaced unisexual spikes. The chance of outcrossing is increased among this group. According to this classification, *C. loliacea* belongs to the group 1.

The principal method of seed dispersal for *C. loliacea*, as for the other wet forest *Carex* species, is by water, e.g. during the spring and autumn overflows or along a stream or a river. The plant may form culms up to 80 cm long and is able to distribute its seeds itself up to this distance. This mode is quite plausible because the populations are often small and composed of compactly spaced, loose tussocks. As *C. loliacea* is a woodland understory species and has during the flowering time very short culms (they elongate later during seed maturation), the potential genetic contact between populations through wind pollination is not likely. According to our observations, *C. loliacea* forms sparse tussocks with very short rhizomes (0.1–6.5 cm), and the possibility for vegetative propagation through the root-cuttings from a parental tussock is very low. Thus, the relatively low genetic diversity in *C. loliacea* could be explained by its fragmented distribution pattern together with predominant selfing.

Bolkovskikh *et al.* (1969) reported the chromosome number for diploid *Carex* species $2n = 16$ or 18 . According to the high chromosome number ($2n = 54$ or 56) published in the literature (Heilborn 1924, Moore & Calder 1964, Novikov 1980), *C. loliacea* is supposed to be a hexaploid. But as the species revealed isozyme electrophoretic patterns typical of a diploid, with no increase in the isozyme number and heterozygosity we may suppose that it is a diploidized polyploid, i. e. functionally diploid. A noteworthy result of our study is that the diploidized polyploid *C. loliacea* revealed an extremely low level of isozyme heterozygosity, which has been found previously in only some sedge species (Waterway 1990, Whitkus 1992), but is characteristic of many highly polyploid homosporous ferns (Haufler & Soltis 1986, Haufler 1987).

The spatial structure of genetic variability has been widely studied on local and geographical scales and was found to be influenced by life form, seed dispersal, geographic range, and breeding system of species (reviewed by Hamrick & Godt 1996). Selfing species show higher allozyme divergence among populations

as opposed to outbreeding species, which have most diversity within populations. Consistent with this general view, the largely selfing *C. loliacea* revealed a distinct geographic differentiation between Polish and Fennoscandian populations with alternate allozymes at the polymorphic MDH-A locus. However, no further differentiation was found between Swedish and Finnish populations.

Evolutionary conservative isozyme loci with limited allelic variation may give plausible evidence for species phylogeographic inferences. For example Cronberg (1998) in his paper about phylogeography of *Sphagnum* spp. used evidence about the geographic distribution of two alleles, *Idh-1A* and *Skd-1B*, for making conclusions about postglacial migration routes of species. The observed pattern of intraspecific geographic differentiation by alternate MDH-A allozymes in *C. loliacea* may also reflect the impact of glaciation in northern Europe. Poland can have been a refugium during the last glaciation from where *C. loliacea* migrated northwards. Northern Poland is today the southernmost area in Europe where climatic conditions are still suitable for the species. Another glacial refugium for northern Europe might have been in the central regions of Russia or Alaska that remained unglaciated during the last Ice Age (Velichko *et al.* 1997). Thus, *C. loliacea* could have migrated to Estonia from two different sources: from the south (Poland) and from the east (Russia), but to Fennoscandia most probably from Russia, because MDH-A1 characteristic of Poland populations was not found in the Fennoscandian populations. Further investigations of local populations in different geographical regions, especially from Russia, would be necessary to elucidate the postglacial phylogeographic history of *C. loliacea*.

Several explanations can be supposed to explain the low genetic diversity of *C. loliacea*. The reduced genetic diversity in small populations may have resulted from inbreeding, genetic drift or bottlenecks (Lande 1988, Young *et al.* 1996). Due to active draining of wet forests, *C. loliacea* suffers from fragmentation of populations and loss of habitat. The populations of *C. loliacea* are often small and widely separated. This results in restricted gene flow among them and may lead to higher probability of self-pollination

or cross-pollination between nearby close genetic relatives within populations, decreasing genetic variation and facing a high risk of extinction. Thus, our data agree with the general view that genetic diversity and population size are often positively correlated, and that small populations frequently have lower genetic diversity than large populations (Brigham 2003, Godt *et al.* 2005). The small fragmented populations in Estonia appeared totally monomorphic at all isozyme loci, whereas larger northeastern and southeastern Estonian populations revealed diallelic polymorphism of MDH-A, indicating the possible importance of the population size. Some other *Carex* species with restricted and fragmented distribution have also found to display low genetic diversity (Hedré 1997, Ford *et al.* 1998). Hooftman *et al.* (2004) showed that habitat fragmentation not only affects the rare species in an ecosystem, but also reduces the survival probabilities of the common species. As *C. loliacea* is in decline throughout its distribution area, it would be beneficial to pay more attention to preserving its natural populations to avoid it becoming very rare or even extinct.

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Allozyme Diversity and Geographic Variation among Populations of the Locally Endangered Taxon *Carex magellanica* subsp. *irrigua* (Cyperaceae)

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Abstract *Carex magellanica* subsp. *irrigua* is a wet habitat taxon that is extinct or declining in the Baltic States and Central Europe, but still quite common in northern areas, in Fennoscandia and Alaska. We investigated the extent of genetic variation within and among populations and geographic regions of this subspecies. Isozyme electrophoresis in polyacrylamide gels was applied to characterize genetic diversity with allozymes as genetic markers. Of the nine putative isozyme loci assessed, five (56%) were found to be polymorphic. The genetic diversity in small and fragmented Estonian populations was lower ($H_e=0.034$) than in larger Fennoscandian and Alaskan populations (average $H_e=0.082$). All standard genetic parameters (A_e , H_o , H_e , P , F_{is} , t) showed the lowest values in Estonian populations. The heterozygosity level in Fennoscandian populations was low ($H_o=0.01$), whereas no heterozygotes were found in Estonian and Alaskan populations. High F_{is} values indicate that *C. magellanica* subsp. *irrigua* is predominantly inbreeding. The main reason for its decline in Estonia is the destruction of suitable habitats. More attention to the protection of Estonian habitats is needed to maintain genetic diversity and stop further decline of this taxon.

Keywords Allozyme variation · *Carex* · Declining taxon · Geographic differentiation · Population genetic structure · Small populations

Introduction

Our knowledge about the relationships between genetic variation, population size and fitness has constantly broadened. Many studies have investigated rare vs common

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species and the effect of population fragmentation on genetic diversity (Cole 2003; Fu and Dane 2003; Leimu and Mutikainen 2005). It has been verified that small and fragmented plant populations suffer from reduction in genetic variation (Durka 1999; Paschke et al. 2002; Mateu-Andres 2004; Šingliarová et al. 2008). In such populations, low genetic variation is expected to be associated with low plant fitness. At the same time, recent studies revealed many exceptional cases of high genetic variation in threatened or geographically restricted species (Neel and Ellstrand 2001; Eckert et al. 2008).

Carex magellanica subsp. *irrigua* (Wahlenb.) Hiitonen is a boreal-montane taxon occurring in the Northern Hemisphere (Hultén and Fries 1986). The other subspecies, *C. magellanica* Lam. subsp. *magellanica*, occurs only in subantarctic regions of South America. The difference between the two subspecies is subtle, being based on different allocation of male and female flowers in the terminal spike (Egorova 1999). Heide (2004) showed that such characters are often induced by environmental conditions and the existence of real subspecies may be questionable.

Carex magellanica subsp. *irrigua* is rare and declining in the Baltic States and in Central Europe, belonging to the Red Data Book List of many countries, but rather common throughout northern Europe (Mossberg et al. 1992; Hämet-Ahti et al. 1998) and North America (USDA, NRCS 2008). In Estonia it is classified as near threatened (NT) and is legally protected as a rare and rapidly declining taxon there (Kukk and Kull 2005). *Carex magellanica* subsp. *irrigua* grows in wetlands. Many wetland species have become vulnerable due to the active human impact. Drainage and forest cutting have induced serious changes in habitats and have degraded their quality. The populations of *C. magellanica* subsp. *irrigua* in Estonia are fragmented, and the amount of suitable habitats for this taxon has declined about 50 percent during the last 40 years (Kukk and Kull 2005), presumably because of extensive drainage. This taxon is sensitive to competition from other species (Cranstone and Valentine 1983), so any activity that alters the hydrology and species composition of sites could affect its habitat quality and cause a decline in abundance. In Latvia, for example, observations have shown that many wetland species, including *C. magellanica* subsp. *irrigua*, rapidly disappear or decline because of drainage (Prieditis 1999). Also in Lithuania the taxon is rare with very small populations that are strongly threatened by drainage (Rašomavičius 2007).

Vollan et al. (2006) studied the geographic distribution of genetic variation of *C. magellanica* from the Southern and Northern Hemisphere using AFLPs. Although they found no hemisphere-specific bands for the species, the populations from the Southern and Northern Hemisphere were genetically well separated. They found that 23.4% of AFLP loci were polymorphic for the species.

Allozymes are reliable and useful markers for studying population genetic structure (Prentice et al. 2006; Oja and Paal 2007; Hedrén 2008). A recent comparative study (Conte et al. 2008) showed that despite the lower polymorphism of allozymes, all estimated values of genetic parameters showed a high congruence between microsatellites and allozymes. The present work applies enzyme electrophoresis to study allozyme variation within and among 22 populations of *C. magellanica* subsp. *irrigua* in Estonia, Fennoscandia and south-central Alaska. Three specific questions were addressed in this study. First, what is the extent and pattern of allozyme variation in *C. magellanica* subsp. *irrigua*? Second, are there differences in the extent of genetic

variation between the small, isolated Estonian populations and large Fennoscandian and Alaskan populations? Third, are there differences in allozyme diversity of *C. magellanica* subsp. *irrigua* in comparison with another declining sedge, *C. loliacea*, studied previously (Kull and Oja 2007)?

Material and Methods

Taxon

Carex magellanica subsp. *irrigua* belongs to the *Carex* subgen. *Carex* (Egorova 1999). The taxon grows in peaty soils of *Sphagnum* bogs, minerotrophic woodland fens, wet meadows, and in quagmires of lakeshores, forming loose clumps with usually 3–6 shoots. It grows along a wide range of the soil pH gradient, but is more commonly found on peatlands that have pH values <6 (Gignac et al. 2004). The taxon forms small clumps and does not spread clonally significantly. Therefore, sexual reproduction should play an essential role for this taxon. The terminal spike is male or gynaeandrous (a spike with upper flowers pistillate and lower staminate) and lateral spikes are female or gynaeandrous (Egorova 1999). One shoot of *C. magellanica* subsp. *irrigua* has 1–4 female spikes and the average seed production is 15 to 29 seeds per spike. The amount of unfertilized seeds might be up to 50% (pers. observations). In general, both cross- and self-pollination may exist in *Carex* species (Aleksiev 1996), but there are no data on the breeding mode of *C. magellanica* subsp. *irrigua*.

Seed Sampling and Germination

The seed material of *C. magellanica* subsp. *irrigua* was collected in summers of 2003–2006 from 22 populations with different population size from Estonia, Finland, Norway, Sweden and south-central Alaska (Table 1, Fig. 1). At all sites, seeds were collected from at least five mother plants at least 2 meters apart from each other.

The seeds were stored in paper bags in the laboratory at approximately 20°C for two months. Before germination, seeds were wet stratified for four months at 4°C in a refrigerator. Germination was carried out in the laboratory at fluctuating day/night temperature of 28/17°C. In total, 468 seed progeny of *C. magellanica* subsp. *irrigua* derived from 22 populations were analyzed (Table 1).

Selected specimens from the studied populations are preserved in the herbarium of the Institute of Agricultural and Environmental Sciences (TAA, Tartu, Estonia).

Allozyme Electrophoresis

Isozymes of total ten enzymes were examined in the preliminary experiments: shikimate dehydrogenase (SKD, EC 1.1.1.25), aspartate aminotransferase (AAT, EC 2.6.1.1), phosphoglucosomerase (PGI, EC 5.3.1.9), peroxidase (PRX, EC 1.11.1.7), phosphoglucomutase (PGM, EC 2.7.5.1), malate dehydrogenase (MDH, EC 1.1.1.37), leucine aminopeptidase (LAP, EC 3.4.11.1), alcohol dehydrogenase (ADH, EC 1.1.1.1.), esterase (EST, EC 3.1.1.2.) and superoxide dismutase (SOD, EC 1.15.1.1).

Table 1 Population codes, geographic origins with coordinates, number of seed progeny analyzed (Nr.) and an approximate number of ramets at the sampled sites (size) of *Carex magellanica* subsp. *irrigua*

Population	Geographic origin	Geographic coordinates	Nr.	size
A1	Baxter Bog, Anchorage, Alaska	61°11'13" N, 149°45'25" W	25	~1000
A2	N of Anchorage, Mirror Lake, Alaska	61°25'23" N, 149°24'36" W	21	>500
A3	Upper Trail Lake, Johnson Trail, Alaska	60°30'36" N, 149°26'00" W	21	~1000
A4	Botlenintnin Lake, Alaska	60°31'10" N, 150°33'14" W	25	>1000
A5	E of Soldotna, Alaska	60°31'32" N, 150°39'21" W	25	>1000
A6	Sterling HGW, Egumen Lake trail, Alaska	60°31'49" N, 150°23'08" W	21	~1000
A7	Wasilla, Big Lake, Alaska	61°32'00" N, 149°49'92" W	23	>1000
A8	Wasilla, quagmire of a small lake, Alaska	61°31'36" N, 149°51'45" W	25	>1000
A9	Wasilla, forest trail, Alaska	61°31'30" N, 149°51'47" W	25	~1000
A10	Nancy Lake, Alaska	61°42'10" N, 150°00'11" W	19	~1000
E1	Ida-Virumaa, Varesmetsa, Estonia	59°07'07" N, 27°27'16" E	25	<100
E2	Jõgevamaa, Endla, Tooma, Estonia	58°51'12" N, 26°13'50" E	21	<20
E3	Võrumaa, Sõmerpalu, Estonia	57°48'12" N, 26°80'12" E	25	500–1000
E4	Jõgevamaa, Endla, Kärde, Estonia	58°51'18" N, 26°14'02" E	18	~300
S1	Vindeln, Örträsk, Sweden	64°09'34" N, 19°06'26" E	20	500–1000
F1	Kuusamo, Juuma, Finland	66°20'28" N, 29°19'47" E	11	500–1000
F2	Kuusamo, Käyla, Finland	66°14'24" N, 29°14'25" E	25	>1000
F3	Kuusamo, Vuosselijärvi, Finland	66°09'38" N, 29°12'06" E	12	~1000
F4	Kuusamo, Rytilampi, Finland	66°23'02" N, 29°18'45" E	19	~1000
F5	Saarijärvi, Finland	62°39'11" N, 24°41'23" E	25	~1000
N1	Nordreisa, Spåkenes, Norway	69°45'46" N, 20°27'49" E	21	unknown
N2	Storfjord, Moalkejavri, Norway	69°15'52" N, 19°55'52" E	16	unknown
total			468	

Six out of ten enzyme systems studied could be interpreted (SKD, PGI, PGM, AAT, PRX, ADH) for allozymes. Zymograms of MDH allozymes showed variation but were hard to interpret and were not taken into account. LAP, EST and SOD also did not give clear bands and were not considered because it was impossible to record them adequately for all individuals. Putative isozymes were numbered sequentially beginning with the allozymes migrating fastest toward the anode.

Enzyme extracts were prepared from leaves of individual seedlings by grinding in 0.3 ml of 0.05 M Tris(hydroxymethyl)aminomethane (Tris)-0.01 M EDTA buffer containing 5 mM cysteine. To increase the viscosity of the extract, 20 mg of sucrose-Sephadex G 200 mixture (4:1) was added. The extracts were subjected to electrophoresis in vertical polyacrylamide gel slabs (120×70×2 mm). The following four gel-buffer systems and two catholytes were applied for different enzymes to achieve better band resolution: gel 1: 7.5% acrylamide, 0.2% N, N'-bisacrylamide (Bis), 0.15 M Tris, and 0.1 M HCl, applied for PGM, PGI, ADH, LAP and PRX; gel 2: 10% acrylamide, 0.15% Bis, 0.15 M Tris, and 0.1 M HCl, applied for AAT; gel 3: 10% acrylamide, 0.3% Bis, 0.15 M histidine, 0.1 M HCl, and 2.5 mM EDTA-Na₂, applied for MDH and SKD; gel 4: 10% acrylamide, 0.2% N, N'-methylene bis-acrylamide

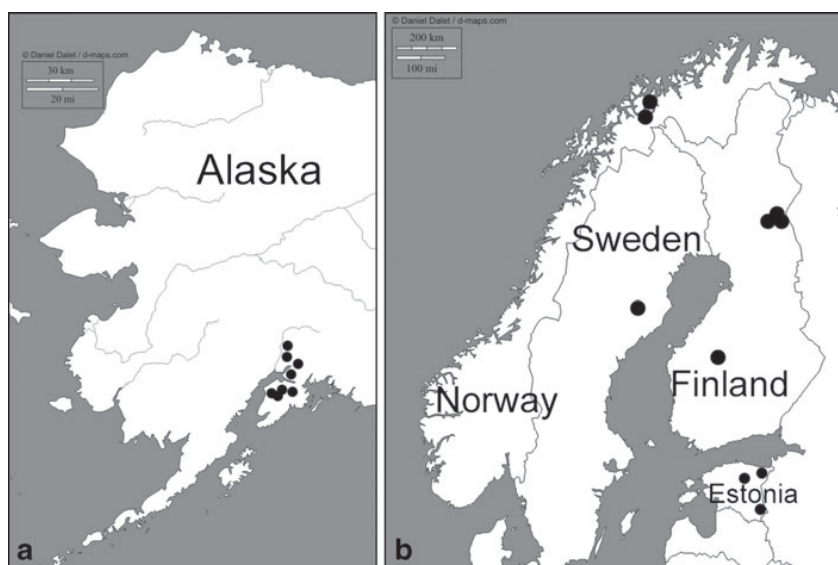


Fig. 1 Sampling localities of *Carex magellanica* subsp. *irrigua* in Alaska (a) and Europe (b)

(Bis), 0.25 M Tris, 0.1 M HCl, applied for EST and SOD. N, N, N', N'-tetramethylethylenediamine (0.05 ml) and ammonium persulfate (1 mg) were added to the gel mixtures to initiate and catalyze their photopolymerization between two daylight fluorescence bulbs for 1 h. The upper catholyte for gels 1, 2 and 4 was 0.08 M glycine with 0.02 M Tris, whereas the lower anode buffer was 0.1 M Tris-acetate with the initial pH about 8.9, and it was used repeatedly as long as the pH remained >7 . For gel 3, the upper catholyte was 0.08 M HEPES with 0.02 M Tris and the lower anode buffer consisted of 0.1 M triethanolamine with 0.02 M acetic acid. Ice-refrigerated electrophoresis was carried out by applying a pulsed current at 15 mA and 20–30 V/cm for about 2–2.5 h until the bromphenol blue marker dye reached the gel end. After electrophoresis, the gels were stained for isozymes using standard histochemical methods (Wendel and Weeden 1989). Banding patterns of zymograms were genetically interpreted according to Wendel and Weeden (1989).

Data Analysis

Allele frequencies, effective number of alleles (A_e), percentage of polymorphic loci (P), observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficient (F_{is}) and fixation index (F_{st}) of populations were calculated using POPGENE (version 1.31) (Yeh et al. 1999). Mean outcrossing rates (t) for populations displaying allozyme polymorphism were estimated by the equation $t = (1 - F_{is}) / (1 + F_{is})$ (Nei and Syakudo 1958). Mean genetic diversity measures (A_e , P , H_o , H_e) were calculated for populations, different geographic groups, and for the subspecies as a whole. Allele frequencies were calculated for each population and were used to calculate Nei's genetic identity (Nei 1978) between populations. These distances

were used in UPGMA and neighbour joining (NJ) cluster analyses to study patterns of between-population variation. UPGMA was performed using the program TFPGA (version 1.3) (Miller 1997), and NJ using program GDA (Lewis and Zaykin 2001).

Results

Isozyme Variation in Carex magellanica subsp. irrigua

Nine putative isozyme loci were clearly resolved, with one locus for SKD, PGI and AAT, and two loci for PGM, PRX and ADH. Additional zones of activity were observed for AAT and PRX, but these bands were indistinct, weak and were not scored. In total, 15 putative allozymes were detected for *C. magellanica* subsp. *irrigua*. The ADH zymograms displayed an invariant three-banded symmetrical phenotype attributed to two invariant isozymes, ADH-A and ADH-B, giving rise to an interlocus hybrid isozyme of intermediate mobility. PGM-A and PRX-B possessed both one invariant and homozygous allozyme. SKD-A was most polymorphic with three different allozymes, comprising three homozygous and three different heterozygous phenotypes. AAT-C, PGM-B, PGI-A and PRX-A had two allozymes each, whereas PGI-A and PGM-B displayed both homozygous and heterozygous morphs. The number of observed heterozygous phenotypes was very low: out of 468 individuals studied, only six individuals displayed heterozygosity in SKD-A phenotypes, two individuals in PGI-A and seven in PGM-B. Two isozymes, PGI-A and AAT-B, revealed region-specific differentiation. All Estonian populations and the southernmost Finnish population F5 showed PGI-A1 and AAT-C1, whereas Alaskan and other Fennoscandian populations showed alternative PGI-A2 and AAT-C2. One Norwegian population (N1) revealed unique PRX-A1 not found elsewhere.

Genetic Variation Within and Between Populations

At the taxon level, five out of nine loci studied (56%) were polymorphic with the average of 2.2 alleles per polymorphic locus. Three alleles (*Pgi-b*, *Prx-b*, *Aat-b*) had frequency over 0.7 and were dominant in most populations. One allele (*Prx-a*) had frequency less than 0.1 and was present in only one Norwegian population (N1). One Estonian population (E2) was monomorphic at all loci. Allele frequencies at polymorphic isozyme loci in populations and for the taxon are given in Table 2.

The effective number of alleles (A_e) ranged between 1.00–1.42 among populations and was 1.14 for the taxon (Table 3). Among the different regions, the Fennoscandian group had the highest value of A_e (1.18) (Table 4). In populations, H_o varied from 0 to 0.031 with a mean of 0.004, whereas mean H_e was 0.073, ranging from 0 to 0.183. Populations from Alaska and Estonia were totally homozygous suggesting an extremely high rate of inbreeding. Fennoscandian populations also revealed great heterozygote deficiency indicating a high level of inbreeding. The outcrossing rate for the taxon was extremely low with the mean value 0.008 indicating predominant selfing. The F_{is} value of *C. magellanica* subsp. *irrigua* was close to one (0.949, Table 5), which is characteristic for inbreeding species. Very

Table 2 Allele frequencies at polymorphic isozyme loci in populations of *Carex magellanica* subsp. *irrigua*. The population codes are the same as those in Table 1

Population	Locus/allele										
	<i>Skd</i>			<i>Pgi</i>		<i>Pgm-2</i>		<i>Aat</i>		<i>Prx-1</i>	
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
A1	0.00	0.44	0.56	0.00	1.00	0.85	0.15	0.00	1.00	0.00	1.00
A2	0.00	0.28	0.71	0.00	1.00	0.05	0.95	0.00	1.00	0.00	1.00
A3	0.05	0.19	0.76	0.00	1.00	0.57	0.42	0.00	1.00	0.00	1.00
A4	0.04	0.28	0.68	0.00	1.00	0.64	0.36	0.00	1.00	0.00	1.00
A5	0.04	0.40	0.56	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00
A6	0.09	0.19	0.71	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00
A7	0.04	0.40	0.56	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00
A8	0.00	0.16	0.84	0.00	1.00	0.52	0.48	0.00	1.00	0.00	1.00
A9	0.12	0.56	0.32	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
A10	0.05	0.10	0.84	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
E1	0.20	0.64	0.16	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00
E2	0.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00
E3	0.04	0.80	0.16	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00
E4	0.00	0.22	0.78	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00
S1	0.00	0.27	0.73	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
F1	0.25	0.33	0.41	0.00	1.00	0.29	0.71	0.00	1.00	0.00	1.00
F2	0.88	0.00	0.12	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
F3	0.88	0.12	0.00	0.00	1.00	0.81	0.19	0.00	1.00	0.00	1.00
F4	0.08	0.40	0.52	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
F5	0.12	0.76	0.12	0.80	0.20	0.00	1.00	1.00	0.00	0.00	1.00
N1	0.28	0.38	0.34	0.00	1.00	0.64	0.36	0.00	1.00	0.44	0.56
N2	0.20	0.35	0.45	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
total	0.14	0.40	0.45	0.25	0.75	0.34	0.66	0.26	0.74	0.02	0.98

high F_{st} (0.666) showed that most of the genetic diversity was between rather than within populations and also supported selfing, as a mating mode for this taxon.

Geographic Distribution of Genetic Variation

The UPGMA and neighbour-joining clustering of populations both showed similar distribution into two major groups with a clear geographic pattern. Estonian populations with one Finnish population formed one cluster. Other Fennoscandian populations and Alaskan populations appeared in two subclusters of the other major cluster (Fig. 2). The appearance of two main clusters on the UPGMA dendrogram reflects genetic differentiation between northern (Alaskan and Fennoscandian) and southern (Estonian) populations, consistent with the differences in the population size and fragmentation level between the regions. Table 4 shows that H_o , H_e , P and A_e are remarkably lower in the southern group

Table 3 Genetic diversity in populations of *Carex magellanica* subsp. *irrigua*. The population codes are the same as those in Table 1

Population	A_e	H_o	H_e	t
A1	1.17	0.000	0.108	0
A2	1.11	0.000	0.077	0
A3	1.20	0.000	0.119	0
A4	1.20	0.000	0.106	0
A5	1.14	0.000	0.076	0
A6	1.09	0.000	0.051	0
A7	1.12	0.000	0.059	0
A8	1.15	0.000	0.087	0
A9	1.15	0.000	0.064	0
A10	1.04	0.000	0.032	0
E1	1.12	0.000	0.059	0
E2	1.00	0.000	0.000	0
E3	1.06	0.000	0.037	0
E4	1.06	0.000	0.039	0
S1	1.12	0.000	0.085	0
F1	1.29	0.009	0.124	0.038
F2	1.03	0.005	0.024	0.116
F3	1.08	0.014	0.060	0.132
F4	1.15	0.000	0.065	0
F5	1.12	0.018	0.081	0.124
N1	1.42	0.031	0.183	0.092
N2	1.19	0.000	0.072	0
Mean	1.14	0.004	0.073	0.008

A_e – effective number of alleles, H_o – observed and H_e – expected heterozygosity, t – outcrossing rate.

with small populations. A significant correlation between population size and H_e was found ($r=0.7$, $P<0.05$).

Difference in the Allozyme Diversity Between Carex magellanica subsp. irrigua and C. loliacea

In our previous paper (Kull and Oja 2007) we studied another declining sedge species, *C. loliacea* that is becoming rare throughout its distribution area. Both *C. magellanica* subsp. *irrigua* and *C. loliacea* have a similar low degree of observed heterozygosity and a high inbreeding coefficient (Table 5). However, genetic diversity in *C. magellanica* subsp. *irrigua*, as is evident from $H_e=0.073$ and $P=55.6\%$, is remarkably higher than in *C. loliacea* ($H_e=0.027$, $P=5.6\%$). The former revealed polymorphism in five and heterozygotes in three isozymes, while *C. loliacea* had polymorphism and heterozygosity in only one isozyme. The distribution of allozyme genetic variation between different geographic districts of *C. magellanica* subsp. *irrigua* is similar to the geographic pattern in *C. loliacea*. As in *C. loliacea*,

Table 4 Distribution of genetic diversity in three different geographic regions, and in the northern group (Fennoscandia + Alaska) of *Carex magellanica* subsp. *irrigua*

Group	A_e	H_o	H_e	F_{is}	F_{st}	P	t
Alaska	1.11	0.000	0.078	1.000	0.352	22.2	0
Estonia	1.06	0.000	0.034	1.000	0.371	11.1	0
Fennoscandia	1.18	0.010	0.087	0.889	0.574	55.6	0.059
Fennoscandia + Alaska	1.16	0.004	0.082	0.951	0.528	55.6	0.025

A_e – effective number of alleles, H_o – observed and H_e – expected heterozygosity, F_{is} – inbreeding coefficient, F_{st} – fixation index, P – % of polymorphic loci, t – outcrossing rate.

populations of *C. magellanica* subsp. *irrigua* from Estonia are genetically differentiated from Scandinavian and Alaskan populations, which are similar to each other (Kull and Oja 2007).

Discussion

Genetic Variation in *Carex magellanica* subsp. *irrigua*

Cole (2003) compared genetic variation in rare and common plants and found that all measures of genetic variability showed reduction in rare species, compared to their common congeners. The mean genetic diversity of *C. magellanica* subsp. *irrigua* ($H_e=0.073$) was considerably lower than for rare species ($H_e=0.142$), reported in the review of Cole (2003). In the southern region, in Estonia, where this taxon is locally rare and declining, the genetic variation was even lower in comparison with other regions. Jonsson (1998) analyzed 41 species of Cyperaceae and found that rhizomatous species show higher levels of within-population genetic variation than caespitose species. Also, Ford et al. (1998) found that caespitose species had a low level of intrapopulational genetic variation. *Carex magellanica* subsp. *irrigua* is rather caespitose and has no special features for long-distance seed dispersal. Restricted seed dispersal results in small spatial clumps of genetically closely related tussocks due to the lack of nonrelative mating partners in the neighbourhood. The observed high mean inbreeding coefficient F_{is} value (Table 5) indicates that *C. magellanica* subsp. *irrigua* is predominantly inbreeding. We speculate that this high inbreeding may not be due to pure autogamous mating mode, because

Table 5 Mean values of allozyme diversity for *Carex magellanica* subsp. *irrigua* and *C. loliacea*. The data for *C. loliacea* are calculated by data from Kull and Oja (2007)

Species	F_{is}	F_{st}	H_o	H_e	P	t
<i>C. magellanica</i> subsp. <i>irrigua</i>	0.949	0.666	0.004	0.073	55.6	0.008
<i>C. loliacea</i>	0.930	0.887	0.0006	0.027	5.6	0.011

F_{is} – inbreeding coefficient, F_{st} – fixation index, H_o – observed and H_e – expected heterozygosity, P – % of polymorphic loci, t – outcrossing rate.

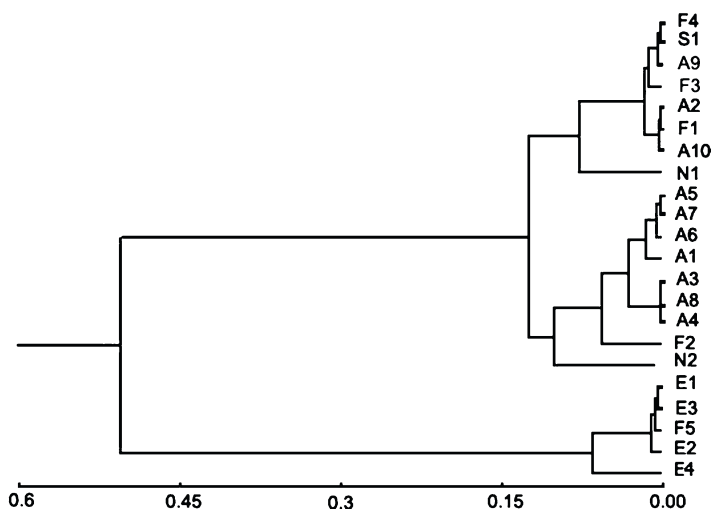


Fig. 2 UPGMA dendrogram based on Nei's genetic distances of *Carex magellanica* subsp. *irrigua* populations (population codes and geographic origins are given in Table 1)

C. magellanica subsp. *irrigua* is presumably a wind-pollinated allogamous taxon, but may be caused by biparental inbreeding or crossing between close relatives.

The taxon has a relatively high chromosome number ($2n = 58$) (Egorova 1999), which suggests polyploidy. But according to the isozyme study it revealed a typical diploid pattern (i.e., one or two alleles per individual). Like in *C. loliacea* (Kull and Oja 2007), we may suppose that *C. magellanica* subsp. *irrigua* is a diploidized polyploid, i.e., functionally diploid.

Genetic Variation Between Populations

Mean genetic differentiation F_{st} for *C. magellanica* subsp. *irrigua* is 0.666, indicating that the level of differentiation between populations is very high and only 33.4% of the diversity lies within populations. Similarly a high fixation index ($F_{st}=0.658$) was found in the *Carex crinita* complex (Bruederle and Fairbrothers 1986). Members of this complex have also caespitose habit and grow in wetlands like *C. magellanica* subsp. *irrigua*. The level of differentiation (F_{st}) between small isolated populations in Estonia is expected to be higher than in continuous abundant populations. However, many common widespread species have revealed the same or even greater genetic differentiation than rare fragmented species (Hamrick and Godt 1989; Gitzendanner and Soltis 2000; Cole 2003). In our study, populations from the central abundant distribution region in Fennoscandia had an even higher level of F_{st} than populations in peripheral areas in Alaska and in Estonia. The high level of differentiation between populations may be caused by the low gene flow over long distances in *C. magellanica* subsp. *irrigua* even between continuous populations because wind pollination of small herbs in closed vegetation is not very effective. A potential explanation for lower value of F_{st} in Estonian populations may be the so-called delayed loss of genetic diversity, i.e., after habitat size is reduced the genetic

differentiation in isolated small populations remains the same for some time after fragmentation (Leblós et al. 2006). Taking into account that the loss of habitats of *C. magellanica* subsp. *irrigua* is quite recent in Estonia, it may be one possible scenario to explain the lower genetic differentiation among the small, isolated Estonian populations than among larger Fennoscandian populations. Our data show that the peripheral Estonian and Alaskan populations of *C. magellanica* subsp. *irrigua* have less genetic variability than central Fennoscandian populations. The lowering of genetic diversity may reduce plant fitness and the potential for adapting to changing environmental conditions and stochasticities. For conservation purposes, it is critical to identify populations with high within-population diversity as well as those with divergent gene pools. In the case of *C. magellanica* subsp. *irrigua*, each population probably represents a set of different autogamous genetic lineages that are important to preserve. Therefore, there is a need to pay close attention to the conservation of *C. magellanica* subsp. *irrigua* in Estonia, which is at risk of anthropogenic disturbance there.

Genetic Variation in Geographic Regions

According to the population size and fragmentation level we can divide the studied populations into two groups. Estonian populations form the southern peripheral group, where populations of *C. magellanica* subsp. *irrigua* are small and fragmented and the taxon is declining (Table 1). In northern areas, in Alaska and Fennoscandia, the taxon is still rather common, and has large populations (pers. observations). UPGMA dendrogram at the regional level revealed clear differentiation between these two groups: southern (Estonian and the southernmost Finnish population) and northern (Fennoscandian and Alaskan populations) (Fig. 2). We found similar distribution for *C. loliacea* studied previously (Kull and Oja 2007), where Fennoscandian and Alaskan populations also formed a separate cluster from Estonian ones. Region-level allelic richness in *C. magellanica* subsp. *irrigua* was highest in Fennoscandia but low in Estonia. Similarly, Tyler (2002) investigated genetic diversity in populations of *C. digitata* throughout the European range and found that the regional allelic richness was highest in Fennoscandia, in the central part of distribution area of the species. Michalski and Durka (2007) obtained similar results studying peripheral and subcentral populations of *Juncus atratus*. We found some heterozygosity in Fennoscandian populations and no heterozygotes in Alaskan and Estonian populations. Based on the distribution map of Hultén and Fries (1986), the geographic distribution range of *C. magellanica* subsp. *irrigua* could be distinguished into two regions: North American and Eurasian. Fennoscandia is the central distribution area of *C. magellanica* subsp. *irrigua* where the taxon achieves highest abundance (Hultén and Fries 1986) accompanied with higher allelic richness, as evident from the present study. The Estonian populations have marginal position in the Eurasian range of the taxon and suffer from habitat fragmentation and population decline, and probably therefore have reduced allozyme polymorphism and heterozygosity. In Central Europe the taxon occurs rarely and grows only in mountains (Ditè and Pukajová 2003). The absence of heterozygotes in Alaskan populations is not so easily understandable. *Carex magellanica* subsp. *irrigua* seems to be common there with numerous large populations (personal observations) and one would expect high

polymorphism and heterozygosity. At the same time, the studied Alaskan populations are situated at the edge of the North American range and may have low genetic diversity because of their marginal position. Hultén and Fries (1986) also indicate that the occurrence of *C. magellanica* subsp. *irrigua* in Alaska and in most parts of North America consists of numerous, but rather isolated populations with a potentially restricted gene flow and a decreased level of genetic polymorphism.

Difference in Allozyme Diversity Between Carex magellanica subsp. irrigua and C. loliacea

Carex magellanica subsp. *irrigua* and *C. loliacea* are both declining in Estonia. Our results show that current level of genetic diversity is quite different in *C. magellanica* subsp. *irrigua* and *C. loliacea* despite their similar caespitose growth, habitat preference and reproduction mode. *Carex magellanica* subsp. *irrigua* maintains more allozyme diversity than *C. loliacea*, with $H_e=0.073$ and 0.027 , respectively. This result could be explained by the contemporary differences in their distribution. *Carex loliacea* is estimated as declining over the whole distribution area, while *C. magellanica* subsp. *irrigua* is still abundant in northern areas. Habitats of *C. loliacea*, wet forests, have suffered from active land use and are declining everywhere, but in Fennoscandia and Alaska there are still sufficiently available quagmires and other suitable habitats for *C. magellanica* subsp. *irrigua*. Their different inflorescence type could also contribute to the different level of allozyme diversity in these two sedge species. *Carex loliacea* has male and female flowers on the same spike, while *C. magellanica* subsp. *irrigua* has male and female flowers on different spikes. The close proximity of male and female flowers may favour geitonogamy and cause lower genetic diversity in *C. loliacea*.

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