DE GRUYTER

Biologia **72**/1: 24—35, 2017
Section Botany
DOI: 10.1515/biolog-2017-0002

The current state of steppe perennial plants populations: A case study on *Iris pumila*

Ivan Yu. Parnikoza[1], Igor O. Andreev[1], Olena M. Bublyk[1], Kateryna V. Spiridonova[1], Joanna Gołębiewska[2], Magdalena Kubiak[2], Anna Kuczyńska[2], Katarzyna Mystkowska[2], Natalia Olędrzyńska[2], Barbara Urasińska[3], Anna Ślęzak-Parnikoza[2], Marcin Górniak[3], Krzysztof Wojciechowski[4], Yakiv P. Didukh[5] & Viktor A. Kunakh[1]

[1]*Institute of Molecular Biology and Genetics NAS of Ukraine, Acad. Zabolotnogo Str. 150, 03143 Kyiv, Ukraine; e-mail: ivan.parnikoza@gmail.com*
[2]*Department of Plant Taxonomy and Nature Conservation, University of Gdańsk, Wita Stwosza Str. 59, PL-80-308 Gdańsk, Poland*
[3]*Department of Molecular Evolution, University of Gdańsk, Wita Stwosza Str. 59, PL-80-308 Gdańsk, Poland*
[4]*Administration of Regional landscape parks of Lublin Vojevodship, Graniczna Str. 4, 20-010 Lublin, Poland*
[5]*M. Kholodny Institute of Botany NAS of Ukraine, Tereshchenkivska Street 2, 01601 Kyiv, Ukraine*

Abstract: A comprehensive study of a typical steppe perennial *Iris pumila* L. were carried out in the central zone of the European part of this species' range, namely in Ukraine. Intraspecific differentiation, population size and isolation degree and its consequences, the threat of human impact were analyzed, as well as ecological amplitude and genetic variation in ISSR markers and selected chloroplast regions were determined. The species was found to have a low intraspecific differentiation that indicates the uniformity of the gene pool in the studied part of the range. Moreover, the results of isolation assessment, population and ecological study of *I. pumila* confirm the potential risk of extinction. A considerable part of the species populations exist as separated patches of rare ecosystems, isolated from the nearest neighbours due to intense plowing of the steppe zone. The generative reproduction is rare. In contrast, ISSR analysis revealed comparably high genetic diversity in all the sampled populations. Furthermore, specific plastid haplotypes were demonstrated in some of them. The inconsistency between the results of population ecological study and the data of molecular genetic analysis indicates that the loss of genetic diversity in the species caused by habitat fragmentation and isolation under increasing anthropogenic pressure is likely to be slower than it would appear judging from the assessment of population parameters, which clearly show negative trends. This result also emphasizes the necessity for integrated approach to assessment of the extinction risk for particular species and careful analysis of all determinants of extinction.

Key words: *Iris pumila*; genetic heterogeneity; genetic erosion; population biology; risk of extinction; steppe zone; Ukraine.

Abbreviations: AMOVA, analysis of molecular variance; CTAB, cetyltrimethylammonium bromide; DMSO, dimethyl sulfoxide; ISSR, Inter-Simple Sequence Repeat; obl., oblast; r-n, raion; vil., village.

Introduction

Formation of Eurasian steppe belt is associated with the climatic factors of the Holocene first of all. Besides, starting from the Neolith, expansion of the steppe biome to the North was promoted by the deforestation of the forests by the first grain growers and cattle herders (Motyka 1946; Bezusko et al. 2011). At the same time, the steppe vegetation spread widely from the initial locations. However, due to the human activity during the XVIII-XX centuries related to the plowing of the steppe for agriculture, the area of steppe was greatly reduced. For example, in Ukraine, unplowed fragments of virgin steppes occupy now only 3% of the steppe zone. The Red Book of Ukraine includes 826 species of vascular plants, of which 33.4% are found only in the steppe biotopes (Parnikoza 2008; Didukh 2009). Steppe biome and steppe plant species are also endangered in other countries of Eurasia (Poradniki 2004). Habitat fragmentation is accompanied by the reduction in habitat size and then in population size. The latter in turn may result in erosion of genetic variation through the loss of alleles by random genetic drift and eventually lead to local population or even species extinction (Young et al. 1996; Young & Clarke 2000). The situation is aggravated by the high degree of isolation of remnant patches of steppe due to increased fragmentation and this significantly reduces or eliminates the exchange of genetic material between populations (Aguilar et al. 2008; Ewers & Didham 2006, Honnay

& Jacquemyn 2007). The above-mentioned indicates a big importance of steppe ecosystems and the need for protecting them for conservation of biodiversity.

Effects of habitat fragmentation on the populations and genetic variation of perennial steppe plants have been investigated to considerably lesser extent compared to plant species of high-mountain, island, polar, and other regions (Wróblewska & Brzosko 2006). However, the numerous studies of the latter species have demonstrated decreased isozyme variability in smaller populations, especially when species have become increasingly rare because of habitat fragmentation and destruction (Lienert 2004). It was shown that habitat fragmentation can result in increased genetic differentiation of populations, when they are isolated and when they lose alleles due to genetic drift (Lienert 2004). Moreover, increased selfing (in plants) and mating among closely related individuals in small populations may result in inbreeding and a reduction in the number of heterozygotes, and consequently lead to reduced fitness of individuals and to reduced viability of the entire population.

The data cited above (for more detail, see also Lienert 2004; Honnay & Jacquemyn 2007) allow to presume that the increased fragmentation of habitat, isolation of remnant populations, and other adverse factors may cause the loss of genetic diversity of steppe perennial plants. To test this assumption the modern approaches to evaluation of genetic erosion can be used, which include analysis of the degree of intraspecific differentiation (presence of races, ecotypes or subspecies); number and isolation of populations, their size, width of the ecological amplitude, frequency of generative reproduction; direct evaluation of genetic divergence by marker loci (isoenzymes or DNA) (Brown et al. 1997). Especially molecular markers can provide evidence to modern status of endangered species (Fisher et al. 2000; Xie et al. 2001). A little is known about intraspecific genetic heterogeneity of clonal plants, like irises. For instance *Iris sibirica* L. demonstrates very low genetic heterogeneity between subpopulations due to habitat fragmentation or other reasons, but gene flow between populations probably exist (Kostrakiewicz & Wróblewska 2008). Genetic characteristics or all other parameters of genetic erosion are virtually unexplored for most of steppe perennial plants of the European steppe zone, like steppe clonal *Iris* species. Especially unknown is the impact of deterioration of populations, their isolation, and decrease in populations' number and size on genetic diversity.

The aim of our study was the comprehensive assessment of the current status of a typical steppe perennial *Iris pumila* L. from the territory of Ukraine as a model species for investigating the consequences of habitat fragmentation for perennial steppe vegetation.

Material and methods

As an object of the study, we have chosen *Iris pumila* L., a typical xerophyte of the European steppe zone and ornamental plant useful for breeding. Ukrainian populations of the species are in the central part of the range of *I. pumila* that stretches from Austria to West Siberia, including the Southern Balkans, Anatolia and the Northern Caucasus (Fig. 1a). However, its abundance is not uniform throughout the range. While the species is common in Hungary and along the periphery of its range in Europe, it is rare in the Czech Republic, Slovakia, and Croatia (Dalmady 1972; Purger et al. 2008; Eliáš 2015; Hoskovec 2007). It also appears to be completely extinct from Southern Poland (A. Cwener, personal communication 2012).

In Ukraine, the species is currently relatively common. Its current range in Ukraine encompasses the steppe zone, northern part of the forest-steppe zone and the Crimea Mountains. The species has been found in a large number of remains of steppe landscapes and on the territory of all reserves in the steppe zone (Vedenkov et al. 1989; Tkachenko et al. 1998; Ostapko 2001; Shelegeda & Shelegeda 2008; Didukh 2009). Importantly, the species is pretty common in the steppe zone, just as in Hungary, while it is rare closer to the verge of the forest-steppe zone, mirroring its scarcity along the borders of its Central European range. In this way, the species status in Ukraine represents a miniature of that in Europe and can be informative on the early of speciation.

In whole 14 populations of *I. pumila* from the steppe zone and forest-steppe zone were examined during the growing seasons of 2004, 2005, 2010–13 (Fig. 1b) to study population and ecological indicators. Due to different accessibility of the populations and our limited technical capabilities, only populations # 1, 3–8, 10–12, and 14 were included in the analysis of chloroplast markers and only populations # 1, 3, 4, and 7 were subjected to ISSR-PCR analysis.

Our study was performed according to the guidelines provided for the assessment of genetic erosion by Brown et al. (1997). At first we analyzed the published data to examine the degree of intraspecific differentiation of *I. pumila* in Ukraine: presence of races, ecotypes or subspecies. This question still remains open, so this part of the study is presented in the Results section like short literature review.

The next part of this paper describes the current status of the populations, especially the size of population, density of clonal individuals and age structure were determined using traditional approaches (Shvets 2004; Parnikoza et al. 2008; Didenko & Shvets 2009; Didenko et al. 2010). In our study, the group of shoots growing from a common rhizome was considered as an individual plant. The density of individuals was studied on 3–5 squares 5 × 5 m and mean value was calculated. For age spectrum analysis we distinguished pre-reproductive stage, i.e. small plants with some leaves and initial root system (seedlings), and reproductive (generative) stage (both flowering and no flowering in current year), i.e. developed plants with a lot of old shoots with leaves on rhizomes, according to detailed studies of this species ontogenesis in Ukraine performed by Shvets (2004) and Didenko et al. (2010). The effectiveness of generative and vegetative reproduction was also studied.

To evaluate ecological amplitude of *I. pumila,* standard synphytoindication method was applied according to Y. Didukh (2011). The main ecological indicators were estimated as follows: soil humidity, variability of damping, soil acidity, soil salt regime, carbonate content in soil, mineral nitrogen content of soil, soil aeration, thermal regime, ombroregime (aridity or humidity), continentality of climate, cryoregime and light. The own geobotanical descriptions of the studied populations and the data from the phytocenological database of the Institute of Botany of NAS of

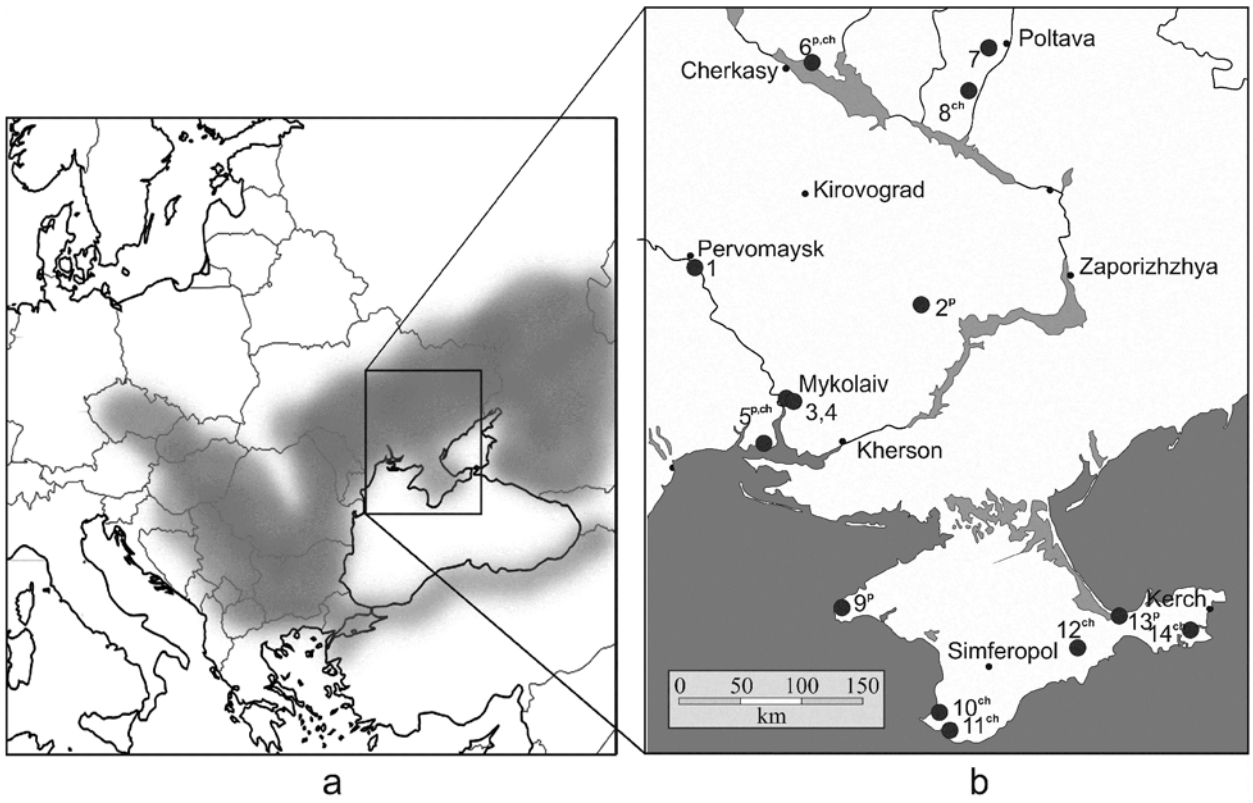

Fig. 1. Distribution of *Iris pumila* in Europe (a) and location of sampled populations of the species in Ukraine (b): 1 – vil. Mygiia (Pervomaisk raion, Mykolaiv oblast); 2 – vil. Zelene (Shyrokovski r-n, Dnipropetrovsk obl.); 3 – Aliaudy (Mykolaiv city); 4 – vil. Kolarovo (Zovtnevyi r-n, Mykolaiv obl.); 5 – vil. Dmytrivka (Ochakiv r-n, Mykolaiv obl.); 6 – vil. Prydniprovske (Chornobai r-n, Cherkasy obl.); 7 – vil. Andriivka (Reshetylivka r-n, Poltava obl.); 8 – Nature Reserve Drabynivka (Kobeliaki r-n, Poltava obl.); 9 – Tarkankut Peninsula, Chornomorski r-n (AR of Crimea); 10 – Inkerman (Sevastopol city, AR of Crimea); 11 – Balaklava (Sevastopol city, AR of Crimea); 12 – Staryy Krym (Kyrovske r-n, AR of Crimea); 13 – Kerch Peninsula (AR of Crimea); 14 – vicinities of Kerch (AR of Crimea). Superscript letter after number indicates that the population was studied partially: 'p' – only population study was done and/or 'ch' – chloroplast sequence analysis was done.

Ukraine were included in the assessment. For each ecological indicator, the maximum and minimum values, weighted mean, and dispersion were then calculated. To examine relationships between the variations in the ecological indicators, linear regression analysis was used.

ISSR-analysis of total DNA and analysis of variation in chloroplast DNA sequences were used to assess genetic diversity of the species. In ISSR-analysis, 40 plants from 4 populations: Andriivka, Aliaudy, Kolarovo, and Mygiia (9–11 plants from each) were examined (Fig. 1b). Due to technical limitations we included in this analysis only four populations, nevertheless we tried to choose the populations from different parts of the region and ones with different size. DNA was extracted from dried leaves by CTAB-method according to Doyle & Doyle (1987). In total, 7 ISSR-primers were used: ISSR-03 – $(AC)_8TT$; ISSR-05 – $(AC)_8TG$; ISSR-59 – $(AG)_8GC$; UBC#810 – $(GA)_8T$; UBC #811 – $(GA)_8C$; UBC #835 – $(AG)_8YC$; UBC #840 – $(GA)_8YT$ (Y = C,T). PCR mixture was 20 μL in volume and contained 20 ng DNA, 0.2 mM dNTP, 1.25 U Taq-polymerase, 0.5–1 μM of primer, 1 × PCR buffer with $(NH_4)_2SO_4$ (Fermentas, Lithuania), and 2 mM $MgCl_2$. As a negative control, standard reaction mixture without DNA was used. Amplifications were performed on a Tertsyk MC2 thermocycler (DNA technology, Russia) in following conditions: 95 °C, 2 min, 35 cycles of 94 °C, 20 s; 53 °C, 30 s; 72 °C, 90 s; 72 °C, 5 min.

PCR products were separated by electrophoresis in 1.5% agarose gel in 1× SB buffer, visualized by staining with ethidium bromide, and photographed under ultraviolet light. Electrophoregrams were presented as a binary matrix, which was used to calculate Jaccard genetic distances between plants, the percentage of polymorphic amplicons (P), Shannon index (S), Nei's unbiased gene diversity (unbiased expected heterozygosity, He), and Nei's unbiased genetic distances between populations.

Analysis of molecular variance (AMOVA) was used to describe the partitioning of genetic diversity between and within two regions (Mykolaiv and Poltava oblasts) and four populations. Genetic parameters were calculated and dendrogram was constructed using FAMD (Schluter & Harris 2006) and GenAlEx software (Peakall & Smouse 2006).

Based on the data of Shaw et al. (2007), the chloroplast DNA markers *trnL-trnF* and *trnG-trnS* were used to study inter- and intrapopulation genetic variation in the species. To amplify plastid sequences, from 2 to 7 plants of each population (except for the population from vil. Zelene) were chosen. Biometra T Gradient and Eppendorf Mastercycler were used. *trnS-trnG* intergenic spacer was amplified using trnS(GCU) and trnG(UCC) primers (Shaw et. al. 2007). The region including *trnL* intron and *trnL-trnF* intergenic spacer was amplified using primers trnL-c and trnL-f described by Taberlet et al. (1991). PCR amplifications were carried out in a total volume of 25 μL containing 1× buffer, 2 mM MgCl2, 0.2 mM dNTPs, 0.2 μM of each

Table 1. PCR conditions for chloroplast markers amplification.

Amplified region	Initial denaturation	Denaturation	Annealing	Elongation	Final elongation	No. of cycles
trnS-trnG	80 °C / 5 min	95 °C / 60 s	50 °C / 60 s	65 °C /4 min	65 °C / 7 min	30
trnL-trnF	94 °C / 4 min	94 °C / 30 s	55 °C / 30 s	72 °C / 2 min	72 °C / 10 min	30

primer, and 1.0 unit of Blue Perpetual DNA polymerase (Eurx, Gdańsk, Poland). PCR condition for all chloroplast regions are described in Table 1. PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Germany). Cycle sequencing was carried out by using the Big Dye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., ABI, Warrington, Cheshire, UK) with the same primers used for PCR amplification. The 10 μL reaction mixture contained 1× sequencing buffer, 1.0 μL of Big Dye terminator with 1.5 μL of 1 μM primer, 100–200 ng of amplified product, and 5% DMSO. Cycle sequencing conditions were as follow: 25 cycles each with 15 s denaturation (94 °C), 5 s annealing (52 °C) and 4 min elongation (60 °C). The sequences were generated on an ABI 3720 automated capillary DNA sequencer in the Genomed LLC (Poland). Both strands were sequenced to assure accuracy in base calling. Finch TV (Geospiza) was used to edit the sequences, and the two complementary strands were assembled by using Auto Assembler (ABI). All sequences were aligned by eye using SeaView v. 4 (Gouy et al. 2010). The chloroplast sequence data were deposited in the GenBank database under the following accession numbers: KU310494-KU310529 (*trnL-trnF* intergenic spacer) and KU310530-KU310541 (*trnG-trnS* intergenic spacer). Due to the low variation of *trnS-trnG* marker revealed after sequencing the accessions from the population of Mygiia and technical limitations only individual plants from each population were analyzed as indicated in Table 5.

Results

Degree of intraspecific differentiation: presence of races, ecotypes or subspecies

G.H.M. Lawrence distinguished *Iris pumila* among other species of bearded iris (subgenus *Iris*, section *Iris* Rod.) on the basis of cytological and systematics data presented by Randalph. *I. pumila,* treated by Lawrence as a type of series *Pumilae*, belongs to a small, evolutionary variable and sufficiently distinguishable group of species, which also includes *I. timofejewii* Woron., *I. glaucescens* Bunge, *I. scariosa* Willd. ex Link (cited according to Sikura & Shisha 2010). Members of the mentioned group differ from others by an intriguing complex of characters: pollen grains have warty exine, seed pods start to break open before maturation, the spathes are connected at the base with other leaves of plant (Sikura & Shisha 2010). All other species of the series do not possess these characters. In 1934, Romanian botanist J. Prodan put hypothesis that true *I. pumila* grows only in Transylvania and every other forms belong to different species or subspecies.

Within the borders of broad range of *I. pumila*, G. I. Rodionenko (cited according to Sikura & Shisha 2010) pointed out the existence of the forms or potential subspecies, which are difficult to recognize using morphological characters. Mainly this is a typical Balkan-Pannonian *I. pumila* ssp. *pumila*. In the east of the European part of Russia, the Northern Caucasus, and Western Siberia, *I. pumila* ssp. *taurica* (Lodd.) Rodion. et Schewcz is a very common plant, whereas *I. pumila* ssp. *aequiloba* (Lĺdeb.) Baker is common in Ukraine from Low Dnieper to Azov sea coast. Not considering the variation in flower colour and other traits, in practice these subspecies are not distinguished (as well as they do not have diagnostic features, e.g. karyological, chromosomal or DNA-markers and other, to distinguish). In the main Ukrainian taxonomic sources (Dobrochaeva et al. 1999; Mosyakin & Fedoronchuk 1999; Yena 2012), this species is mentioned without subspecies. In the same way this species is specified in the Red Data Book of Dnipropetrovsk oblast (Kucherevskyi 2001, 2004). In the Red book of Azov sea region (Ostapko & Kolomyichuk 2012), two subspecies are mentioned for Ukraine according to opinion of G.I. Rodionenko, however their distinctive characters and borders of distribution are not specified. Furthermore, according to the World checklist of selected plant families, these subspecies are not considered as valid now and regarded as synonyms of *I. pumila* ssp. *pumila*.

It was noticed that across the wide range of *I. pumila*, various forms occur, which are differed in some morphological traits: narrow or wide leaves, flowering shoot with single or multiple flowers, wide range of flower color, up to 19 forms were counted (Ostapko & Kolomyichuk 2012), and in biology: early flowering and late flowering forms, more or less hydrophilic, nevertheless they are not taxonomically important (Sikura & Shisha 2010).

It should be mentioned that physical appearance of all the accessions of *I. pumila* sampled from the studied populations located in steppe zone and forest-steppe zone of Ukraine matched identification keys mentioned for the species in the Manual (Dobrochaeva et al. 2009).

Range of distribution, degree of isolation, abundance and size of populations, frequency of generative reproduction

The *I. pumila* range is now highly fragmented and reduced due to intense human activity. The size and degree of isolation of *I. pumila* populations vary significantly in Ukrainian part of the range. The species is very rare in the forest-steppe zone. There are only a few isolated populations reported in Kyiv and Poltava oblast. Here *I. pumila* was found in the fragments of meadow steppe communities along the upper part of slopes of river valleys (Chopyk et al. 1998; Bairak & Stetsiuk 2005; Bairak et al. 2006). In natural steppe

Table 2. Main characteristics of some investigated populations of *I. pumila*.

Population	Andriivka	Prydniprovske	Dmytrivka	Aliaudy	Kolarovo	Mygiia	Tarkhankut Peninsula	Kerch Peninsula
Year of assessment	2012	2012	2010	2010	2010	2010	2010	2010
Total grass cover, %	100	100	50	45–50	60	90–100	50	70
Cover of *I. pumila*, %	1	10	1–5	1–5	1	1–5	1	1
Population size, individuals	~ 50	~ 1000	~ 100	~ 40	~ 50	> 1000	~ 50	1–5
Density, ind./m^2	1.3±0.3/0.5	4.7±0.2/0.4	4.5±0.3/0.7	1.6±0.3/0.6	2.6±0.3/0.3	9.8±0.4/0.8	1.8±0.2/0.3	1.2±0.2/0.3
Presence of: pre-reproductive plants (seedlings)	10	20	20	5	10	20	0	0
reproductive plants, %,	90	80	80	95	90	80	100	100
particularized clumps of plants	not estimated	+	+	+	–	–	–	+

part of Ukraine, this is a typical species. As a result of disintegration of initially uninterrupted steppe zone area into smaller steppe fragments, populations of *I. pumila* are strongly isolated from each other. For now, most of these areas have been preserved just because they are not suitable for agriculture (see Parnikoza 2011).

The greatest part of unplowed steppe fragments is preserved in Luhansk and Donetsk oblasts, and in Crimea, so it is believed that the largest populations of *I. pumila* are concentrated right there (Biodiversity Support Program 1999; Bokov 1999; Parnikoza 2008). In Crimea, this species occurs in steppe fragments in the central part of Peninsula, mainly at the Perekop isthmus and the coasts of Sivash (Ostapko & Kolomyichuk 2012), Kerch and Tarkhankut Peninsulas, as well as in fragments of the Crimea Mountains flat steppes, known as Yaylas, and on the slopes of Crimea South Coast.

The size of sampled populations varies within a wide range (Table 2). The studied populations on Kerch Peninsula are represented by small groups of several individuals each. Some forest steppe populations (Andriivka) and steppe ones: Aliaudy, Kolarovo and Tarkhankut Peninsula consist of only a few dozen of plants. Steppe population in Dmytrivka consists of about 100 plants. Populations from Prydniprovske (forest-steppe) and Mygiia (steppe) are the largest and consist of near 1000 or more than 1000 individual plants; however the size of these populations obviously has been also reduced due to agricultural activity.

Age structure analysis of all sampled populations (Table 2) proves the predominance of reproductive individuals. Pre-reproductive plants are very rare in the populations and can be found mostly on the peripheries of large clumps. The sampled populations can be classified as incomplete-membered with right-handed distribution pattern (dominance of reproductive plants).

In some population we found the cases of particularized clumps of plant. An increase in the size of individual clumps was often accompanied by the particularization (fragmentation) of clusters of individual shoots originating from a common rhizome that can be regarded as a sign of senility.

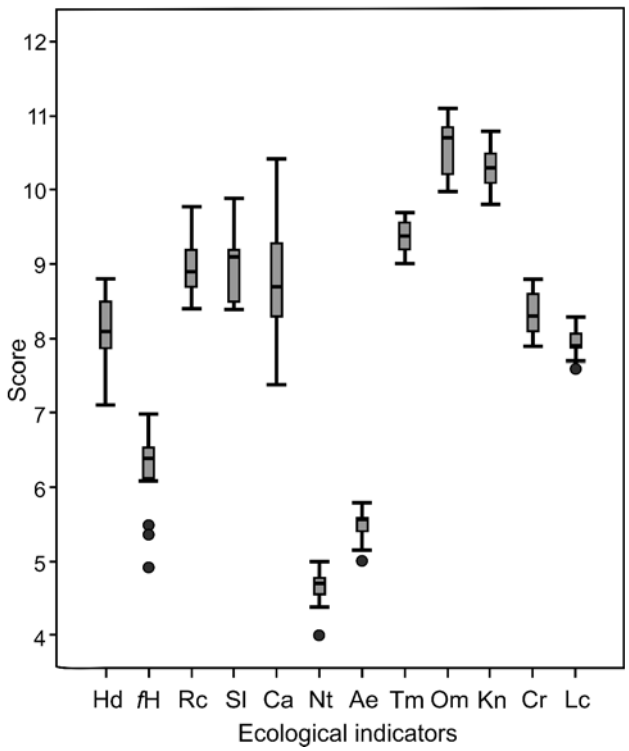

Fig. 2. The range of the most important ecological factors in habitats of *I. pumila*. Abbreviations: Hd – soil humidity, *f*H – variability of damping, Rc – soil acidity, Sl – total salt regime, Ca – carbonate content in soil, Nt – nitrogen content in soil, Ae – soil aeration, Tm – thermal climate, Om – ombroregime (aridity or humidity), Kn – continentality of climate, Cr – cryoregime, Lc – light (Didukh 2011).

Ecological amplitude

Phytoindication analysis of studied populations of *I. pumila* from the forest-steppe and steppe zones revealed that the species has narrow ecological amplitude (Fig. 2). Ecological factors within the species habitats such as soil moisture regime, soil acidity, soil salt regime, carbonate content in soil, humidity, frost conditions have larger ranges, while soil aeration, nitrogen content of soil, and light have narrower ranges and thus are limiting conditions for the species.

We also found statistically significant correlations between the variations of some ecological factors in

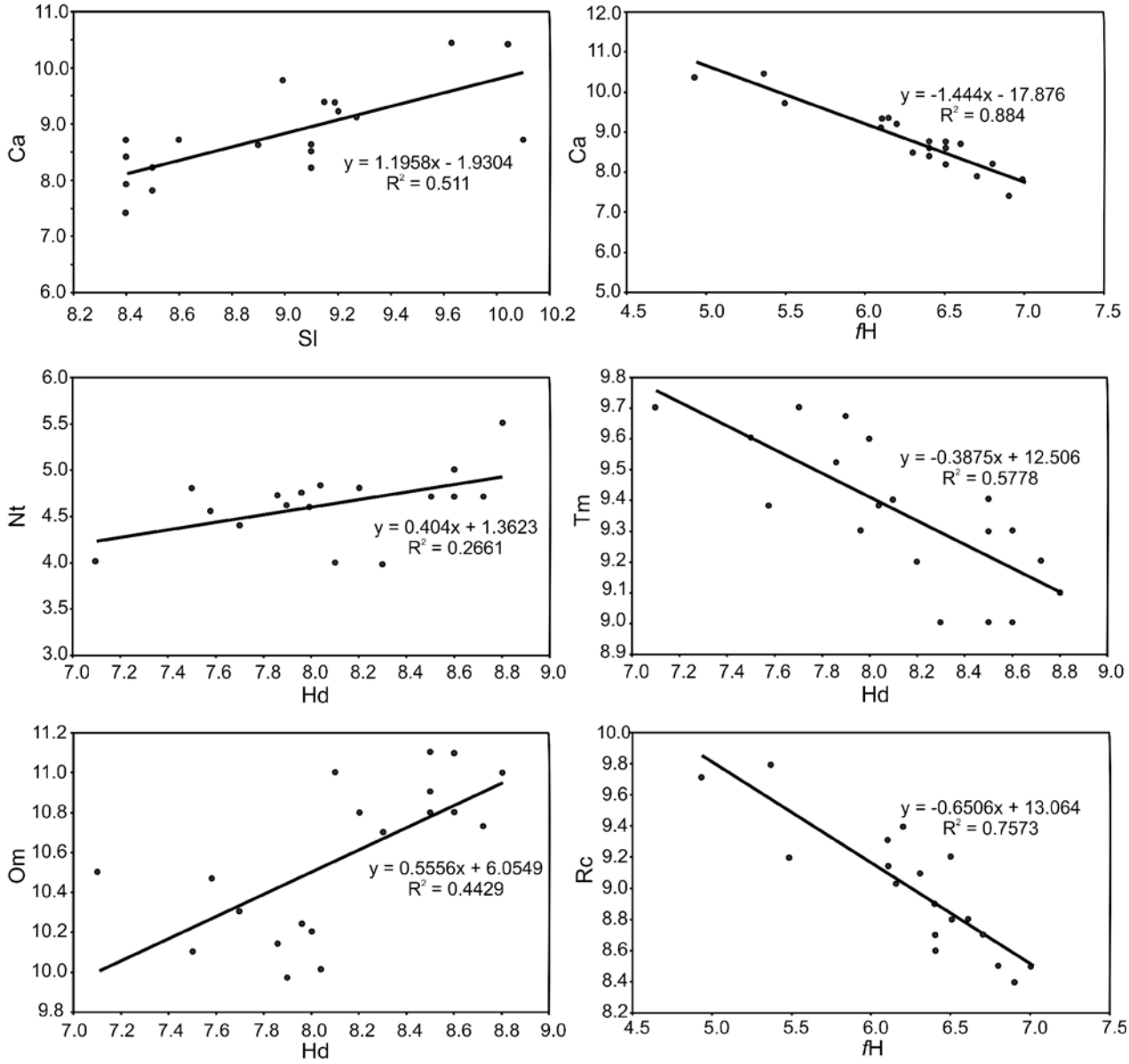

Fig. 3. Statistically significant correlations between the variations of the ecological factors in habitats of *I. pumila*. Abbreviations are the same as above in Fig. 2.

plant communities where *I. pumila* occurs, which are shown in Fig. 3. Positive correlations were found between following pairs of the factors: strong correlation between soil salt regime (Sl) and carbonate content in soil (Ca), moderate correlation between ombroregime (Om) and soil humidity (Hd), and weak correlation between soil humidity (Hd) and nitrogen content (Nt). Strong negative linear correlations were observed for another three pairs of factors: variability of damping (fH) and carbonate content in soil (Ca), soil humidity (Hd) and thermal regime (Tm), soil acidity (Rc) and variability of damping (fH).

Genetic heterogeneity

The results of PCR-analysis of genetic diversity of 40 *I. pumila* accessions from four populations (Aliaudy, Andriivka, Kolarovo, and Mygiia) using 7 ISSR-primers showed a high level of genetic variation (Table 3). The populations involved in the analysis have different sizes and are located in various parts of the range (see Fig. 1b and Table 2 for the information about location and characteristics of the populations). Nevertheless we found no statistically significant differences between the populations in terms of major indices of genetic diversity except for Mygiia. The population of Mygiia significantly differs from all of the others by a higher level of genetic variation.

The Jaccard genetic distance values calculated on the basis of the ISSR-data were used to construct a dendrogram revealing genetic relationship of the individuals from studied populations. A higher level of genetic similarity was shown between the plants from Aliaudy and Kolarovo populations. They formed one common group on the dendrogram, while the plants from Mygiia and Andriivka populations formed two separated clusters (Fig. 4). Mygiia and Andriivka popula-

Table 3. Main parameters of genetic variation of *I. pumila* based on ISSR-analysis.

Population	Total number of amplicons	Percentage of polymorphic amplicons, %	Nei unbiased gene diversity (unbiased expected heterozygosity, He)	Shannon index (S)	Jaccard genetic distances between plants (Dj), %	Mean Jaccard genetic distances between plants (Dj), %
Mygiia	135	63.4	0.171 ± 0.012	0.261 ± 0.017	43.5–75.6	61.2
Aliaudy	112	50.5	0.135 ± 0.012*	0.205 ± 0.017*	44.1–70.4	57.5
Kolarovo	113	48.5	0.122 ± 0.012*	0.189 ± 0.016*	38.3–63.5	51.5
Andriivka	107	49.5	0.127 ± 0.012*	0.195 ± 0.017*	43.8–72.1	60.0
Mean value	117	52.9	0.139 ± 0.006	0.212 ± 0.008	38.3–75.6	57.6
Sum matrix	194	97.9	0.171 ± 0.011	0.287 ± 0.014	38.3–83.8	69.2

* – significantly different from Mygiia population ($p < 0.05$)

Table 4. Nucleotide sequences of two polymorphic fragments of non-coding region of chloroplast genome of *I. pumila* L. identity in sequence is indicated by a dot; a dash indicates a nucleotide gap.

Haplotype	Polymorphic fragments
	trnS-trnG intergenic spacer (636–693 positions)
H1	GCAAAGGAAAGCATATATATAATAATATAATCGAA**CTTT**----TTATGTTTTTTATGT
H2	..........----..............
H3	..........CTTT..............
	trnL-trnF intergenic spacer (430–487 positions)
H1	CCAATAAAAAGTCCATTTTATTTCCTA-----**ACTAT**TTATATTC-TTTTTTTTTTTC
H2	..........-----.....T.....
H3	..........ACTAT.....-.....

tions were also the most similar in allele's frequencies, as well as Nei's genetic distance between them was minimal (0.011) as compared to the distances between the other populations, which lie in the range from 0.036 for Mygiia and Aliaudy to 0.052 for Andriivka and Mygiia populations.

Analysis of molecular variance (AMOVA) showed that 75% of the total genetic variation was attributable to differences within populations, while variations among populations and between regions (Poltava and Mykolaiv region) were relatively low (17% and 8%, respectively) despite significant geographical distance between them.

Combined use of the *trnS-trnG* and *trnL-trnF* cpDNA markers resulted in an alignment of 1686 base pairs (bp), which revealed 3 variable sites (including two duplications) (Table 4). *trnL-trnF* marker was found to be more variable than *trnS-trnG*. *trnL-trnF* intergenic spacer has two polymorphisms: a variation in length of polyT repeat and a duplication of 5-bp fragment, whereas only a duplication of 4-bp fragment was found in the *trnS-trnG* marker. Three different haplotypes were identified in 36 accessions of *I. pumila* sampled from 8 populations.

Haplotype H1 was the most frequent and found in all studied population except for the population of Inkerman. Haplotype H2 when compared to H1 and H3 has additional one base repeated [poly(T)] within marker *trnL-trnF*. It was found in the accessions from Inkerman populations and also from the vicinities of Kerch. Haplotype H3 diverged by two duplications, one per each analyzed marker (Table 5). It is very interesting that the latter haplotype was found only in the Mygiia population sample. Haplotype H1 was also revealed among the plants of this population.

Discussion

According to current data, there is no reason to subdivide *I. pumila* in Ukraine into lower taxonomic units. The diversity of forms, which are sometimes perceived as subspecies, may be the result of high genetic variation within the species' populations (Tarasjev et al. 2009). This, incidentally, gives no basis to discuss the origin of subdivision of the species into lower taxa. As it was previously mentioned, this is one of the signs of genetic erosion (Brown et al. 1997). As suggested by Sikura & Shysha (2010), presumable hybrid origin of *I. pumila* from Mediterranean taxa *I. attica* Boiss. et Heldr. (*I. pumila subsp. attica* (Boiss. & Heldr.) K. Richt. according to World checklist of selected plant families) and *I. pseudopumila* Tineo, which was accompanied by genomic merger of full chromosome sets derived from each of these species, may have long-term consequences, such as increased genomic instability, and thus may account also for the morphological plasticity of the species. Such plasticity may be the reason for distinguishing subspecies by some authors. The data of our ISSR and plastid markers analysis also do not provide support to distinguish any subspecies of *I. pumila* in Ukraine.

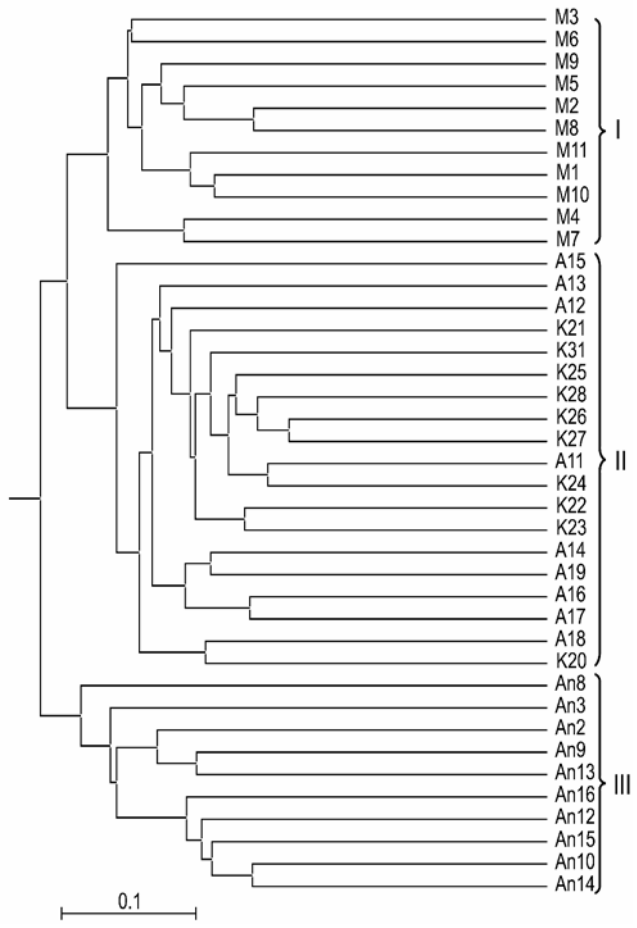

Fig. 4. Dendrogram of genetic distances between *I. pumila* plants from four populations (M – Mygiia, A – Aliaudy, K – Kolarovo, An – Andriivka) constructed by UPGMA method according to Jaccard genetic distances based on the data of ISSR-analysis. Roman numerals indicate individual clusters.

Moreover the results of the assessment of the range of distribution and degree of isolation, size of populations, frequency of generative reproduction and ecological amplitude of *I. pumila* habitats confirm the potential risk of extinction. A considerable part of the species populations exist as separated patches, isolated from the nearest neighbours due to intensive plowing. Therefore, the relative rarity of the species in the forest-steppe zone in comparison with steppe zone is obviously resulted from the more intensive habitats destruction and isolation due to human activity in past. The relatively better situation is also observed in the whole steppe part of the range of the species in Ukraine.

In addition to the isolation, the other anthropogenic factors are equally important, especially such as soil erosion and landslides at the sea and estuary coasts in the Black Sea region, where the species grows in a narrow coastal strip. Moreover, the process of forestation, burning grasses, using steppe lands as pastures or landfill sites, extraction of minerals, and other physical factors that contribute to destruction of steppe communities and soil disturbance are among anthropogenic factors that can have the crucial negative impact on the sampled populations. Digging plants in the wild for the use as an ornamental culture have also to be mentioned. All these impacts may be especially dangerous because of the lack of the data on the dynamics of *I. pumila* populations in the major part of the current range of distribution (Parnikoza et al. 2007; Fedyaeva et al. 2011).

The populations sampled in our study are typical, but not the largest among the described for *I. pumila*. In our research we have found that the species reaches the maximum plant density on slopes of ravines and gorges, which are unsuitable for agriculture. This matches with the observations of other authors, who likewise mentioned rocky steppes and dense petrophytic or sand steppes as habitat of *I. pumila*. In fragments of virgin dense steppes, density of the species is significantly lower (Fedyaeva et al. 2011).

The most abundant populations of *I. pumila* occur at the Crimean Azov sea coast. Especially in the Sivash region or in Mountain Crimea the plant cover reaches 10%, like in Mygiia population in our study. At the Northern Azov sea coast (Donetsk oblast), especially in Bilosaraiska kosa reserve, plant density in a study plot of 100 m^2 reaches 124 specimens. *I. pumila* populations on the territory of Krasnodar oblast of Russia are most abundant, here the plant density reaches 19–93 individuals per m^2 (Ostapko & Kolomyichuk 2012).

In all forest-steppe communities, populations of *I. pumila* are relatively less abundant. In our study, the populations from this zone have density 1–5 plants per m^2 with the species cover of 1–10 %. In Poltava oblast, 17 habitats of the species were found, where populations composed of 10-20 clumps with the species cover of 1–2 % (Bairak et al. 2006).

In our study as well as in other reports, the characteristic feature of the species is the presence of populations with the predominance of reproductive individuals. On the other hand, in some instances a different situation can be observed. For example, in Poltava oblast, populations were reported, where all developmental stages were present (full-membered populations) (Bairak et al. 2006). Such populations were found in steppe fragments in Rostov and Krasnodar oblasts of Russia (Ostapko & Kolomyichuk 2012). In age spectrum of Rostov oblast populations the ratio of virginal (non-flowering repoductive), generative (flowering reproductive) and senile (post-reproductive) plants was close to 2:4:1 (Fedyaeva et al. 2011). Most likely it is due to a local reduction in environmental impact of human activity. However, it is obvious even without looking at the age spectrum that generative reproduction of the species to a large extent depends on the climatic conditions of a particular year. This significantly limits replenishment of populations by pre-reproductive individuals. So, in rich grass-forb steppe communities grown on calcareous soils, the frequency of viable seed formation does not exceed 33% in the years with favourable climatic conditions (Sluginova 2008). Life strategy of this species is to maintain survival reproductive individuals along with occasional generative reproduction in favourable years.

Table 5. Distribution of different haplotypes according to chloroplast markers *trnL-trnF* and *trnS-trnG* in *I. pumila* populations.

Population	Accession number	Haplotype			GenBank accession numbers	
		H1	H2	H3	*trnL-trnF* intergenic spacer	*trnS-trnG* intergenic spacer
Andriivka	An3	+			KU310494	
(Poltava obl.)	An21	+			KU310495	
Mygiia	M2	+			KU310496	KU310530
(Mykolaiv obl.)	M7	+			KU310497	KU310531
	M8			+	KU310498	KU310532
	M9	+			KU310499	KU310533
	M10			+	KU310500	KU310534
	M11			+	KU310501	KU310535
	M14			+	KU310502	KU310536
Aliaudy & Kolarovo	A13	+			KU310503	
(Mykolaiv obl.)	A14	+			KU310504	
	A19	+			KU310505	
	K27	+			KU310506	
	K28	+			KU310507	
	K31	+			KU310508	KU310537
Dmytrivka	2-6	+			KU310509	
(Mykolaiv obl.)	2-13	+			KU310510	
	2-18	+			KU310511	
	2-21	+			KU310512	
	4-1	+			KU310513	
	4-10	+			KU310514	KU310538
	5-1	+			KU310515	
	5-3	+			KU310516	
Inkerman	N2		+		KU310517	
(AR of Crimea)	N4		+		KU310518	
	N7		+		KU310519	KU310539
Balaklava	N4	+			KU310520	
(AR of Crimea)	N7	+			KU310521	
	N9	+			KU310522	
Staryy Krym	N2	+			KU310523	KU310540
(AR of Crimea)	N7	+			KU310524	
The vicinities of Kerch	N1	+			KU310525	
(AR of Crimea)	N2	+			KU310526	
	N3	+			KU310527	
	N5		+		KU310528	
	N11	+			KU310529	KU310541

Phytoindication analysis of studied populations of *I. pumila* revealed that the species has a narrow ecological amplitude. The narrowest are the ranges of soil aeration, nitrogen content of soil, and light. This means that the changes in these factors are limiting for *I. pumila* distribution. The latter, in turn, indicates substantial susceptibility of the species to human impact that may lead to increased nitrates content of soil, decreased soil aeration (trampling effects), and succession resulted in altered light conditions, which is especially important for the forest-steppe zone.

The species also displays a narrow range of tolerance to soil humidity. This gives evidence of the known fact that *I. pumila* belongs to euxerophytes (Kucherevskyi 2001) or mesoxerophytes both in the forest-steppe zone and the steppe zone (Tarasov 2005; Travlieiev 2010). This means that its habitats include exclusively dry and temperate dry localities.

Of particular importance are climatic factors, which depend on precipitation, such as humidity, continentality, and temperature (thermal and cryo-regime), along with the chemical properties of soil, which strongly depend on the abovementioned factors, as shown in Figure 3. Therefore the changes of these ecological variables may indirectly cause a biotope shift.

The majority of non-affected virgin dry steppe communities, where *I. pumila* plants grow, are very sensitive to human impact on surrounding areas. Therefore, they are listed as rare or are included into Ukrainian list of protected communities: Green Book of Ukraine (Didukh 2009). Furthermore, these xerothermic communities are protected according to European

Habitat Directive (Poradniki 2004). This additionally indicates a vulnerability of the species.

However, genetic analysis does not provide any evidence of reduced genetic diversity within individual populations and significant divergence among populations of *I. pumila*. Analysis of molecular variance (AMOVA) showed that total genetic variation was attributable mainly to differences within populations, while variations among populations and between regions were relatively low despite significant geographical distance between them. This is consistent with the data of the study of another xerotermic species *Iris aphylla* L. in Poland: RAPD-analysis of 84 genotypes from three distant localities revealed a high level of genetic variation within the populations and low genetic differentiation between them (Wróblewska et al. 2003).

As far as *I. pumila* genetic diversity is concerned, analysis of own and literature data shows that the general level of genetic variation of the species is similar or higher than that of other species of *Iris* genus (Artyukova et al. 2001; Kozyrenko et al. 2004; Kozyrenko et al. 2009; Arafeh et al. 2002; Makarevitch et al. 2003; Wróblewska et al. 2003; Wróblewska & Brzosko 2006; Wang et al. 2009). The species is a perennial plant that forms long-living mature clones and this provides an opportunity to preserve initial genetic heterogeneity for a long time (Aguilar et al. 2008; Wróblewska et al. 2003). Restricted renewal of populations under the influence of human activity results in a slowdown of accumulation of differences between populations even in conditions of strong isolation.

Thus this inconsistency between the data of population ecological study and the results of molecular genetic analysis can be a good illustration of the need to implement integrative approach for the assessment of the extinction risk for a particular species and careful analysis of all the available symptoms. Researched populations do not significantly differ in terms of major indices of genetic diversity. Only population of Mygiia differs from all of the other by a higher heterogeneity. Presumably, this fact is related to the larger size of Mygiia population, which exceeds 1000 individuals, while the other populations comprise no more than 40–200 plants (see Table 2), since it is known that the level of genetic variation correlates positively with population size (Leimu et al. 2006). However, the study of *I. aphylla* and some other species show that genetic diversity may be independent of population size (Wróblewska et al. 2003).

The results of cluster analysis presented in Fig. 4 indicate a positive correlation between genetic and geographical distances between populations (the distance between Aliaudy and Kolarovo populations is approximately 1.5 km, whereas the rest of populations are separated by hundreds of kilometers). It seems that the exchange of genetic material between populations through cross-pollination and seed dispersal is possible only at such small distances.

Interestingly, Andriivka population located at the north margin of distributional range of *I. pumila* in Ukraine does not differ significantly in the level of genetic diversity from the populations of similar size from the central part of the range. According to current knowledge, marginal populations are expected to show lower genetic diversity compared to the central ones due to less favourable conditions for reproduction and survival and genetic drift. On the other hand, the requirement to adapt to more variable environment at the margin of the distributional range may contribute to an increased genetic variation of peripheral population (Safriel et al. 1994). This is close to the findings reported for marginal population of *I. aphylla* in Poland (Wróblewska et al. 2003).

The most plausible explanation for the results of genetic analysis is that the comparably high level of diversity within *I. pumila* populations and the low population divergence have been preserved from the times when the species was widespread throughout the steppe and partially the forest-steppe region in Ukraine and populations shared a common gene pool. Similar low level of genetic heterogeneity is shown for isolated Poland populations of wet-meadow *I. sibirica* (Kostrakiewicz &Wróblewska 2008).

Maintenance of the initial level of genetic diversity in conditions of continuous reduction and fragmentation of habitats obviously has been possible due to high longevity of reproductive individuals. According to the literature data, outcrossing, clonal, long-lived, and endangered species exhibit relatively high variation within populations, whereas inter-populational variation is rather low (see in Wróblewska et al. 2003). Furthermore, even occasional sexual reproduction within populations might enrich genetic variation from time to time. Another potential source of maintenance of high genetic diversity is the probable amphidiploid origin of *I. pumila* (Sikura & Shysha 2010). This is confirmed by the analysis of the plastid markers variation. Occurrence of a specific haplotype H3 (2 duplications) among the plants of Mygiia population probably indicates the molecular synapomorphy. But an increase of sample size is required to prove the specificity of this haplotype for Mygiia population.

Considering comparably high genetic diversity of isolated populations of *I. pumila*, one cannot also exclude potential contribution of other mechanisms of accumulation of additional genetic heterogeneity. So, our study of a typical steppe perennial *I. pumila* does not provide undisputable answer about the risk of genetic erosion for the species. The inconsistency between the data of molecular genetic analysis and other indices of genetic erosion studied here indicates that the loss of genetic diversity in the species caused by habitat fragmentation and isolation under increasing anthropogenic pressure is likely to be slower than it would appear judging from the assessment of population parameters, which clearly show negative trends.

According to the risks observed for *I. pumila*, which can be considered as an indicator of well-preserved steppe communities, some substantial measures should be taken to protect the populations of

the species, such as creation of new natural reserves in places of its habitats, especially on the margins of the range in the forest-steppe zone and in steppe areas highly populated by the species. These conclusions will perhaps hold for other populations from the European part of the species' range. As concerns to the other countries experience, an issue of the inclusion of *I. pumila* into the Red Data Book of Ukraine can be considered to reinforce its protection.

Acknowledgements

The research was carried out in the frame of memorandum of collaboration between Gdańsk University of Polish Academy of Sciences and Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine. The study was carried out with partial financial support from the NAS of Ukraine through the Targeted interdisciplinary programme of scientific research "Fundamentals of molecular and cellular biotechnology" and joint research project of NAS of Ukraine and Polish Academy of Science "Adaptive strategy of mutual survival of organisms in extreme environmental conditions". We also thank to A. Cwener, V. Shevchyk, and O. Poronnik for helping with the preparation of the manuscript.

References

Aguilar R., Quesada M., Ashworth L., Herreriasdiego Y. & Lobo J. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol. Ecol. **17:** 5177–5188.

Arafeh R.M.H., Sapir Y., Shmida A., Iraki N., Fragman O. & Comes H.P. 2002. Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (Iris sect. Oncocyclus = the royal irises) along an ecogeographical gradient in Israel and the West Bank. Mol. Ecol. **11:** 39–53.

Artyukova E.V., Kozyrenko M.M., Ilyushko M.V., Zhuravlev Yu.N. & Reunova G.D. 2001. Genetic Variability of *Iris setosa*. Mol. Biol. (Mosk). **35:** 134–138.

Bairak O.M. & Stetsiuk N.O. 2005. Atlas ridkisnkh ta znykayuchkh rosln Poltavshchini. Verstka, Poltava, 248 pp.

Bairak O.M., Shevel I.M., Hrytsai I.A., Kryvoruchko T.V. & Nechytailo V.I. 2006. Botanichniĭ zakaznik Drabinivka. Verstka, Poltava, 81 pp.

Bezusko L.G, Mosiakin S.L., Bezusko A.G. 2011. Zakonomirnosti ta tendentsiyi rozvytku roslynnoho pokryvu Ukrayiny u pizn'omu pleĭstotseni ta holotseni. Alterpress, Kyiv, 488 pp.

Biodiversity Support Program. 1999. Priority setting in conservation: A new approach for Crimea. Washington, D.C., 257 pp.

Bokov V.A. 1999. Perspektivi sozdaniya Edinoĭ prirodookhrannoĭ seti Kryma. Crimean educational pedagogical publishing house, Simferopol, 192 pp.

Brown A., Young A., Burdon J., Christidis L., Clarke G., Coates D. & Sherwin W. 1997. Genetic indicators for state of the environment reporting, Australia: State of the Environment Technical Paper Series (Environmental Indicators), Department of the Environment, Sport and Territories, Canberra, 30 pp.

Chopyk V.I., Bortniak M.M., Voitiuk Yu.O. et al. 1998. Konspekt flory Seredn'oho Prydniprov"ya. Fitosotsiotsentr, Kyiv, 139 pp.

Dalmady J. 1972. Iris species on Slovenski Kras area. Nature Prot. **3:** 64–65.

Osoblivosti ontohenezu *Iris pumila*, pp. 246–247. In: Materialy naukovoyi konferentsiyi "Bilolohiya: vid molekuly do biosfery", November 17-21, 2009, Kharkiv.

Didenko I.P., Shvets T.A. & Tomashevska N.P. 2010. Ekolohotsenotychni osoblivosti *Iris pumila* L. v Kirovohrads'kiĭ oblasti, pp. 206. In: Aktual'ni problemy botaniky ta ekolohiyi. Materialy mizhnarodnoyi konferentsiyi molodykh uchenykh, September 21–25, 2010, Yalta, Simpheropol.

Didukh Ya.P. 2011. The ecological scales for the species of Ukrainian flora and their use in synphytoindication. Phytosociocentr, Kyiv, 176 pp.

Didukh Ya.P. 2009. Zelena kniha Ukrainy. Altpress, Kyiv, 448 pp.

Didukh Ya.P. 2009. Chervona kniha Ukrayiny. Roslynnĭ svit. Globalconsulting, Kyiv, 900 pp.

Dobrochaeva D.N., Kotov M.I., Prokudin Yu.N., et al. 1999. Opredelitel' vysshikh rasteniĭ Ukrainy. Fitosoctsiotsentr, Kiev, 548 pp.

Doyle J.J. & Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19:** 11–15.

Ewers R.M. & Didham R.K. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biol. Rev. **81:** 117–142.

Fisher M., Musi R., Prater D., Peintinger M., Van Kleunen M., Shmidt B. 2000. RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). Am. J. Bot. **87**: 1128–1137.

Fedyaeva V.V., Shmaraeva A.N. & Shyshlova Zh.N. 2011. Monitoring populyatsiĭ *Iris pumila* L. v Rostovskoĭ oblasti. Nauchnye vedomosti Belgorodskogo gosudarstvennogo universiteta. Estestvennie nauki. **15:** 326–331.

Gouy M., Guindon S. & Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. **27:** 221–224.

Kostrakiewicz K. & Wróblewska A. 2008. Low genetic variations in subpopulations of an endangered clonal plant *Iris sibirica* in Southern Poland. Ann. Bot. Fenn. **45:** 186–194.

Kozyrenko M.M., Artyukova E.V. & Zhuravlev Yu.N. 2009. Independent species status of *Iris vorobievii* N.S. Pavlova, *Iris mandshurica* Maxim., and *Iris humilis* Georgi (Iridaceae): Evidence from the nuclear and chloroplast genomes. Russ. J. Genet. **45:** 1394–1402.

Kozyrenko M.M., Artyukova E.V., Boltenkov E.V. & Lauve L.S. 2004. Somaklonal'naya izmenchivost' *Iris pseudacorus* L. po dannym RAPD- i tsitogeneticheskogo analiza. Russ. J. Biotechnol. **20 (2):** 13–23.

Kucherevskyi V.V. 2001. Atlas ridkisnykh ta znykayuchykh roslin Dnipropetrovshchyny, Phytosociocentr, Kyiv, 360 pp.

Kucherevskyi V.V. 2004. Konspekt flory Pravoberezhnoho stepovoho Prydniprov"ya, Prospect, Dnipropetrovsk, 292 pp.

Leimu R., Mutikainen P., Koricheva J. & Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? J. Ecol. **94:** 942–952.

Hoskovec L. 2007. Zvláště chráněné druhy rostlin České republiky. http://botany.cz/cs/chranene-rostliny/ (accessed 11.11. 2015).

Eliáš jun. P. 2015. Červený zoznam výtrusných a kvitnúcich rastlín Slovenska. http://botany.cz/cs/ohrozene-rastliny-slovenska/ (accessed 11.11.2015).

Lienert J. 2004. Habitat fragmentation effects on fitness of plant populations – a review. J. Nat. Conserv. **12**: 53–72.

Makarevitch I., Golovnina K., Scherbik S. & Blinov A. 2003. Phylogenetic relationships of the Siberian Iris species inferred from noncoding chloroplast DNA sequences. Int. J. Plant Sci. **164:** 229–237.

Mosyakin S.L. & Fedoronchuk M.M. 1999. Vascular plants of Ukraine: A nomenclatural checklist. M.G. Kholodny Institute of Botany, Kyiv, 346 pp.

Motyka J., 1946. Step środkowo-europejski. Acta Soc. Bot. Pol. **17:** 46.

Ostapko V.M. & Kolomyichuk V.P. 2012. Krasnaya kniga Priazovskogo regiona. Sosudistie rasteniya. Altapress, Kyiv, 267 pp.

Ostapko V.M. 2001. Raritetnyĭ florofond Yugo-Vostoka Ukrainy (khorolohiya). Lybed, Donetsk, 121 pp.

Parnikoza I. 2008. Zberezhennya ukrayins'koho stepu: shcho mozhna zrobiti vzhe s'ohodni? pp. 53–62. In: Zagorodniuk I.V. (ed.), Rare theriofauna and its conservation, **9**, Luhansk.

Parnikoza I. 2011. Deyaki refuhiumy stepovoyi bioty ta mistsezrostannya ridkisnikh roslin v okolytsyakh mist Kryvy Rih ta Dnipropetrovs'k, pp. 31–40. In: Naukovi pratsi Luhans'koho prirodnoho zapovidnika. Roslinniĭ ta tvarinniĭ svit ta ĭoho okhorona. The flora and fauna, and conservation], Issue 2, Luhansk.

Parnikoza I.Yu., Shevchenko N.M., Bogomaz M.V. & Shevchenko M.S. 2007. The influence of grass burning on the dynamics of *Pulsatilla nigricans* Stork population of the regional landscape park "Lysa Gora" (Kyiv), pp. 4–5. In: Szhmalej S.A., Rusina L.Yu., Akimov I.A. (eds), Falz-Fein Readings. PP Vyshemyrskyi, Kherson.

Parnikoza I.Yu., Shevchenko M.S., Inozemtseva D.M., Vasyliuk O.V. & Shevchenko O.S. 2008. Raritetna flora (Okhorona, vivchennya, reintroduktsiya). Kyiv ecological and cultural centre, Kyiv, 132 pp.

Peakall R. & Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. **6:** 288–295.

Poradniki ochrony siedlisk i gatunków. Natura 2000 – podrecznik metodyczny, Murawy kserotermiczne. T. 3. 2004. Warszawa, 244 pp. http://natura2000.gdos.gov.pl/uploads/download/219 (accessed 31.07.2016)

Purger D., Csiky J., Topić J. 2008. Dwarf iris, *Iris pumila* L. (Iridaceae), a new species of the Croatian flora. Acta Bot. Croat. **67:** 97–102.

Safriel N.U., Volis S. & Kark S. 1994. Core and peripheral populations and global climate change. Isr. J. Plant Sci. **42:** 331–345.

Schluter P.M. & Harris S.A. 2006. Analysis of multilocus fingerprinting data sets containing missing data. Mol. Ecol. Notes. **6:** 569–572.

Shaw J., Lickey E.B., Schilling E.E. & Small R.L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. Am. J. Bot. **94:** 275–288.

Shelegeda V.I. & Shelegeda O.R. 2008. Ridkisni ta znikayuchi roslini Zaporiz'koyi oblasti. Tandem-art-studia, Zaporizhzhia, 96 pp.

Shvets T.A. 2004. Osoblivosti ontohenezu predstavnikiv riznikh sektsiĭ rodu Iris L. v umovakh kul'turi. Visnik Lvivskoho universitetu. Seriia biolohichna. **36:** 203–206.

Sikura I.I. & Shisha E.N. 2008. Genus *Iris* L. (Iridaceae). Znania Ukrainy, Kiev, 195 pp.

Sluginova I.S. 2008. Semennaya produktyvnost' nekotorikh vidov rasteniĭ melovikh obnazheniĭ basseĭna Polnoĭ. Izvestiia vuzov. Severo-Kavkazskiĭ region. Estestv. Nauki. **146:** 75–79.

Taberlet P., Gielly L., Pautou G. & Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. **17:** 1105–1109.

Tarasjev A., Klicaric N.B., Stojkovic B. & Avramov S. 2009. Phenotypic plasticity and between population differentiation in *Iris pumila* transplants between native open and anthropogenic shade habitats. Russ. J. Genet. **45:** 1078-1086.

Tarasov V.V. 2005. Flora Dnipropetrovs'koyi ta Zaporiz'koyi oblasteĭ. Sudinni roslini. Bioloho-ekolohichna kharakteristika vidiv: Monohrafiya. Publ. House of DNU, Dnipropetrovsk, 276 pp.

Templeton A.R., Crandall K.A. & Sing C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics. **132:** 619–633.

Tkachenko V.S., Didukh Ya.P., Henov A.P. et al. 1998. Ukrayins'kiĭ pryrodniĭ stepoviĭ zapovidnik. Roslynniĭ svit. Fitosotsiotsentr, Kyiv, 232 pp.

Travlieiev A.P. 2010. Chervona Kniha Dinpropetrovs'koyi oblasti (Roslynniĭ svit). VKK "Blans-Klub", Dnipropetrovsk, 500 p.

Vedenkov E.P. 1989. Flora zapovidnika "Askaniya Nova". Moscow, 50 pp.

Wang K., Kang J., Zhou H., Sun Y., Yang Q., Dong J. & Meng L. 2009. Genetic diversity of *Iris lactea* var. *chinensis* germplasm detected by inter-simple sequence repeat (ISSR). Afr. J. Biotechnol. **8:** 4856-4863.

World checklist of selected plant families. http://apps.kew.org/wcsp/qsearch.do (accessed 11.11.2015).

Wróblewska A., Brzosko E., Czarnecka B. Nowosielski J. 2003. High levels of genetic diversity in populations of *Iris aphylla* L. (Iridaceae), an endangered species in Poland. Bot. J. Linn. Soc. **142:** 65–72.

Wróblewska A. & Brzosko E. 2006. The genetic structure of the steppe plant *Iris aphylla* L. at the northern limit of its geographical range. Bot. J. Linn. Soc. **152:** 245–255.

Yena A.V. 2012. Prirodnaya flora Krymskogo poluostrova. N. Oreanda, Simpheropol, 232 pp.

Xie Z., Lu Y., Ge Song, Hong D and Li F. 2001. Clonality in wild rice (*Oryza rufipogon*, Poaceae) and its implications for conservation management. Am. J. Bot. **88:** 1058–1064.

Received April 29, 2016
Accepted October 26, 2016