

Population structure of *Liparis loeselii* (L.) Rich. in relation to habitat conditions in the Warta River valley (Poland)

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Abstract. The paper presents results of a study on the population structure of the orchid *Liparis loeselii* growing in the Warta River valley peat bog in Myszków (Woźnicko-Wieluńska Upland, S. Poland). Individuals of *L. loeselii* occurred in patches of *Menyantho trifoliatae-Sphagnetum teretis* and *Eleocharitetum pauciflorae* – associations. The demographic structure of *L. loeselii* populations was described by variations in plant height, the length and width of leaves and the number of flowers of the constituting individuals. It was observed that habitat conditions such as conductivity, pH and the percentage cover of tall perennials had the strongest effect on the structure of *L. loeselii* populations.

Key words: orchid, meta-population, rare species, fen, ecological site “Przygiełka” in Myszków

1. Introduction

Human activity causes habitat fragmentation and a decrease in the habitat area. This, in turn, causes a decrease in population abundance (Eriksson 1996). Small isolated populations are more vulnerable to extinction because of low genetic diversity (Oostermeijer *et al.* 2003). Peat bogs and wetland areas, on the one hand, are European habitats which undergo changes very quickly but, on the other hand, in peat bogs and wetland habitats many rare and endangered species may occur. Proper management and nature protection of rare and endangered species is possible only if their ecology, demography and genetics is well known and their processes are well understood (Oostermeijer *et al.* 2003). Currently, there is still a great deal that needs to be studied. Our knowledge and understanding of processes regarding population dynamics enables assessment and prediction of the number of individuals in the population of plant communities (Eriksson 1996). Studies of rare species demography provide important data about critical life stages and calculation of population growth rates (Harvey 1985; Crone *et al.* 2011). Relevant data

influencing protection effectiveness are also provided by research conducted on, for example, relative proportions of individuals in different ontogenetic stages of their life cycle (Oostermeijer *et al.* 1994). In this study, the research focuses on *Liparis loeselii* (L.) Rich., a small orchid, which occurs in Europe and North America. This orchid remains the only representative of the *Liparis* genus in Poland and also in whole of Europe. The number of its individuals is declining in its overall distribution in Europe, including in Poland (Kucharski 2014; Oostermeijer & Hartman 2014). Approximately, 300 localities of the species were recorded in Poland. Unfortunately, according to Kucharski (2014), half of the recorded localities no longer exist. *L. loeselii* has been given “endangered” species status in Central Europe (EN category) (Schnittler & Günther 1999), while in Poland, this species has been placed in the Polish Red Book of Plants in the category “vulnerable species” (VU) (Kucharski 2014), and on the “Polish Red List”, it has been placed in category E, i.e. critically endangered (Zarzycki & Szelağ 2006). In the Silesian Province, *L. loeselii* is considered to be endangered (EN) (Parusel & Urbisz 2012). This orchid species is

also protected by Polish Law (Regulation 2014) as well as by International Law, the Berne Convention (1979) and Council Directive (1992). Some *L. loeselii* populations growing in Poland are part of a national monitoring system (Kucharski 2010). Demographic monitoring conducted on the *L. loeselii* populations in the United Kingdom, on dune slack complexes (Jones 1998) and a fen (Wheeler *et al.* 1998) indicates that populations of this orchid are short-lived and rapidly expanding or disappearing as changes in site conditions take place (Jones 1998).

Due to high vulnerability of *L. loeselii* both in Poland and Europe, there is lack of data about conditions in which the species occurs as well as data about the population structure of this species. This study was undertaken in order to gather information on these population aspects in order to enable proper management and protection of this species.

The natural transition mire “Przygielka” in Myszków has the largest population of *L. loeselii* on a natural bog in the Silesian Province (Halabowski & Błońska 2015). This locality of *L. loeselii* became seriously endangered in 2014, and, therefore, it was decided to conduct this study there with the aim of characterizing the population at the newly found location.

This paper presents a spectrum of phytocoenoses and habitats in which *L. loeselii* occurs, and attempts to determine the impact of different habitat conditions on the population structure of the species in the Warta River valley. The conservation status of this particular population of the species is also assessed.

2. Material and methods

2.1. Study species

Liparis loeselii (L.) Rich. is a small, heliophilous perennial plant of the Orchidaceae family with an erect, straight and triangular stem (Kłosowski & Kłosowski 2006). The inflorescence bears small, inconspicuous flowers producing fruit in the form of an elevated bag. Two leaves usually grow from pseudobulbs (Szlachetko 2001). It is a geophyte (Zarzycki *et al.* 2002), in the early stages of development entirely dependent on mycorrhizal fungi; however, adults can sometimes be fully autotrophic (Procházka & Velíšek 1983; Pawlikowski 2004). This orchid is a weak competitor, has CSR life strategy, and generative propagation predominates over vegetative. Flowers usually are self-pollinated, and only occasionally small wasps fly to them (Zarzycki *et al.* 2002; Pawlikowski 2004), but it is not clear up to now if wasps are passive or active visitors. In Poland, *Liparis loeselii* is recognized as a species occurring with high fidelity in plant communities representing the *Caricion davallianae* alliance and locally the *Orchido-*

Schoenetum nigricantis association (Głazek 1992; Matuszkiewicz 2008). *Liparis loeselii* (L.) Rich. most often occurs on bogs rich in calcium carbonate (Pawlikowski 2004), rarely in transitional mires, short sedge acid fen wet meadows on sandy soils and in *Cladietum marisci* phytocoenoses (Piękoś-Mirkowa & Mirek 2003). It often occurs in anthropogenic habitats such as quarries, gravel and sand pits (Czylok & Rahmonov 1996; Czylok 1997; Dubiel & Gawroński 1998; Bzdon & Ciosek 2006; Pisarczyk 2006; Bzdon 2009; Błońska 2010; Molenda *et al.* 2012). In the region of Silesia, the species occurs most frequently in habitats created as a result of human activity, mainly on wetlands developing on old, currently unexploited sand pits, in areas with discharge of mineral-rich groundwater that infiltrated through the Triassic rock (Czylok 1997; Błońska 2010; Molenda *et al.* 2012). In the Silesian Province, it rarely occurs in natural peat bogs, and only forms small populations (Olesiński & Sendek 1980; Bernacki & Nowak 1994; Nowak 1999; Pisarczyk 2006; Błońska 2013).

2.2. Study area

The study was conducted in July 2014 in Myszków, on the transition mire located in the valley of the Warta River in northern part of the town (N 50° 35' 21", E 19° 17' 24") (Fig. 1). This bog has been subject to legal protection as an ecological site “Przygielka” since 2002 (Regulation 2002). It is located in the Woźnicko-Wieluńska Upland (Kondracki 2011). The upland is composed of Mesozoic (Triassic and Jurassic) rocks

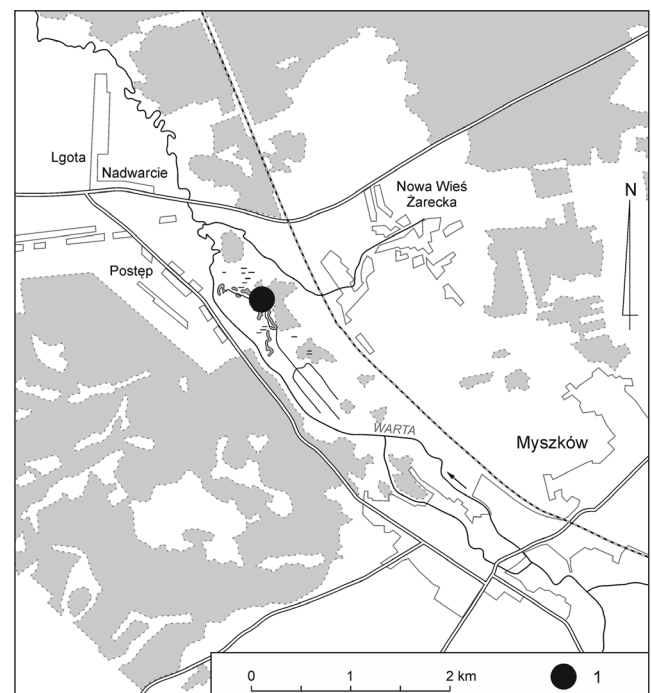


Fig. 1. Location of the studied population of *Liparis loeselii* in the ecological site “Przygielka” in the Warta River valley in Poland

consisting of limestone, sandstone, marl and clay. Lowering of the Upper Warta was established in little resistant ore-bearing clays of Middle Jurassic age. In the Quaternary, sands and glacial clay of varying thickness were deposited on clays (Dulias & Hibszer 2004). In the Warta valley and its affluents, peat soils, created under anaerobic conditions with large amounts of water and high accumulation of organic matter occur. Among them, there are muck-mineral, silty-peat and peat soils with maximum thickness of up to 4 m, with peatland vegetation, mainly transient (Myga-Piątek *et al.* 2003). The average annual rainfall reaches here 700-750 mm (Gil 2007). The average annual temperature ranges from 7.5-8°C (Miroszewski *et al.* 2010).

2.3. Field and laboratory studies

In vegetation patches of 16m² area, lists of plant species occurring together with fen orchids were recorded. All species cover was evaluated in the modified 13-point Braun Blanquet scale (Chmura 2014). Vegetation relevés were arranged in a phytosociological table. The syntaxonomic status of the species was adopted from Matuszkiewicz (2008) and Ratyńska *et al.* (2010), the names of vascular plants follow Mirek *et al.* (2002), and of mosses (Ochyra *et al.* 2003).

Population and habitat studies were carried out in smaller plots with an area of 4 m². Altogether, nine randomly distributed sample plots were set up in the area covering the entire *Liparis* population distribution on the peat bog "Przygiełka" in Myszków. From each recorded plot, water samples for further analyses of physico-chemical properties were collected. Electrical conductivity [$\mu\text{S}\cdot\text{cm}^{-1}$], pH, redox potential [mV], dissolved oxygen [%], concentration of minerals and nitrogen in the form of nitrate ions [$\text{mg}\cdot\text{dm}^{-3}$] were directly analyzed using the multi-parameter analyzer Professional Plus of YSI. Analyses of calcium, magnesium, sulfate and phosphate concentrations [$\text{mg}\cdot\text{dm}^{-3}$] were performed with a spectrophotometer (DR 5000 Hach Lange). For each sample plot, environmental factors were determined as they might influence occurrence and parameters of *Liparis* individuals according to the methodology of national monitoring system (Kucharski 2010). The analyzed vegetation traits included: height of the herb layer [cm], a place to germinate [%] (e.g. % of gaps not covered by plants and mosses), thickness of organic matter (mean from 20 measurements [cm]), habitat water content (defined in 5-point scale, where 1 means desiccated habitat and 5 water stagnant on the surface); separately cover by tall herbaceous perennial plants [%] (e.g. cover of herbaceous plants that could cause shadow to *L. loeselii* individuals), shrub cover [%] (e.g. cover of shrub and trees), herb cover layer [%], moss cover layer [%]. We only included environmental factors that are also used in the national plant species

monitoring scheme (Kucharski 2010).

In addition, *L. loeselii* individuals were counted in sample plots, specifying whether they were generative, vegetative or juvenile. The recognition of age structure makes it possible to determine population structure (Kucharski 2010). We also measured morphological features of a series of *Liparis* individuals using an electronic caliper with an accuracy of 0.1 mm. We performed the following six measurements on 70 flowering individuals: – plant height [mm], – length of the largest leaf [mm], – width of the largest leaf [mm], – inflorescence length [mm], – number of flowers, and – number of fruits.

The fruit set was also calculated, defining it as the percentage of flowers that developed ripe fruit. All morphological measurements were performed non-destructively, due to the fact that *L. loeselii* is legally protected (Regulation 2014).

Moreover, an assessment of habitat and population state was conducted, based on guidelines for species monitoring (Kucharski 2010).

2.4. Numerical and statistical analysis

Variability analysis was performed on the basis of morphological features of flowering plants. Variability of environmental factors was also analyzed. For each analyzed trait, basic statistics were provided: average, standard deviation, minimum and maximum. The calculations were conducted using Statistica 10. A Principal Component Analysis (PCA) was applied in order to show which features of plants were responsible for the diversity of *Liparis* individuals. In order to show the impact of environmental variables on the characteristics of individuals, CCA analysis was performed (Canonical Correspondence Analysis). Moreover, a Monte Carlo permutation test was performed. These analyses were performed with the assistance of CANOCO 4.5 software (ter Braak & Šmilauer 2002).

3. Results

3.1. Phytocoenotic spectrum

Individuals of *L. loeselii* occurred in patches of *Menyantho trifoliatae-Sphagnetum teretis* and *Eleocharitetum pauciflorae* association (Table 1). These patches were quite rich in species (27-42 plant species). Herb and moss layer was well-formed in both types of phytocoenoses. The main constituents of these phytocoenoses were calciphilous species, character species of the *Caricetalia davallianae* order, as well as *Scheuchzerio-Caricetea nigrae* class. Physiognomy of the *Menyantho trifoliatae-Sphagnetum teretis* phytocoenoses was formed by massively occurring *Menyanthes trifoliata*, and *Eleocharis quinqueflora*

Table 1. Floristic composition and structure of phytocoenoses with the participation of *Liparis loeselii* in Myszków

Relevé identification number	1	2	3	4	5	6	7	8	9	Frequency [%]	Cover Coefficient	
Field ID no. of relevé	23	22	1	123	2	1	21	124	18			
Cover shrub layer [%]	10	10	5	20	40	-	5	5	10			
Cover herb layer [%]	100	100	90	90	90	80	90	90	80			
Cover moss layer [%]	80	80	30	60	80	30	80	70	80			
Area of relevé [m ²]	16	16	16	16	16	16	16	16	16			
Number of vascular plants in relevé	35	31	21	24	31	27	35	35	28			
Number of mosses in relevé	6	7	6	5	6	9	7	5	5			
Number of all species in phytosociological relevé	41	38	27	29	37	36	42	40	33			
Plant community	<i>Menyantho trifoliatae-Sphagnetum teretis</i>					<i>Eleocharitetum pauciflorae</i>						
*<i>Liparis loeselii</i>	c	1	2	1	2	1	1	2	1	1	100	133
Ch. <i>Scheuchzerio-Caricetea nigrae</i>, *<i>Caricetalia davallianae</i>												
<i>Menyanthes trifoliata</i>	c	60	50	80	40	90	5	10	10	20	100	4056
<i>Eriophorum angustifolium</i>	c	10	10	10	40	10		5	2	2	89	1113
<i>Carex nigra</i>	c	10	10	10	20	2		5	20	10	89	1088
<i>Comarum palustre</i>	c	2	10	10	20	5	5	10	5	5	100	800
<i>Hydrocotyle vulgaris</i>	c	1		1	2	5	10	5	5	5	89	425
<i>Juncus articulatus</i>	c	2	2	1	2	2		2	2	2	89	188
* <i>Eleocharis quinqueflora</i>	c	10				1	80	40	50	20	67	3350
<i>Agrostis canina</i>	c	1		1			2	1	5	5	67	250
<i>Triglochin palustre</i>	c						10	10	2	5	44	675
* <i>Valeriana simplicifolia</i>	c	1	1		5		2	2			56	220
<i>Viola palustris</i>	c	1		2				1	5	1	56	200
* <i>Epipactis palustris</i>	c	2	2					1		1	44	150
<i>Carex diandra</i>	c			30				20		2	33	1733
<i>Rhynchospora alba</i>	c		10						5	2	33	567
* <i>Carex lepidocarpa</i>	c	1	2			5					33	267
* <i>Carex flava</i>	c	2					2		1		33	167
* <i>Carex pulicaris</i>	c					5					11	500
* <i>Carex davalliana</i>	c		2						5		22	350
<i>Drosera anglica</i>	c	1									11	100
* <i>Carex dioica</i>	c					1					11	100
<i>Sphagnum contortum</i>	d	20	20	5		10	5	10	20	30	89	1500
* <i>Scorpidium scorpioides</i>	d						2		5		22	350
<i>Sphagnum teres</i>	d	5					2				22	350
<i>Cinclidium stygium</i>	d	2	5				2				33	300
<i>Hamatocaulis vernicosus</i>	d		2	1	5	2					44	250
* <i>Campylium stellatum</i>	d						1	2			22	150
Sporadic species: <i>Carex echinata</i> 8(5)												
Ch. <i>Oxycocco-Sphagnetea</i>												
<i>Oxycoccus palustris</i>	c		20		10	1	5	10	10	20	78	1086
<i>Drosera rotundifolia</i>	c	1	1			1	1	1	2	1	78	114
<i>Sphagnum fallax</i>	d					2	2	30	20	20	56	1480
<i>Aulacomnium palustre</i>	d		2		5			5			33	400
Ch. <i>Molinio-Arrhenatheretea</i>												
<i>Galium uliginosum</i>	c	2	1	2	2	1	2	2	1	10	100	256
<i>Lythrum salicaria</i>	c	1		1	2	5	2	1	2		78	200
<i>Lysimachia vulgaris</i>	c	2	1			1		5	1		56	200
<i>Carex hirta</i>	c		5							5	22	500
<i>Succisa pratensis</i>	c				2			5			22	350
<i>Molinia caerulea</i>	c				2		2		2		33	200
<i>Cirsium palustre</i>	c		1			1		1			33	100
<i>Prunella vulgaris</i>	c						1		1	1	33	100
<i>Potentilla anserina</i>	c		1					1			22	100
<i>Sanguisorba officinalis</i>	c						1			1	22	100
Sporadic species: <i>Equisetum palustre</i> 3(2); <i>Epilobium palustre</i> 2(1); <i>Filipendula ulmaria</i> 5(1); <i>Climacium dendroides</i> d 3(2)												
Ch. <i>Phragmitetea</i>												
<i>Peucedanum palustre</i>	c	1	2	2	1		2	2		2	78	171
<i>Scutellaria galericulata</i>	c		1	1		1		1	1	2	67	117

Relevé identification number	1	2	3	4	5	6	7	8	9	Frequency [%]	Cover Coefficient	
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Cover moss layer [%]	80	80	30	60	80	30	80	70	80			
Area of relevé [m ²]	16	16	16	16	16	16	16	16	16			
Number of vascular plants in relevé	35	31	21	24	31	27	35	35	28			
Number of mosses in relevé	6	7	6	5	6	9	7	5	5			
Number of all species in phytosociological relevé	41	38	27	29	37	36	42	40	33			
Plant community	<i>Menyantho trifoliatae-Sphagnetum teretis</i>					<i>Eleocharitetum pauciflorae</i>						
<i>Typha latifolia</i>	c			2		2	10		30	44	1100	
<i>Carex rostrata</i>	c			20	5	1		5		44	775	
<i>Phragmites australis</i>	c	10	1							22	550	
<i>Carex elata</i>	c	2		2		10				33	467	
<i>Carex gracilis</i>	c	1				2				22	150	
Sporadic species: <i>Lysimachia thyrsiflora</i> 4(5); <i>Carex vulpina</i> 3(2); <i>Alisma plantago-aquatica</i> 2(1); <i>Glyceria fluitans</i> 6(1)												
Other species												
<i>Alnus glutinosa</i>	b	1	5	1	10	30		1	2	10	89	750
<i>Alnus glutinosa</i>	c	5	1	1		1	2	1		5	78	229
<i>Betula pubescens</i>	b	2			20	5			2		44	725
<i>Betula pubescens</i>	c	2	1					2			33	167
<i>Pinus sylvestris</i>	b	2				1		2	2		44	175
<i>Pinus sylvestris</i>	c	2			1				1		33	133
<i>Salix cinerea</i>	b	1		5							22	300
<i>Salix cinerea</i>	c	2	2	1	2	2			1	5	78	214
<i>Carex panicea</i>	c	10	5		5	2	5	2	5		78	486
<i>Potentilla erecta</i>	c	2	2		1	1	2	5	1		78	200
<i>Lycopus europaeus</i>	c	1						1	1	2	44	125
<i>Mentha aquatica</i>	c	1	1			1		1	2		56	120
<i>Utricularia vulgaris</i>	c					10	1	5			33	533
<i>Linum catharticum</i>	c				1		1			10	33	400
<i>Eupatorium cannabinum</i>	c						2		1	5	33	267
<i>Briza media</i>	c	2					2	2			33	200
<i>Carex viridula</i>	c	2				1					22	150
<i>Dactylorhiza incarnata</i>	c				2				1		22	150
<i>Utricularia minor</i>	c							1	1		22	100
<i>Limprichtia revolvens</i>	d	30	40	5	30	50	10	20	20	20	100	2500
<i>Rhynchospora squarrosus</i>	d	20	5	2	10	10	5	5	5	10	100	800
<i>Calliergonella cuspidata</i>	d	1	5	10	10	5	2	5		2	89	500
Sporadic species: <i>Frangula alnus</i> b 1(2); <i>Betula pendula</i> b 2(5); <i>Danthonia decumbens</i> 2(1); <i>Drosera ×obovata</i> 1(1); <i>Sphagnum fimbriatum</i> d 6(1)												

Explanations: date 24-25. 06. 2014; locality Ecological site "Przygielka"; * – species from the *Caricetalia davallianae* order

occurred in *Eleocharitetum pauciflorae* patches with large coverage. In the studied patches, some species of more acidic habitat like *Eriophorum angustifolium*, *Carex nigra* or *Oxycoccus palustris* were also numerous. Apart from typical bog species, also species characteristic of other classes of vegetation, mainly of *Molinio-Arrhenatheretea* and *Phragmitetea* classes were recorded in the studied vegetation patches. Tall perennial species belonging to meadow vegetation reached high cover in some patches, directly shading the (micro)habitat of *Liparis*. Shrub and tree layers did not reach high coverage.

3.2. Habitat spectrum

L. loeselii occupied a relatively well hydrated habitat, where water was visible on the surface after low pressure. It was characterized by pH ranging from 6.5 to 6.96 and electrical conductivity from 161.5 to 400.9 $\mu\text{S}\cdot\text{cm}^{-1}$. Average calcium concentration was 28.37 $\text{mg}\cdot\text{dm}^{-3}$, and magnesium ions – 7.06 $\text{mg}\cdot\text{dm}^{-3}$. Water sampled from the *Liparis* habitat contained an average of 33.22 $\text{mg}\cdot\text{dm}^{-3}$ of sulphate ions, while concentrations of nitrate (5.94 $\text{mg}\cdot\text{dm}^{-3}$) and phosphate (0.17 $\text{mg}\cdot\text{dm}^{-3}$) were relatively small (Table 2). All the analyzed water properties showed high variability.

Table 2. Characteristics of habitat of the *Liparis loeselii* study population

Parameter	Minimum	Maximum	Mean	Standard deviation
pH (median)	6.50	6.96	6.62	0.14
Dissolved oxygen [%]	23.00	55.00	41.11	9.56
Electrical conductivity [$\mu\text{S}\cdot\text{cm}^{-1}$]	161.50	400.90	244.51	68.79
Concentration of minerals [$\text{mg}\cdot\text{dm}^{-3}$]	105.30	260.65	158.44	45.06
Redox potential [mV]	169.70	197.80	182.61	9.85
Concentration of nitrate nitrogen N-NO_3^- [$\text{mg}\cdot\text{dm}^{-3}$]	4.79	10.27	5.94	1.70
Concentration of calcium ions Ca^{2+} [$\text{mg}\cdot\text{dm}^{-3}$]	11.60	43.70	28.37	9.39
Concentration of magnesium ions Mg^{2+} [$\text{mg}\cdot\text{dm}^{-3}$]	0.80	13.40	7.06	5.04
Concentration of sulphates SO_4^{2-} [$\text{mg}\cdot\text{dm}^{-3}$]	22.00	77.00	33.22	16.84
Concentration of phosphates PO_4^{3-} [$\text{mg}\cdot\text{dm}^{-3}$]	0.06	0.45	0.17	0.11
Height of a herb layer [cm]	30.00	50.00	37.78	8.33
Cover of tall perennial herbs [%]	0.00	40.00	12.22	14.6
Cover of shrubs and trees [%]	0.00	40.00	15.83	14.29
Place to germinate/bare soil [%]	0.00	20.00	11.11	7.41
Degree of hydration	3.00	4.00	3.44	0.53
Leaf thickness [cm]	0.00	2.00	1.11	1.05
Cover shrub layer [%]	0.00	40.00	12.22	14.60
Cover herb layer [%]	60.00	90.00	75.56	13.33
Cover moss layer [%]	20.00	100.00	53.33	25.98

L. loeselii individuals grew in places where average height of dense litter sward was nearly 40 cm, tall perennials covered from 0 to 40% of the surface and shrubs about 15%. Coverage of the herb layer was 60-90% and moss layer covered 20-100% of the area (Table 2).

3.3. Morphological differentiation and population structure

Flowering adult individuals reached an average height of almost 134 mm, but the range of this trait was considerable (from 52.6 to 247.6 mm). The length and width of the largest leaf were also highly variable, ranging between 35 and 207.7 mm and 11 and 36 mm, respectively. The mean number of flowers per inflorescence was 8, and ranged from 2 to 23. The number of set fruits was significantly lower with averaged value of 2.56. That was the reason for a very small percentage of fruiting individuals, reaching an average of

34%, although there were individuals which did not effectively initiate establishing fruits, as well as those for which the success of fruiting reached 100% (Table 3). *L. loeselii* individuals from Myszków showed relatively little variation in terms of metric traits (small gradient on the axes of PCA). The first two axes of the PCA explain 89% of the variance in the data set. The first axis accounted for more than 74% of the variance and was correlated with plant height, leaf length and width and the number of flowers. The second axis, explaining 15% of the variance, was correlated with fruit set (Fig. 2).

In total, we counted 154 individuals in 9 plots, among them 111 vegetative and 37 generative, although the size of the entire population of fen orchid was estimated at more than 200 individuals, which occurred across the area of approx. 3000 m². Calculations showed that 24% of individuals flowered (out of 154 analyzed). The number of juveniles and damaged individuals was negligible.

Table 3. Characteristics of *Liparis loeselii* individuals

Feature	Minimum	Maximum	Mean	Standard deviation
Plant height [mm]	52.60	247.60	133.96	39.05
Inflorescence length [mm]	13.00	115.80	46.10	22.21
Longest leaf length [mm]	35.00	207.70	103.81	33.04
Widest leaf width [mm]	11.00	36.00	21.06	5.46
Number of flowers	2.00	23.00	8.07	4.09
Number of fruits	0.00	10.00	2.56	1.95
Fruit set [%]	0.00	100.00	34.43	22.84

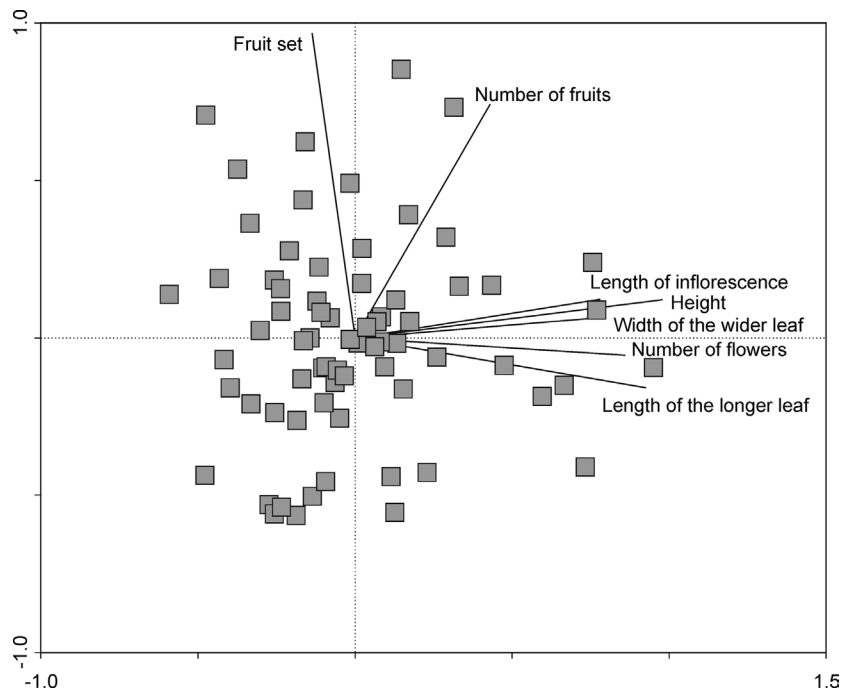


Fig. 2. Principal Component Analysis (PCA) of morphological measurements of *Liparis loeselii* individuals in the Warta river valley population in Poland

3.4. Impact of environmental conditions on inter-individual characteristics

Of the 19 analyzed environmental factors, 7 had a significant effect on individual characteristics of *L. loeselii* (Monte Carlo test). The most significant was electrical conductivity, which correlated negatively with

fruiting success and positively with plant height and leaf length. The pH and overgrowing by tall perennial herbs significantly positively affected the number of flowers and inflorescence length (Fig. 3). The first two axes of the CCA explained more than 97% of relationships between traits and environmental conditions.

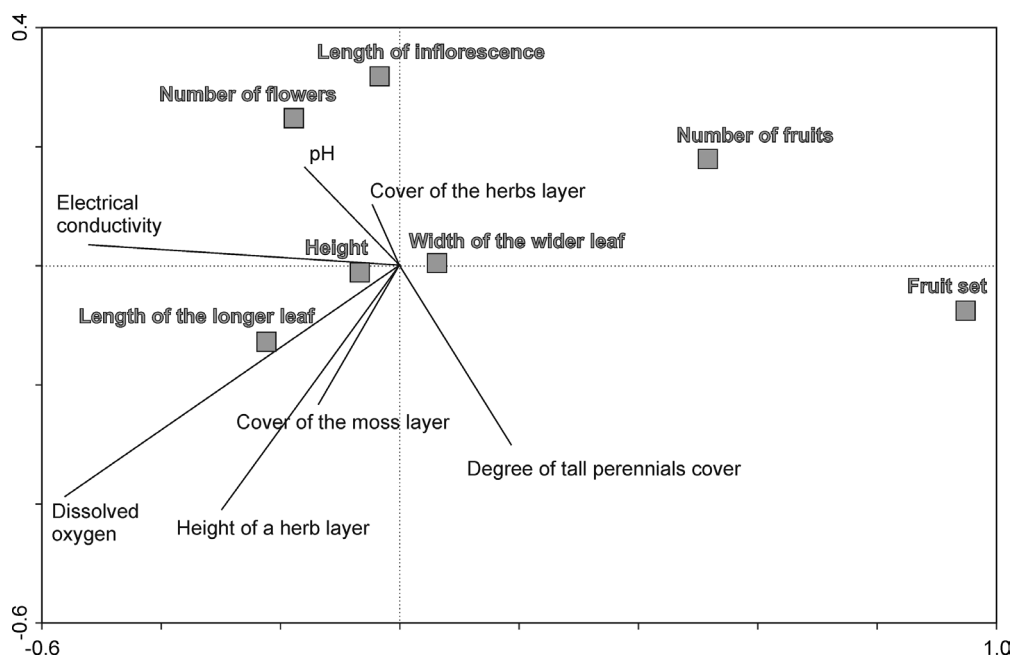


Fig. 3. Canonical Correspondence Analysis (CCA) showing the relationship between the characteristics and conditions of *Liparis loeselii* habitat in the Warta river valley in Poland

3.5. Evaluation of population status and habitat

The number of *L. loeselii* individuals, presence of generative as well as health condition (no damaged or injured individuals) allowed to consider the state of the *L. loeselii* population on the “Przygiełka” ecological site as appropriate according to the requirements of the national monitoring of plant species. In contrast, the state of habitat of this species according to the criteria set out in the guidelines of the national monitoring of plant species was unsatisfactory. This was due to: sward height and relatively large coverage of high perennial and expansive species such as *Phragmites australis*, *Typha latifolia*, *Eupatorium cannabinum* and some species of tall sedges in some patches; all the listed species shaded the habitat of fen orchid. Among current possible negative impacts on the *L. loeselii* population and habitat, one should include: a gradual succession of vegetation and trampling by people visiting the place.

4. Discussion

Average values of all analyzed *Liparis* specimen traits from Myszków fell within typical ranges given in the keys for plant recognition and descriptions of the species (Szafer *et al.* 1988; Rutkowski 2011; Wheeler *et al.* 1998; Szlachetko 2001, Pawlikowski 2004; Kłosowski & Kłosowski 2006; Oostermeijer & Hartman 2014). However, they were much higher compared to the results obtained by Nacz & Minasiewicz (2010) from Gdańsk Pomerania. This pattern applied to all analyzed traits with the exception of the success of fruiting. Although individuals of fen orchid from Myszków developed an average of nearly twice as many flowers compared to individuals from Gdańsk Pomerania (Nacz & Minasiewicz 2010), they established fewer fruits and the studied individuals were nearly half as successful in fruiting than individuals described from other localities. The maximum values of all the measured parameters were higher than those reported in literature (Szafer *et al.* 1988; Rutkowski 2011; Wheeler *et al.* 1998; Szlachetko 2001; Pawlikowski 2004; Kłosowski & Kłosowski 2006; Urban 2013; Oostermeijer & Hartman 2014).

There is little data on what the habitat requirements of *L. loeselii* are. It is known that it most often grows on peat soils, with neutral or alkaline to only weakly acidic water reaction (6.6–7.6–7.8) (Pawlikowski 2004; Kucharski 2014). Habitat of fen orchid in Myszków does not differ from the one reported in the literature in terms of pH. In terms of average values of pH and electrical conductivity, the analyzed habitat of *L. loeselii* did not differ from those of Poreba near Zawiercie (Błońska 2013), although the maximum value of the second parameter almost twice exceeded the value

from Poreba. It was also similar to the habitat where *L. loeselii* occurred on moors in the Netherlands in terms of coverage of moss layer, a place where the orchid to germinate most likely and thickness of litter. Coverage of herb and shrubs layer, as well as pH, were higher in Myszków than in the Netherlands (Oostermeijer & Hartman 2014).

The analyzed population was dominated by generative adults. Demographic studies on *L. loeselii* show that generative adults have the best chance of emergence in the next season (McMaster 2001). They are the least susceptible to changeable environmental conditions. Wheeler *et al.* (1998) showed that the population stability of *L. loeselii* is possible, despite short life span and high mortality of juveniles, due to rapid development of the new generation from seeds. The possibility of renewal from seeds is crucial for the survival of this species. The population of fen orchid from Myszków had a relatively large proportion of generative individuals, but on the other hand, it showed signs of disorders associated with dominance of adults and a small share of juveniles, which might indicate difficulties in recruitment of seedlings and/or mortality of juveniles and problems with renewing of the population.

Information about how the habitat conditions affect the health of populations and fen orchid individuals is rare. It is known that *L. loeselii* is a heliophilous species (Pawlikowski 2004). It is also a typical species of early successional stages (Jones 1998; Wheeler *et al.* 1998), it grows most abundantly in habitats with sparse vegetation on moist calcareous soils, where, in natural conditions, there is no competition of tall herbaceous plants (McMaster 2001). The study of Jones & Etherington (1992) showed that *L. loeselii* appeared between 12 and 15 years after initial vegetation establishment of the dune slacks.

The orchid started to decline when *Salix repens* shrub, developed a dense vegetation cover. Oostermeijer & Hartman (2014) recorded *L. loeselii* on dunes and bogs, which were generally characterized by taller vegetation with higher cover than in initial habitats of dune slacks. These authors attributed *L. loeselii* occurrence to the fact that they occur on sites with higher open areas than in the dunes. Therefore, tall perennials, such as common reed (*Phragmites australis*) or bulrush (*Typha latifolia*) may threaten the population of *L. loeselii* individuals. In the studied site in Myszków, the *L. loeselii* population may be endangered because of a highly expansive reed expanding species into the peat bog vegetation. In addition, our results also showed a negative impact of perennial species abundance on the number of flowers in the fen orchid. It was shown that abiotic factors were also important and influenced survival and population density and other population features of this orchid. Oostermeijer & Hartman (2014), studying bogs

in the Netherlands, revealed that there was a positive correlation between the number of individuals and pH and negative correlations between the thickness of the litter layer and number of individuals. In our study, no statistically significant correlations with these habitat parameters were found (Spearman correlation, not shown). Water level is an important factor influencing the condition of individuals of *L. loeselii*. This species is dependent on good hydration of its habitat and a decline in hydration adversely affects leaf size (Roze *et al.* 2014). When changes in the water table, floods or dry periods are temporary, the species does not flower and survives in the vegetative stage. When the changes are permanent, however, the population may disappear after three or four years. In some studies, a reduction in the number of individuals was observed much earlier (Jersakova & Kindlman 2004). Roze *et al.* (2014) reported the same observation. It is to be expected, therefore, that also in Myszków, any reduction in water level will be detrimental to the species. Recent dry and hot summers increase the likelihood of such events. As determined by the authors of the Report of the IPCC (2007, 2013), increasing temperature extremes and heavy rains may cause either dehydration or flooding of peat bogs. According to the climate model, heat waves as these recently observed are likely to become more frequent, longer and more intense (Kiktev *et al.* 2003; Meehl & Tebaldi 2004; Beniston *et al.* 2007; Kürbis *et al.* 2009; Pongrácz *et al.* 2013).

Although the current status of fen orchid population in Myszków is satisfactory, it is necessary to conduct regular monitoring, both of the population as well as environmental conditions. The previous study showed that population life span of the species was very short, between 5 and 15 years and most of the population would disappear from the vegetation patch in the period between 4 and 8 years from its colonization (Eriksson 1996; Oostermeijer & Hartman 2014). Research conducted by Oostermeijer & Hartman (2014) suggests that *L. loeselii* must have highly dynamic meta-populations. According to the meta-populations theory, a species can exist as a system of groups of connected populations between different vegetation patches, habitats, fragments, so protection of species meta-populations should focus on keeping balance between colonization and extinction processes (Hanski 1999). The population genetic study conducted across Europe revealed that genetic differentiation between populations was generally low, and genetic variation within populations relatively high for a self-pollinating species (Pillon *et al.* 2007). These results suggest that a mechanism enabling dispersion among populations and habitat fragments must exist, e.g. multiplied foundation of a population. This might be possible due to a far distance dispersing system used by this orchid. The study conducted in the

Netherlands proved that colonisation and a new population can be founded in a long distance (>25 km). On Dutch Wadden islands, seed migration between islands almost > 100 km away was recorded (Pillon *et al.* 2007; Vanden Broeck *et al.* 2014).

In our study, the *L. loeselii* population closest to those growing in “Przygiełka” in Myszków was located in a protected area of Poreba near Zawiercie (Błońska 2013) which was about 15 km south. However, the Poreba population was low in species and it could hardly be a population source for the “Przygiełka” in Myszków *L. loeselii* locality. In the radius of 50 km from the “Przygiełka” in Myszków population, other 15 localities of *L. loeselii* were found (Błońska 2010) and this might be a population source for the “Przygiełka” in Myszków population. Among the above-mentioned 15 locations, the one located in Dąbrowa Górnicza (25 km away from Myszków) was probably initially the population source for our study object.

Such distance is not too far for orchid seeds to be dispersed, particular since the wind here blows mostly from the south supporting such dispersal.

The Dąbrowa Górnicza population developed in a primary habitat on an open cast sand pit which was left abandoned since the end of the 20th century. This population is currently the richest in individuals in southern Poland. About several thousand *L. loeselii* individuals were recorded (Kucharski 2014). Taking all the above into consideration, the “Przygiełka” in Myszków population is not isolated, there are neighboring populations and potential habitats (e.g. the Warta valley and the Warta river tributary as well as other appropriate habitats often of anthropogenic origin) which might be colonized by *L. loeselii* individuals creating a population constituting part of the above-mentioned meta-population of the region.

However, because of short life span, fragmentation, changes of habitat patches and high rates of local extinction crucial for the meta-population survival is existence and/or creation of initial open habitats. Such open habitats might be created by peat removal enabling *L. loeselii* seeds to establish (Eriksson 1996; Oostermeijer *et al.* 2003, Oostermeijer & Hartman 2014).

In order to protect *L. loeselii* meta-population, it is important to maintain the existing population as long as possible. This can be achieved on condition that the existing habitats are left unchanged which requires occasional mowing or removing shrubs and, definitely, leaving the hydrological conditions unchanged (Grootjans *et al.* 2002). Persistence of the *L. loeselii* population will depend on proper management of the area under consideration. This objective can be reached thanks to application of appropriate protection support tools and regular monitoring of the habitat conditions and population characteristics.

Acknowledgements. We are grateful to the anonymous Reviewer for constructive comments to our manuscript. This work was financially supported by Ministry of Science and

Higher Education in years 2010-2013 (grant No. N N305 384938).

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