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GENETIC DIVERSITY OF *LIPARIS LOESELII* IN LATVIA

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According to the Global Strategy for Plant Conservation, 75% of endangered species should be preserved in ex situ *collections till 2020. The genus* Liparis *has a lot of recognised taxons, but only one species,* Liparis loeselii *(L.) Rich., grows in Europe.* L. loeselii *is a rare and endangered orchid species occurring in Europe. In Latvia* L. loeselii *is classified as the third category of endangered and protected species. To develop the best conservation strategy, the knowledge concerning the genetic differences of protected plants in a particular area is crucial. For this purpose, the genetic diversity of* L. loeselii *populations from different Latvian habitats was tested. The inter-retrotransposon amplified polymorphism method (iPBS) was used for population genetic diversity evolution. In total, 54 accessions from nine habitats were collected and analysed.* L. loeselii *leaves have a high content of phenols that reduce the quality of extracted DNA. It was found that the percentage of polymorph loci varied among the populations of* L. loeselii *growing in different habitats; some of the populations were genetically homogeneous. The genetic diversity levels of* L. loeselii *populations are related with the population age and the growing conditions.*

Key words: *orhids, protected species, iPBS method.*

INTRODUCTION

In 2002, the Global Strategy for Plant Conservation (GSPC) was added to the UN Convention on Biological Diversity (CBD), signed in Rio de Janeiro in 1992. Latvia is one of the 180 countries that has joined the CBD and accepted GSPC, including the statement about the role of Botanical Gardens in the conservation of wild flora. To protect the rare and endangered orchid species in the nature, a detailed biological research is required; one of the consequential aspects is the population analysis of genetic diversity. The results of these investigations make the grounds for elaboration of the strategy for plant species protection, including, *in vitro* culture methods to conserve the diversity of rare plant populations. *In vitro* propagation not only provides genetic material for broader research and repatriations, but also makes possible investigation of plant life cycle development (Wotavova-Novotna, 2007). A good knowledge of the genetic diversity and population structure is a necessary prerequisite for the conservation of species, since it reflects the status and survival potential of populations (Lande, 1988; Dixon *et al*., 2003). Conservation of biodiversity requires evaluation of the conservation value of populations, species and ecosystems, and determination of priorities at each scale. The species is the most commonly used measure for biodiversity and the main unit in conservation (Purvis and Hector, 2000), and conservation of wild species requires good knowledge of species peculiarities and differences from their closest relatives (Vane-Wright *et al*., 1991). This requires the use of molecular markers that can also reveal the dispersal capacity of species and their infraspecific structure (Ouborg *et al*., 1999; Soltis and Gitzendanner, 1999).

Liparis loeselii (L.) Rich. is a rare and endangered orchid occurring in Europe and North-East America. *L. loeselii* is classified as the third category of endangered and protected species in Latvia. In its area *L. loeselii* occupies preserved fen and wet meadow areas. Environmental and climate changes, as well as anthropogenic factors, have dramatically influenced the *L. loeselii* habitat, which has led to the decrease of species occurrence. In addition to the habitat age

and vegetation, abiotic factors such as water level and pH also have been mentioned in the literature as important factors influencing the survival and population density of this orchid. The population life span of the species was generally very short — between 5 and 15 years (Oostermeijer and Hartman, 2014). *L. loeselii* is a perennial plant with pseudobulb, two leaves in the adult plant, and a central inflorescence with up to twenty green scentless flowers. Vegetative reproduction is achieved through the development of one or two small pseudobulbs from an adult one. Most of these species are subtropical epiphytes. The growth of *L. loeselii* is epiphytic and can be rooted in the upper layer of the soil as well in ditches, in the moss deck, and on the surface of the tree trunks (Case, 1987; Wheeler *et al*., 1998).

The presence of *L. loeselii* depends on the required specific soil conditions in particular microhabitats. A negative effect of the soil organic matter was found on the survival of *L. loeselii* populations: the extinct populations were associated with sites with high content of organic matter (Grootjans *et al*., 2017). Accumulation of organic matter in the soil alters many soil properties, such as soil moisture and pH, and, consequently, has a large impact on mineralization rates (Berendse *et al*., 1998). The high concentration of Fe had an indirect effect on increased accumulation of organic matter in soil. Therefore, high iron concentrations might imply indirect impact on the decline of orchid populations (Grootjans *et al*., 2017). In Latvia, pH levels in soils of microbiotopes with presence of *L. loeselii* populations varied from acid (pH 4.41) to basic (pH 8.75) (Roze *et al*., 2015).

It has been described that *L. loeselii* can be both a self-pollination and cross-pollination plant (Catling, 1980). *L. loeselii* has generally appropriate levels of polymorphism (Pillon *et al*., 2007). Genetic diversity and structure of this species in northwest France and the United Kingdom were investigated using amplified fragment length polymorphisms (AFLPs). Clonality and autogamy are common in *L. loeselii*, and moderating to important variability within populations was found. It was shown that populations from dune slacks and fens should be managed separately and that geographically distant populations may be similar (Pillon *et al*., 2007).

Different methods are used for investigation of the genetic diversity of populations. Early developed AFLPs method provide the possibility to study multilocus markers that have already shown their usefulness in population genetics studies of rare or endangered species (Vos *et al*., 1995; Travis *et al*., 1996). Recently very topical for population genetic diversity evaluation has been the use of universal methods like inter-retrotransposon amplified polymorphism (IRAP) method. Many retrotransposons' features make them very appealing as the basis of molecular marker systems (Kalendar *et al*., 1999; Schulman, 2007). An important category of large changes to the genome is insertion of retrotransposons at new loci. Many scientists have worked on the retrotransposon life cycle and the role of retrotransposons in genome evolution. The large central part of the retrotransposon encodes the proteins needed for reverse transcription, packaging into virus-like particles, and integration back into the genome. Both, the overall structural features, as well as the basic stages of the life cycle, are shared by retrotransposons and retroviruses (Frankel and Young, 1998; Kumar and Bennetzen, 1999; Kim *et al*., 2004). For other molecular marker systems, the emergence of retrotransposon-based methods followed the basic research that demonstrated their ubiquity and activity in the plants (Flavell *et al*., 1992; Grandbastein, 1992; Voytas *et al*., 1992; Suoniemi *et al*., 1998).

The retrotransposon-based technique is anonymous and producing fingerprints from multiple sites of retrotransposon insertion in the genome (Schulman *et al*., 2004). Retrotransposon integration sites represent joints between the conserved LTR ends and flanking, essentially random, genomic DNA. Most retrotransposon-based marker systems use PCR to amplify a segment of genomic DNA surrounding this joint (Kalendar *et al*., 2010). A retrotransposon-based primer can create up to 50 loci, which, in comparison with other types of markers, is one of the most essential advantages to use them for detection of species diversity. The sole retrotransposon method has been designed to detect polymorphism in the integration of an element at a particular locus is RBIP (Retrotransposon-Based Insertion Polymorphism) (Flavell *et al*., 1998). As a result of their general applicability, simplicity of implementation, and genotype resolution, retrotransposon-based marker systems have been widely applied in evolutionary and genetic diversity studies (Feschotte *et al*., 2002; Schulman *et al*., 2004; Kalendar and Schulman, 2006; Kalendar *et al*., 2010).

The aim of this study was estimation of the genetic diversity of *L. loeselii* population in Latvia by the universal retrotransposon-based molecular marker method (Kalendar *et al*., 2010).

MATERIALS AND METHODS

Habitats. Orchids *Liparis loeselii* for genetic diversity analyses were chosen from different parts of Latvia. *L. loeselii* plants were described in their natural range. Habitats with *L. loeselii* were classified, based on characteristic plant species found within the habitat (Auniņš, 2013; Directive 92/43/EEC).

Plant material and DNA extraction. Fifty-four samples from nine habitat regions of Latvia were collected for molecular analyses in June 2010 (Fig. 1). The populations in localities considered as very small (number of plants 1–10): Bog Pēterezers and Kaņieris NT; small (10–30): Lake Silabebri and Lake Dreimaņi; middle (30–50): Engure near Lepste and Kaņieris; large (50 and more): Lake Būšnieki and gravel pit "Šalkas". A part (~10×10 mm) of *L. loeselii* leaves without insect damages were used for DNA extraction. Leaves were collected in plastic Petri plates with moist filter paper. Plates were put into cool box with isolated ice elements, then the leaves were dried at 45 ^{o}C for 16 hours,

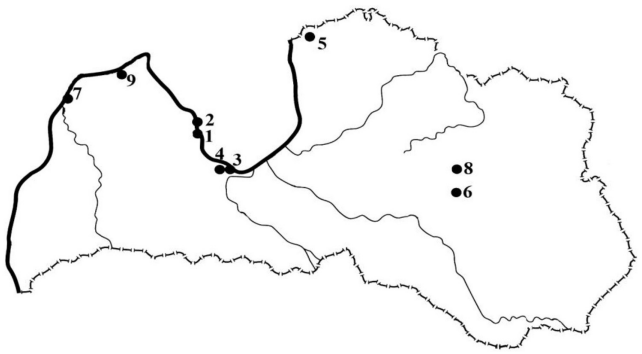

Fig. 1. The map of different areas of habitat regions of Latvia, where samples of *L. loeselii* leaves for molecular analyses were collected. 1, Engure Orchid Trail (OT); 2, Engure near Lepste; 3, Kaņieris; 4, Kaņieris Nature Trail (NT); 5, "Šalkas" gravel pit; 6, Lake Silabebri; 7, Lake Būšnieki; 8, Lake Dreimaņi; 9, Bog Pēterezers.

Table 1

INVESTIGATED *L. LOESELII* HABITATS

No.	Habitat regions of Latvia	Number of plants
1	Engure OT	16
2	Engure near Lepste	15
3	Lake Kaņieris	5
4	Lake Kaņieris NT	2
5	Gravel pit "Šalkas"	3
6	Lake Silabebri	1
7	Lake Būšnieki	2
8	Lake Dreimaņi	7
9	Bog Pēterezers	3

after that hermetically sealed in plastic Petri plates and kept in darkness. Two types of *L. loeselii* leaves for DNA isolation were used — from dried leaves and from green leaves of *in vitro* plants (seeds were collected from Bog Pēterezers in 2007), grown in the Laboratory of Plant Ecophysiology of the National Botanic Garden of Latvia. In total, 54 accessions from nine habitats were used for molecular analyses (Table 1). Samples being from rare and protected species, the number of plants in some habitats was limited.

Five DNA extraction replications were made using two different methods — Saghai-Maroof (1984) and modified method of Friar (2005): (1) DNA was extracted from plants using 1% CTAB (cetyltrimet-ammonium bromaide) buffer, with 1.25% mercaptoethanol, precipitate DNA with cold chloroform–izoprophanol (24 : 1) mix (Saghai-Maroof *et al*., 1984); (2) isolation of DNA using 2% w/v CTAB, with added 1% w/v PVP-40 and 2 µl mercaptoethanol, double precipitation of DNA with ice-cold 95% ethanol and overnight incubation at minus 20 °C (Friar, 2005).

The quality of isolated DNA (addition of RNA and phenols) was controlled by using Thermo Nanodrop-1000 and Ependorf BioPhotometer. Determination of DNA quality was assessed by electrophoresis on agarose gel (1.5% concentration of agarose gel, 70 V, 1 h – 1 h 30 min). Gel was put into 1 × TAE buffer liquid float, which previously had been added colour, 20 µl· l^{-1} ethidium bromide 10% solution, colouring time 40 min in stirring mode; after that the samples were put in distilled water float for 10 min in stirring mode. Visualization of gel with UVItec Limited STX-20.M and documentation of agarose gel with digital camera was performed.

Two polymorphic iPBS primers —
2079 (AGGTGGGCGCCA) and
2415 (CATCGTAGGTGGGCGCCA) were selected from Kalendar primer collection (Kalendar *et al*., 2010) for genetic analysis of Latvian population of *L. loeselii*. DNA amplification was performed in Gene Amp® PCR System 9700 thermocycler under following conditions PCR appropriates: denaturation 95 °C/3 min, then 30 cycles (denaturation 95 °C/45 s, to anneal primer 50 °C/40 s, elongation 68 °C/60 s) and finish elongation 72 °C/10 min and 4 °C soaking. DNA dilution amount for one reaction was 4 µl and PCR mixture (MIX for 50 DNA samples: purified H_2O/861.25 µl; 10× Tag buffer/125.0 µl; dNTPs/25.0 µl; primer 125.0 µl; Dream Tag polymerase 12.5 µl; Pfu polymerase 1.25 µl) total volume was 25 µl.

Analyses of PCR product quality by electrophoresis on agarose gel were performed (in each box of PCR plate added 5 µl 6 × Mass Loading Gel Solution and 25 µl PCR product, 1.5% concentration of agarose gel, 1 h, 90 V).

The results of genetic analysis of *L. loeselii* populations were analysed using POPGENE Version 1.31 and NTSYSpc 2.1 software.

RESULTS

Molecular analysis using retrotransposon markers requires good quality DNA (high molecular weight DNA free of RNA, protein and phenol contaminants) in a concentration range 60–100 ng/µl (Kalendar *et al*., 2010). *L. loeselii* has a high level of phenols in leaves that decreases the quality of extracted DNA (Arditti, 1992). DNA extraction by Friar (2005) method with some modifications gave the possibility to obtain large amounts of high quality DNA, concentration range 6.0–187.0 ng/µl. Isolation of DNA from dried and green leaves gave the similar DNA concentrations. Only high-quality DNA materials were used for PCR.

In total, two polymorphic iPBS primers (Kalendar *et al*., 2010) produced 50 loci (in amplified DNA fragments there were 23 loci, produced by primer 2415, and 27 loci, produced by primer 2079), that were used for examination of genetic diversity in Latvian population of *L. loeselii*. Both primers produced a high amount of clear and easy visible bands (Belogrudova *et al*., 2012). The percentage of polymorphic loci varied among the populations of *L. loeselii* in different habitats: Engure OT — 32 loci (percentage of polymorphic loci is 64%), Engure near Lepste — 42 loci (84%), Lake Dreimaņi — 28 loci (56%), Bog Pēterezers — 24 loci (48%).

A dendrogram (Fig. 2) shows the genetic similarity of the analysed plants growing in nine habitats (Tables 2 and 3).

Table 2

THE GENETIC DISTANCE BETWEEN *LIPARIS LOESELII* PLANTS FROM DIFFERENT HABITATS

Habitat	Engure OT	Engure near Lepste	Lake Kaņieris	Lake Kaņieris NT	"Šalkas" gravel pit	Lake Silabebri	Lake Būšnieki	Lake Dreimaņi
Engure near Lepste	0.068							
Lake Kaņieris	0.306	0.300						
Lake Kaņieris NT	0.222	0.198	0.396					
'Šalkas" gravel pit	0.374	0.338	0.092	0.446				
Lake Silabebri	0.317	0.325	0.610	0.654	0.654			
Lake Bušnieki	0.138	0.213	0.313	0.301	0.478	0.616		
Lake Dreimaņi	0.076	0.125	0.280	0.293	0.333	0.183	0.203	
Bog Pēterezers	0.101	0.085	0.186	0.182	0.244	0.534	0.110	0.171

The dendrogram consists of four main groups; two groups consist of similar cluster pairs: Engure OT — Engure and Kaņieris — "Šalkas" gravel pit. Individuals of Kaņieris and "Šalkas" are geographically distant, but orchids from those habitats are genetically similar. Geographically distant habitats, and a separate cluster of branches, which are different from the other orchids, were analysed. Other habitats of orchids, according to their geographical breakdown decomposition, suggest *L. loeselii* population of the low degree of structuring. In some habitats unique loci of orchids were found (Table 4): from Engure OT two unique loci – No. 4 and No. 13; No. 13 has a very high frequency — 50 %. One more unique locus, No. 16, with rather high frequency — 29% was presented in the habitat by the Lake Kaņieris. The cluster (Fig. 3) of summarised results does not show sample grouping according to the sample collection places, it presents two big, quite separate cluster group branches, where the first one includes plants from the habitats at Lake Engure and also one from Lake Būšnieki. The second cluster branch includes plants that were collected in places with changing water level (Engure OT, Engure near Lepste, Lake Dreimaņi, Lake Silabebri). Also, there can be distinguished a small cluster sub-branch with low-structure division, which includes mutually similar individuals of *L. loeselii*

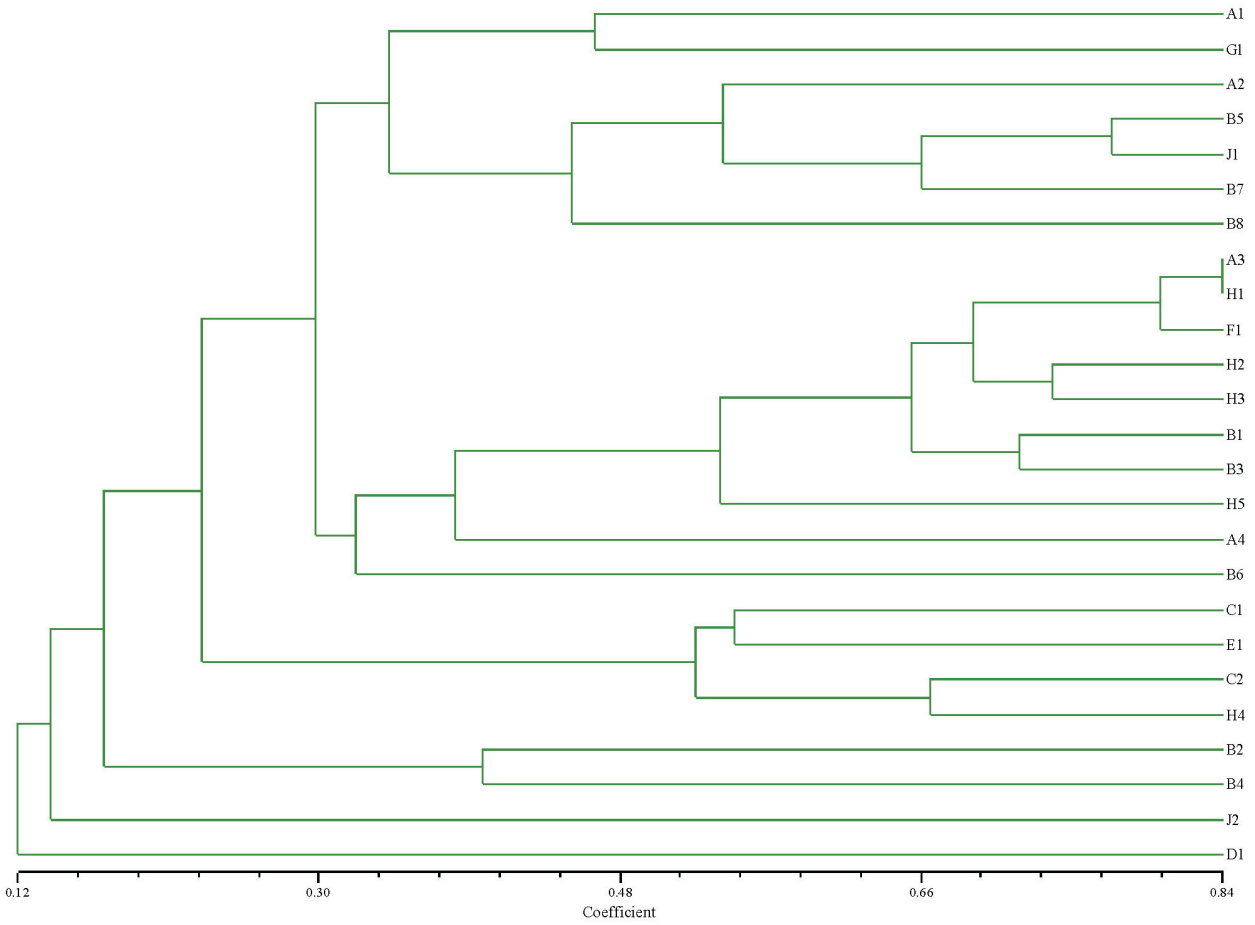

Fig. 2. Dendrogram of genetic similarity of *Liparis loeselii* plants within the population: A, Engure OT; B, Engure near Lepste; C, Lake Kaņieris; D, Lake Kaņieris NT; E, "Šalkas" gravel pit; F, Lake Silabebri; G, Lake Būšnieki; H, Lake Dreimaņi; J, Bog Pēterezers.

Table 3

HABITATS AND ITS CHARACTERISTIC SPECIES

No.	Inspected habitats	Classification's No. of *European Union* protected *habitats*	Characteristic species
1.	Orchid Trail in Engure	7230 Alkaline fens	*Cladium mariscus, Phragmites australis, Schoenus ferrugineus, Utricularia sp.*
2.	The east lakeshore of Lake Engure by Lepste	7210* Calcareous fens with *Cladium mariscus* and species of the Caricion davallianae	*Cladium mariscus, Phragmites australis, Schoenus ferrugineus, Utricularis sp.*
3.	The lakeshore of Lake Kaņieris	7210* Calcareous fens with *Cladium mariscus* and species of the Caricion davallianae	*Cladium mariscus, Phragmites australis, Schoenus ferrugineus, Utricularia* sp.
4.	Nature Trail by Lake Kaņieris	7230 Alkaline fens	*Myrica gale, Phragmites australis, Schoenus ferrugineus, Pedicularis palustris*
5.	Gravel pit near the city Ainaži ("Šalkas")	7230 Alkaline fens	*Carex flava* group*, Equisetum variegatum, Phragmites australis, Primula farinosa*
6.	Lake Silabebri	7140 Transition mires and quaking bogs	*Carex lasiocarpa*, *C. rostrata, Comarum palustre, Menyanthes trifoliata, Peucedanum palustre, Phragmites australis, Thelypteris palustris*
7.	West part of Lake Būšnieki	7140 Transition mires and quaking bogs	*Comarum palustre, Eriophorum polystachion, Menyanthes trifoliata, Myrica gale, Phragmites australis, Thelypteris palustris, Utricularia sp.*
8.	Peninsula in Lake Dreimaņi (Svētes)	7230 Alkaline fens	*Carex lasiocarpa, Cladium mariscus, Eriophorum polystachion, Phragmites australis, Schoenus ferrugineus, Utricularia sp.*
9.	Humid dune slack Pēterezers (Bog Pēterezers)	2190 Humid dune slacks	*Carex lasiocarpa*, *Drosera anglica, Eriophorum polystachion, Menyanthes trifoliata, Oxycoccus palustris, Rhynchospora alba, Thelypteris palustris, Utricularia sp.*

Table 4

UNIQUE LOCI IN POPULATIONS OF *LIPARIS LOESELII* GROW IN DIFFERENT HABITATS

Loci No.	Habitats	Gene frequency (%)
4	Engure OT	13.4
13	Engure OT	50.0
16	Lake Kaņieris	29.2

from different habitats (Lake Kaņieris, "Šalkas" gravel pit), which were formed at the former gravel deposit.

Individuals from Lake Kaņieris and "Šalkas" gravel pit population habitat are geographically distant, but genetically mutually similar, and this can be seen also by the genetic distance (Table 2), whith values varying from 0.068 to 0.092. The branch of the eighth group cluster forms a set of genetically distinctly different individuals, which characterise individuals from Lake Silabebri habitat. Values of orchid's genetic distance in this habitat vary from 0.317 to 0.654.

DISCUSSION

To genetically analyse the orchids from different habitats, first habitat plant communities with *L. loeselii* description and classification were surveyed. There are described nine habitats corresponding to four of the protected habitats of the European Union: 2190 (Humid dune slacks) – 1, 7140 (Transition mires and quacking bogs) – 2, 7210* (Calcareous fens with *Cladium mariscus* and species of the *Caricion davalianae* – 2, 7230 (Alkaline fens) – 4 (Auniņš, 2013).

Genetic polymorphism of populations can be influenced by many factors, including the history of population, diversity of ecological factors etc. (see, e.g., Hartl, 1988). In Latvia, populations of *L. loeselii* provided age differences which are depending from the peculiarities of the habitat formation. Our results show that the populations of *L. loeselii* growing in abandoned Šalkas gravel pit and Kaņieris NT were genetically homogeneous. These populations have formed rather recently. Extraction from Šalkas gravel pit was started in the 1994. Information about complete extraction of gravel on both parts of gravel pit is missing, but in 2009 the population of *L. loeselii* had successfully colonised this habitat. It is known that the flowering specimens of *L. loeselii* were detected in Germany four years after the completion of the quarry (Prochazka and Velisek, 1983). The opening of nature trails in wetlands, followed by reduction of the cover of trees and perennial herbaceous plants, creates suitable conditions for the growth of *L. loeselii* as it was found in Kaņieris NT.

According to our results the highest polymorphism was detected in the oldest populations of *L. loeselii* — Lake Engure near Lepste (84%), Engure OT (64%), Lake

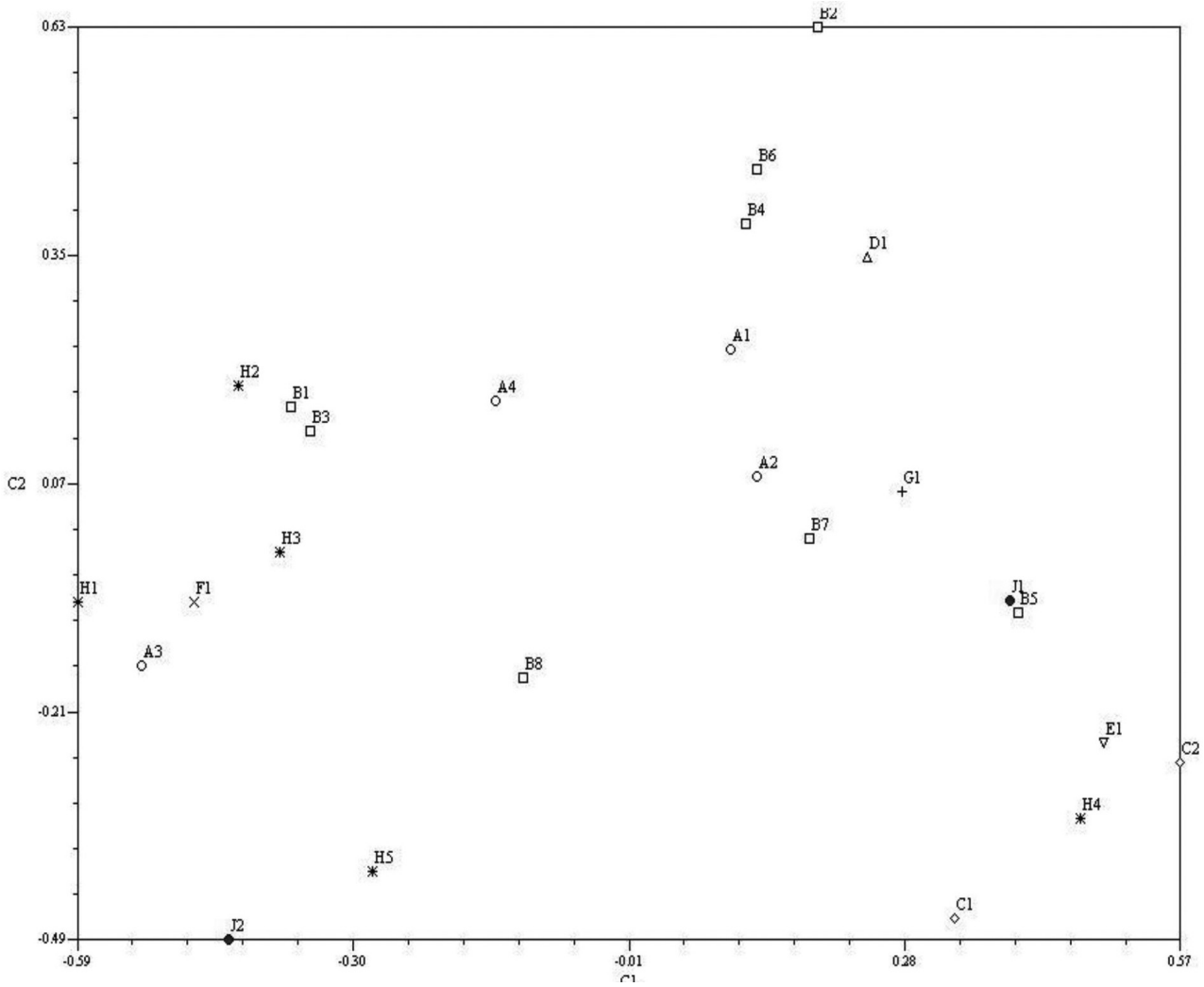

Fig. 3. Dispersion in the population of genetically different individuals of *Liparis loeselii*.

A, Engure OT; B, Engure near Lepste; C, Lake Kaņieris; D, Lake Kaņieris NT; E, "Šalkas" gravel pit; F, Lake Silabebri; G, Lake Būšnieki; H, Lake Dreimaņi; J, Bog Pēterezers.

Dreimaņi (56%), Bog Pēterezers (48%) and Lake Kaņieris (20%). The habitats of these localities are formed by gradual overgrowing of the lake or topographical depression near the lake. It provides an opportunity to create a new metapopulation of *L. loeselii*. It can be assumed that in a period of more than a hundred years (herbarium RIG material was collected near Lake Engure in 1906 and near Lake Kaņieris in 1897) population can accumulate mutations and increase polymorphism.

The effects of environmental factors on population polymorphism are best illustrated by studies in the population at Lake Būšnieki. This population also is located in the habitat that was formed by gradual overgrowing of the lake. In addition, the habitat is at the stage when the alkaline fen is transformed into a transition mire and is at a further stage of succession. Regardless of the age of population at Lake Būšnieki, the population was genetically homogeneous. Important environmental factors limiting growth and reproduction of *L. loeselii* include water level fluctuations and the competition as a result of succession (Rasmussen, 1995; Wheeler *et al*., 1998; Grootjans *et al*., 2006; Oostermeijer and Hartman, 2014).

In the ecological studies of Latvian populations of *L. loeselii* the most favourable growth conditions for *L. loeselii* was found in the habitats with partially water saturated substrate with balanced water supply and with the vascular plant cover which does not exceed 80%. The locality at Lake Būšnieki is characterised by these optimal growth conditions (Megre *et al*., 2018). It is possible that the fluctuating water that was detected in the habitats of populations of. *L. loeselii* at Lake Engure near Lepste, Engure OT, Lake Dreimaņi, Bog Pēterezers, and Lake Kaņieris (Megre *et al*., 2018), also has stimulated polymorphisms of this population.

Survey results do not show any correlation between the polymorphism and population size. The percentage of polymorphic loci varied among the populations of *L. loeselii*, in different habitats some of populations were genetically homogeneous. In genotyping of orchids from two habitat populations unique loci were found. The populations were clus-

tered mainly according to habitat type than to geographical location and distance between habitats.

It follows that the inner genetic diversity or plant genetic similarity of *L. loeselii* from different habitats is probably connected with the age of habitat and growing conditions.

There are few studies on the genetic diversity of populations whose habitats, like those of *L. loeselii*, are fragmented or isolated. There are different observations in some case studies (Hensen and Oberprieler 2005, Stöcklin *et al*., 2009). Although generally after fragmentation of populations the genetic drift led to variability decrease (Hartl, 1988) later on some other abovementioned factors like the age of populations and ecological diversity can accelerate the genetic diversity. Therefore, the population size should be taken into consideration in context with other factors influencing genetic polymorphism.

It is necessary to collect the different genetic material for *ex situ* conservation made in two directions — in collections of botanic gardens in field conditions and *in vitro.* Different genetic individuals collected of fen orchid, make the first conservation *in vitro* and the newly obtained regenerants transplant *ex vitro* — in established habitat models in botanic garden. There can be a possibility to test the surviving process in half-natural conditions and interaction with the characteristic and accompanied species like *in situ*. This competence contributes restock of the extinct or endangered species anew in natural habitats. However, it is not possible to argue about the adaptation to different growing conditions but should be determined by the genetic differences of population. The hypothesis can be confirmed or disproved by further growth monitoring of other similar circumstances of *L. loeselii* ecological studies.

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LIPARIS LOESELII ĢENĒTISKĀ DAUDZVEIDĪBA LATVIJĀ

Augu saglabāšanas globālās stratēģijas programmas ilgtermiņa plāns paredz, ka līdz 2020. gadam būtu jāsaglabā 75% no apdraudētām sugām *ex situ* kolekcijās. *Liparis* ģints ietver daudz sugu, bet tikai viena no tām — *Liparis loeselii* (L.) Rich. aug Eiropā. Latvijā *L. loeselii* tiek klasificēta kā trešā apdraudēto un aizsargājamo sugu kategorija. Lai varētu izstrādāt vislabāko aizsardzības stratēģiju, ļoti svarīgas ir zināšanas par aizsargājamo augu bioloģiskajām īpatnībām un to ģenētiskajām atšķirībām. Tika noteikta ģenētiskā daudzveidība *L. loeselii* populācijām no dažādiem Latvijas reģioniem. Šim nolūkam tika izmantota iPBS metode, kas balstās uz retrotranspozonu izvietojuma salīdzinājumu. Kopumā tika izanalizēti 54 paraugi no deviņiem dažādiem Latvijas reģionu biotopiem. *L. loeselii* augu lapās ir augsts fenolu saturs, kas samazina iegūtās DNS kvalitāti. Tika konstatēts, ka polimorfo lokusu daudzums procentuāli atšķīrās dažādos biotopos augošajām *L. loeselii* populācijām; dažas populācijas bija ģenētiski viendabīgas. *L. loeselii* ģenētiskās daudzveidības atšķirības ir saistītas ar populācijas vecumu un to augšanas apstākļiem.