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POPULATION GENETIC STRUCTURE OF *IRIS PUMILA* L. IN UKRAINE: EFFECTS OF HABITAT FRAGMENTATION

Olena Bublyk[1], Igor Andreev[1]*, Ivan Parnikoza[1,2], Viktor Kunakh[1]

[1]*Institute of Molecular Biology and Genetics of NAS of Ukraine, Akad. Zabolotny str., 150, Kyiv, 03143, Ukraine*

[2]*National Antarctic Scientific Center of Ukraine, Boulevard Tarasa Shevchenka 16, 01601 Kyiv, Ukraine*

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Habitat fragmentation is one of serious threats to biodiversity of nature in today's world. The present study of a typical steppe species *Iris pumila* L. (Iridaceae) has analyzed the impacts of geographical isolation and population size on genetic diversity and population structure in conditions of habitat fragmentation. The key indices of population genetic variability calculated from the ISSR markers data were on average as follows: Shannon diversity index (S) – 0.188; unbiased Nei's gene diversity (H_e) – 0.123; and the average measure of Jaccard's genetic distances between individuals within populations – 58.4%. Although the largest population had significantly higher values of S and He, the small and marginal populations also showed a comparable level of variation. Most of the genetic variation of *I. pumila* was distributed within the populations. A strong correlation was found between Nei's genetic distances and geographic distances between the populations. According to the Bayesian analysis, genetic structure of the populations was highly homogeneous; however, the presence of admixed genotypes indicated the possibility of gene flow between the populations at present.

Keywords: conservation, genetic polymorphism, habitat fragmentation, ISSR markers, population genetics

INTRODUCTION

The development of human civilization has led to the conversion of vast areas of wilderness to croplands that caused fragmentation of the habitats of numerous species and changed natural ecosystems (Sala et al., 2000). To date, the fragmentation of natural habitats is considered one of the main threats to biodiversity (Hobbs and Yates, 2003; Haddad et al., 2015).

Habitat fragmentation has three major consequences for plant populations: the loss of suitable habitat, reductions in population size, and increasing spatial isolation between remnant populations (Klank et al., 2012). These changes often cause a reduction in effective population size and adversely affect ecological and genetic processes (Aguilar et al., 2008; Ewers and Didham, 2006). In small isolated populations, genetic drift and inbreeding are unavoidable due to the small effective population size and limited gene flow between isolated populations. The consequence is the loss of genetic diversity, which, in turn, translates into reduced viability and evolutionary potential of populations (Aguilar et al., 2008; Ewers and Didham, 2006).

Steppe ecosystems are among the most threatened in Europe due to the anthropogenic destruction and fragmentation of steppe patches (Cremene et al., 2005); however, publications that focus on the impact of habitat fragmentation on steppe vegetation are not numerous (Dembicz et al., 2018; Heinicke et al., 2016; Wódkiewicz et al., 2016). A recent review by Kajtoch et al. (2016)

* Corresponding author, e-mail: i.o.andreev@imbg.org.ua

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showed a low genetic variation in populations of plant species related to dry grasslands in Eastern Central Europe: 94% of the studied plant species (15 out of 16 taxa for which the data were available) had some or all of their populations characterized by low diversity, while highly diverse populations were found in 38% of taxa. These differences in the genetic variation can be partly attributed to the biology and ecology of the studied species.

Iris pumila L. (Iridaceae) is an ornamental plant species valuable for breeding, which inhabits the European steppe. Ukrainian populations of the species are in the central part of its range that stretches from Austria to West Siberia, including the Southern Balkans, Anatolia and the Northern Caucasus (Sikura and Shisha, 2010). Before the 19th century, the natural habitat of the species within the territory of modern Ukraine covered, probably, the southern part of the forest-steppe zone and the entire steppe zone along with the Crimea Mountain range. The range of the species has been significantly reduced and fragmented because of large-scale ploughing of the steppes that began in the mid-eighteenth century (Moon, 2013). Currently, the species populations are exposed to strong anthropogenic pressure that caused a reduction in the number of individual localities over the past decades, and now *I. pumila* is protected in many areas of Ukraine (Andriyenko and Peregrym, 2012).

In Ukraine, *I. pumila* has not yet been studied in sufficient detail. To date, there is only one monograph of the species of *Iris* (Sikura and Shisha, 2010) which mentioned *I. pumila*, and a few isolated publications on its individual localities (see in Parnikoza et al., 2017). There are only two studies on genetic diversity of this species (Dembicz et al., 2018; Twardowska et al., 2015). The consequences of a reduction in the size of populations and their isolation on population genetic structure of the species are still unknown. Furthermore, *I. pumila* is a typical steppe perennial, therefore this study will improve our understanding of the effects of human activities on steppe species, for which information on genetic diversity is still limited, compared to other groups of plants (Wroblewska et al., 2006).

The aim of the work was to determine the level of genetic variability and population genetic structure of *I. pumila* in conditions of habitat fragmentation.

MATERIALS AND METHODS

Iris pumila L. (Iridaceae) is a typical xerophyte of the European steppe zone, a perennial, clonal, pollinated by insects, and strictly-outcrossing plant species with low seed dispersal ability (Parnikoza et al., 2017). It is an allopolyploid with a chromosome number 2n = 32 supposedly originated from natural hybridization between *I. attica* (2n = 16) and *I. pseudopumila* (2n = 16) (Randolph and Mitra, 1959).

Plant material was collected from five *I. pumila* populations that were spread throughout the species range in Ukraine from the southern Crimea to the northern limits of the range (Fig. 1). In total, 49 specimens (9–11 plants of each population) were sampled by randomly selecting one sample per genet. Young leaves were collected and dried in

Fig. 1. Location of the studied populations of *Iris pumila* in Ukraine: M – Mygiia, the vicinity of vil. Mygiia, Pervomaisk raion, Mykolaiv oblast, E 30°58', N 48°02'; A – Aliaudy, the vicinity of Mykolaiv city, E 32°03', N 47°01'; K – vil. Kolarovo (renamed into Karavelove in 2016) Vitovskyi r-n, Mykolaiv obl., E 32° 02', N 47°00'; An – vil. Andriivka, Reshetylivka r-n, Poltava obl., E 34°15', N 49°33'; B – Balaklava, Sevastopol city, the AR of Crimea, E 33°37', N 44°30'.

silica gel. The population size was estimated by counting adult rhizomatous clones or young plants produced from seeds (whenever they were present). The studied populations are described in detail by Parnikoza et al. (2017).

DNA was isolated from silica gel dried leaves using CTAB-method with chloroform extraction by Doyle and Doyle (1987).

For PCR-analysis, 7 Inter-Simple Sequence Repeat (ISSR)-primers were used that have been previously shown to be efficient for the evaluation of genetic variation in *I. pumila* (Bublyk et al., 2013). The primers are listed in Table 1. The 20 μl PCR mixture contained: 20–30 ng of genomic DNA, 0.2 mM of each dNTP (Fermentas, Lithuania), 1.25 U Taq DNA-polymerase (Fermentas, Lithuania), 1 μM of a primer, 1 × PCR buffer with $(NH_4)_2SO_4$ and 2.5 mM $MgCl_2$ (Fermentas, Lithuania). Reaction mix was layered with a drop of mineral oil to avoid evaporation. As a negative control for amplification, a reaction mixture containing sterile water instead of DNA was used. PCR amplification was performed in a Tertsyk MC2 thermal cycler (Biotechnology, Russia) under the following conditions: 95°C – 2 min., 35 × (94°C – 20 s, 53°C – 30 s, 72°C – 90 s), 72°C – 5 min.

TABLE 1. The sequences of ISSR-DNA primers used to assess genetic variability in *Iris pumila* (Y = C, T).

No.	Primer	Nucleotide sequence (5'-3')
1	UBC#03	$(AC)_8TT$
2	UBC#05	$(AC)_8TG$
3	UBC#59	$(AG)_8GC$
4	UBC#810	$(GA)_8T$
5	UBC#811	$(GA)_8C$
6	UBC#835	$(AG)_8YC$
7	UBC#840	$(GA)_8YT$

The PCR products were separated by electrophoresis in 1.5% agarose gel in 1 × SB buffer (5 mM $Na_2B_4O_7$, pH 8.5) at 90 V (4 V/cm) for 5 hours, then visualized by staining with ethidium bromide and photographed under UV light.

Each PCR reaction was repeated at least twice to verify the reproducibility and only distinct, reproducible fragments were scored. The amplification products were scored for the presence (1) or absence (0) of each band across all genotypes, and the data were used to generate a binary data matrix. From the resulting binary matrix, Jaccard's genetic distances between plants were calculated and then used to perform principal coordinate analysis (PCoA) with FAMD software (Schluter and Harris, 2006).

The indices of genetic variability: percentage of polymorphic bands (P), Shannon index (S), Nei's unbiased gene diversity (expected heterozygosity, H_e), and Nei's unbiased genetic distance between populations were calculated using GenAlEx 6.5 (Peakall and Smouse, 2006). The same software was used to test the relationship between the matrices of pairwise genetic distances and geographic distances between plants and between populations using Mantel test (Mantel, 1967) with 999 permutations. An analysis of molecular variance (AMOVA) was performed to determine thepartitioning of total genetic variation between the three regions (Mykolayiv oblast, Poltava oblast, and the Autonomous Republic of Crimea), between the populations and between genotypes within populations.

The genetic structure of populations was analyzed by the software Structure 2.3.4 (Pritchard et al., 2000) using the admixture model with correlated allele frequencies in the parental populations (K) and without prior population information. To determine the most likely number of genetic clusters (K) ten independent runs were performed for the K from 1 to 8 with a burn-in period of 50,000 replicates and sampling period of 300,000 replicates. To infer the genetic structure of populations for the most probable K value, an analysis was performed with a burn-in period of 100,000 and a sampling period of 1,000,000 replicates.

RESULTS

The PCR analysis of *I. pumila* plants collected from five natural populations that were spread throughout the species range in Ukraine (Fig. 1) was performed using 7 ISSR primers (Table 1). An example of PCR-amplification profile is shown in Fig. 2. For the total sample of plants from all populations, 222 fragments were scored, 97.8% of which were polymorphic (Table 2). Percentages of polymorphic fragments within individual populations ranged from 39.6% (Balaklava) to 56.3% (Mygiia), with an average of 46.0%. The values of

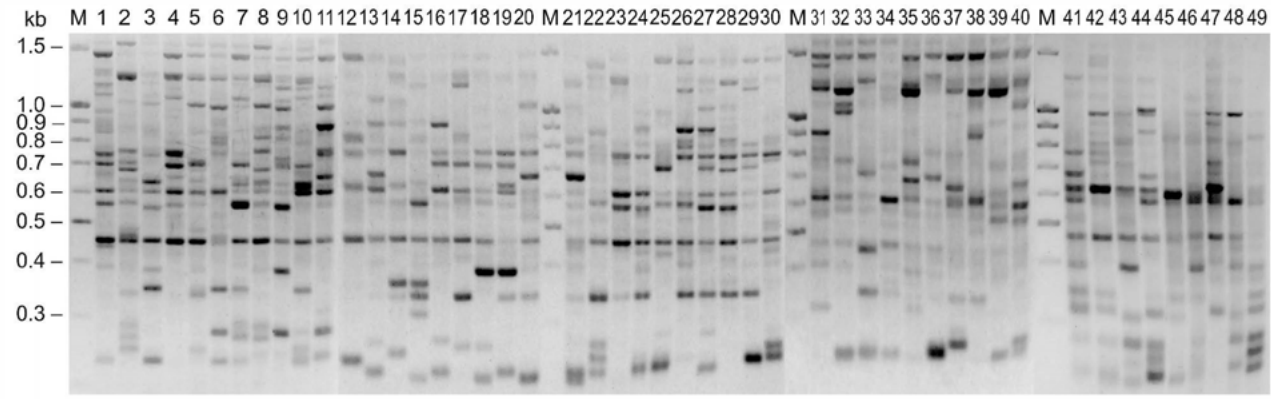

Fig. 2. An example of PCR-amplification profiles generated by ISSR primer UBC#840 from *Iris pumila* plants of different populations: Lanes 1–11 – plants from Mygiia population; 12–20 – Aliaudy; 21–30 – Kolarovo; 31–40 – Andriivka; 41–49 – Balaklava; and lane M is molecular size marker "100 bp Ladder".

this indicator in the remaining three populations were close to the average of all populations. Bands unique to individual populations were found. The greatest number of them was in Balaklava population (18.0%) and the smallest number was in Aliaudy population (2.6%).

The indices of *I. pumila* genetic variability calculated from the ISSR markers data were as follows: Shannon diversity index (S) – 0.230–0.171 with an average of 0.188; unbiased Nei's gene diversity (expected heterozygosity) – 0.111–0.150 with an average of 0.123; and the averaged Jaccard's genetic distances between individuals within populations – 55.0–61.9% with an average of 58.4%. The values of the Shannon index (S) and unbiased Nei's gene diversity (H_e) were significantly higher in Mygiia population, whereas in the rest of the populations their values were very similar. Kolarovo and Balaklava populations were found to have a lower value of the averaged Jaccard's genetic distance between plants (about 55%), compared to the rest of the populations (60%).

For the total sample of 49 *I. pumila* plants (intraspecies variability) genetic variability indices were higher than for individual populations (percentage of polymorphic bands, averaged Jaccard's genetic distance, and Shannon's index) or approximately equal to the highest value (unbiased Nei's gene diversity) (Table 2).

Principal coordinate analysis (PCoA) of the Jaccard's distance matrix revealed four groups of the samples (Fig. 3). Three of these groups are composed of the individuals from one of the populations: Mygiia, Andriivka or Balaklava. The

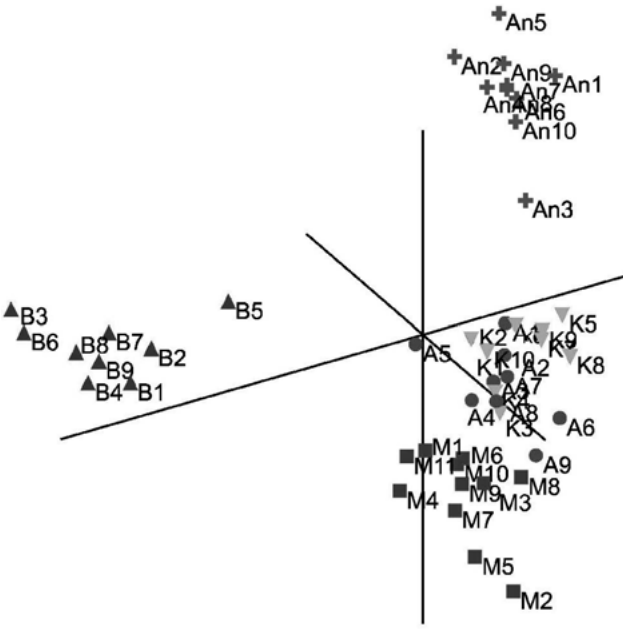

Fig. 3. Principal coordinate analysis (PCoA) of the Jaccard's distance matrix calculated from the data of ISSR-analysis for *Iris pumila* individuals from five populations (M – Mygiia, A – Aliaudy, K – Kolarovo, An – Andriivka, B – Balaklava).

fourth group includes samples from Aliaudy and Kolarovo populations, thus indicating their close genetic relatedness.

Genetic relationships among populations were assessed using Nei's unbiased genetic distances (Table 3). It was found that Aliaudy and Kolarovo

TABLE 2. Indices of genetic variability for studied *Iris pumila* populations.

Population	N	TB	Bun (%)	P (%)	S	H_e	Dj (%)	Djav (%)
Mygiia	>1000	137	12.4	56.31	0.230 ±0.016	0.150 ±0.011	43.55–75.61	61.9
Aliaudy	~40	116	2.6	46.85	0.190 ±0.016	0.125 ±0.011	46.67–73.97	59.8
Kolarovo	~50	116	7.8	44.59	0.178 ±0.015*	0.116 ±0.011*	43.08–67.11	55.0
Andriivka	~50	106	11.3	42.79	0.171 ±0.015*	0.111 ±0.011*	41.67–72.06	60.0
Balaklava	~200	100	18.0	39.64	0.171 ±0.016*	0.115 ±0.011*	40.82–72.86	55.3
Average data		115	10.3	46.04 ±2.83	0.188 ±0.007	0.123 ±0.005	–	58.4
Total sample data		222	–	97.75	0.261 ±0.012	0.152 ±0.009	40.82–86.36	68.5

N – population size; TB – total amount of scored bands; Bun – percentage of unique bands; P – percentage of polymorphic bands; S – Shannon's Index; H_e – Nei's unbiased gene diversity (expected heterozygosity); Dj – Jaccard's genetic distances between plants; Djav – average Jaccard's genetic distances between plants; * – significantly different from Mygiia population ($p < 0.05$).

TABLE 3. Nei's unbiased genetic distances calculated from the data of ISSR analysis (below diagonal) and geographic distances (km) between *Iris pumila* populations (above diagonal).

	Mygiia	Aliaudy	Kolarovo	Andriivka	Balaklava
Mygiia	–	140	141	295	443
Aliaudy	0.036	–	1.5	327	304
Kolarovo	0.041	0.011	–	328.5	305
Andriivka	0.052	0.040	0.044	–	565
Balaklava	0.061	0.043	0.050	0.063	–

populations are the most genetically similar, whereas Mygiia and Balaklava populations are the most genetically distant ones.

The relationship between the pairwise genetic distances and spatial distances between individuals was furthermore evaluated for each individual population using the Mantel test with 999 permutations (Mantel, 1967). The correlation coefficients between the pairwise Jaccard's genetic distances and spatial distances between individuals were as follows: Mygiia – -0.041 ($p = 0.405$), Aliaudy – -0.101 ($p = 0.275$), Kolarovo – 0.313 ($p = 0.009$), Andriivka – 0.309 ($p = 0.081$), and Balaklava – 0.056 ($p = 0.428$). These results suggest a weak significant positive correlation for Kolarovo population and lack of significant correlation for the rest of the populations. The correlation coefficient between the matrices of Nei's genetic distances and spatial distances between the populations was 0.900 ($p = 0.01$), indicating a significant positive relationship between genetic and spatial distances.

An analysis of molecular variance (AMOVA) showed that 76% of the total molecular variance was attributed to within population genetic heterogeneity, while 24% was among populations. This is typical of cross-pollinated species, to which *I. pumila* belongs. A three-level hierarchical analysis of molecular variance taking into account the three regions, Mykolayiv and Poltava oblasts, and the Autonomous Republic of Crimea, found that proportion of genetic variation accounted for by the differences at the regional scale, by variation among populations, and by heterogeneity within populations were 11%, 16%, and 73%, respectively.

To determine the genetic structure of *I. pumila* populations, the STRUCTURE 2.3.4 software was used (Pritchard et al., 2000). We analyzed the probability that the sampled individuals came from K ancestral populations (K = 1–8), and established K = 4 as the best value of K for the input data (results not shown). Thus, the Bayesian analysis, like cluster analysis, revealed four groups of the individuals and confirmed the common origin of Aliaudy and Kolarovo populations (Fig. 4). The remaining three populations had independent origins. The analysis revealed two genotypes of Aliaudy and Andriivka populations with a substantial estimated membership fraction in Mygiia population (11.4% and 12.7%) which suggests the probability of common origin of ancestors of these individuals and individuals from Mygiia populations. However, it should be noted that all individuals demonstrated a small proportion of the genome originated from other inferred populations.

DISCUSSION

GENETIC DIVERSITY OF THE SPECIES

In the present study, ISSR markers were applied to assess genetic diversity and genetic structure of five populations of vulnerable steppe plant species *I. pumila* from Ukraine. The average values of genetic diversity parameters were as follows: Shannon diversity index (S) – 0.188, unbiased Nei's gene diversity (H_e) – 0.123, and the mean percentage of polymorphic bands (P) – 46.0%. These values were lower than those determined by Dembicz et al. (2018) for 14 populations of *I. pumila* in Kherson Region of Ukraine (mean H_e = 0.233; mean P = 58.6%) (Dembicz et al., 2018). However, this difference may be attributed to some extent to different markers used in this study.

It is known that genetic diversity and genetic structure are mostly determined by life history traits of the species: life form (annual or perennial), breeding system (the ratio of sexual and asexual reproduction), seed dispersal mechanism, evolutionary history (hybridization and/or polyploidization); but also depend on climatic conditions and anthropogenic pressure (Hamrick and Godt, 1996). The studied species *I. pumila* is a rhizomatous outcrossing perennial. Its seeds are dispersed by gravity close to the mother plant and may be occasionally dispersed by animals or water (Sikura and Shisha, 2010). There are a number of *Iris* species with a similar biology and ecology that

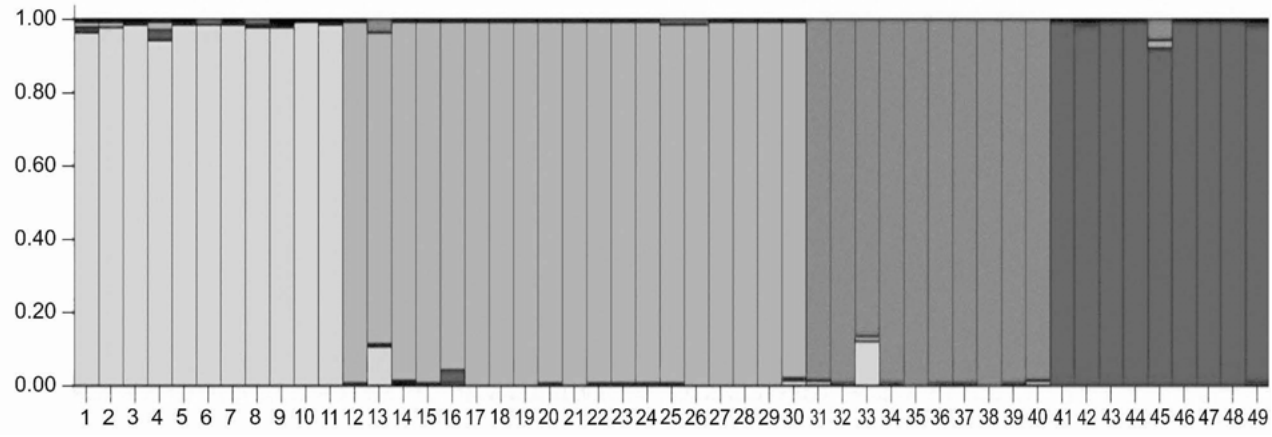

Fig. 4. Genetic structure of *Iris pumila* populations inferred from Bayesian clustering method (Pritchard et al. 2000). Each accession is represented in a plot by a single vertical bar partitioned into grey-shaded segments, which represent estimated membership fraction of individuals in one of the four clusters. The numbers below the bars indicate the origin of the corresponding individuals as follows: 1–11 – Mygiia; 12–20 – Aliaudy; 21–30 – Kolarovo; 31–40 – Andriivka; 41–49 – Balaklava.

allowed us to compare our results to the data on genetic variation in these species. Some of these species show lower values of genetic variation indices, compared to *I. pumila*, while the others are similar to it.

For example, for three Far East populations of *Iris setosa*, the percentage of polymorphic bands determined by the RAPD-analysis was 35% and genetic similarity was 0.89–0.97 (Artyukova et al., 2001). According to the results of RAPD-analysis obtained by another group, the populations of *I. aphylla* from the northern limit of the range also had somewhat lower values of genetic variation than *I. pumila*: P = 30.6%, I = 0.146, and He = 0.097 (Wroblewska and Brzosko, 2006). The genetic distances between 12 Siberian species of *Iris* ranged from 0.16 for related species *I. ruthenica* and *I. uniflora* to 0.76 for *I. bloudowii* and *I. pseudacorus*. The mean interspecific genetic distance was 0.32 (Makarevitch et al., 2003), which is even lower than the average pairwise genetic distance between the *I. pumila* individuals of the same population determined in our study (see Table 2).

Nevertheless, RAPD-analysis of intrapopulation variability of three Far East species *I. vorobievii*, *I. mandshurica* and *I. humilis* showed the results comparable with those in our study. The percentages of polymorphic bands in three species were 32.5%, 31.3%, and 48.1%, respectively; the coefficients of gene diversity were 0.104, 0.108, and 0.168; and Shannon's indices were 0.158, 0.161, and 0.251 (Kozyrenko et al., 2009). A similar level of genetic variation was detected also in *I. pseudacorus*: P = 61%, H_e = 0.227, and genetic distances ranged from 0.108 to 0.377, with an average of 0.3095 (Kozyrenko et al., 2004). For nine populations of two closely related Mediterranean endemics *I. haynei* (3 populations) and *I. atrofusca* (6 populations), the average values were: the percentage of polymorphic bands – 74.2%; Shannon's index – 0.388, unbiased Nei's gene diversity – 0.258 (Arafeh et al., 2002). For 24 specimens of *I. lactea* var. *chinensis*, each of which was collected from an individual population located in China, South Korea, Russia, or Kazakhstan, ISSR-analysis revealed a high level of variability: the percentage of polymorphic loci was 79% and genetic similarity ranged from 0.400 to 0.929 with an average of 0.592 (Wang et al., 2009).

The analysis of published data showed that the genetic variation of *I. pumila* is approximately the same or higher than that of other *Iris* species with the same biology, both at the intrapopulation and intraspecific levels. This may indicate that, despite having the status of vulnerable, the species currently has sufficient genetic diversity to survive if its natural habitats are kept in the current condition.

GENETIC STRUCTURE AND DEGREE OF POPULATION DIFFERENTIATION

The plants under study formed four groups in the PCoA plot, each of which includes the accessions from the same locality with the exception of the samples from Aliaudy and Kolarovo populations, which were grouped in one cluster (Fig. 3). The latter is in good agreement with the geographic distance between these populations, which is only 1.5 km, the rest of the pairwise distances between populations amount hundreds of kilometres. Obviously, gene flow between *I. pumila* populations through pollen and seed dispersal is possible only at such small distances.

Analysis of Nei's unbiased genetic distances between populations also showed that Aliaudy and Kolarovo populations are the most related. In general, the resulting picture of genetic relationships between the populations agrees well with their spatial distribution: the correlation coefficient between the genetic and geographic distances was 0.9. This indicates the significant effect of isolation by distance on the genetic structure, the occurrence of gene flow between populations that proportionally decreases with increasing distance, and lack of significant effects of genetic drift, which may change the distribution of genetic variation by the decrease of variation within populations and the increase of differentiation between populations (Ellstrand and Elam, 1993). At the same time, we could not find a correlation between the spatial distances and genetic distances between plants within the same population.

The Bayesian analysis of genetic structure of the sampled populations based on allele frequencies of individual PCR loci also revealed four groups formed according to the geographical location, one of which combined the plants from Aliaudy and Kolarovo populations (Fig. 4). The results of the population structure analysis indicate a high homogeneity in the *I. pumila* populations studied. Minor estimated membership fractions, indicative of the relatedness to the populations other than the original one, may point to free exchange of genetic information between populations in the past, before

the habitat fragmentation. Furthermore, there were individual plants with membership coefficients in the populations other than the original that exceeded several percent. These data suggest that the exchange of genetic material between distant populations occurs at the present time as well.

Most of the genetic variation of *I. pumila* is within populations, and the differentiation between the populations and regions is relatively low, despite the considerable geographical distances separating them. Analysis of molecular variance (AMOVA) showed that the between-population component of genetic variation is only 24%, whereas the within-population component is 76%. Such a pattern of genetic variation is typical of outcrossing perennial plants (Hamrick and Godt, 1996), such as *I. pumila*. This is also consistent with data of Dembicz et al. (2018) who found that most of the *I. pumila* molecular variance (82%) was distributed within populations, whereas genetic differentiation among populations was moderate. In our study, the latter portion was larger which can be attributed to the larger distances between populations.

The three-level hierarchical analysis including additionally three regions: Mykolayiv and Poltava oblasts and the Autonomous Republic of Crimea revealed that 11% of the genetic variation was among the regions, 16% of the variation was among populations, and 73% was found within populations.

The genetic differentiation of plant populations reflects interactions amongst a range of different processes, including the long-term evolutionary history of the species (e.g., shifts in distribution, habitat fragmentation and population isolation), mutation, genetic drift, mating systems, gene flow and selection (Schaal et al., 1998). The genetic similarity of geographically distant populations of *I. pumila* may be due to a number of factors, including the same directional selection pressure, the gene flow between populations, and their common origin. Transfer of genetic material between populations via dispersal of pollen or seed over such long distances is hardly probable, but theoretically can occur through continuous exchange of genes between closely located geographically intermediate populations, mainly via pollen by insect pollinators. It can be easily imagined in the natural steppe part of Ukraine, where *I. pumila* is a typical species, although its populations are isolated from each other as a result of fragmentation. But apparently this process also takes place in the forest-steppe zone, where the species is very rare and only a few isolated populations have been reported in Kyiv and Poltava oblast (Parnikoza et al., 2017). According to Mills and Allendorf (1996), one to ten migrants per generation are sufficient to slow down the genetic divergence of populations caused by the gene drift.

THE EFFECTS OF HABITAT FRAGMENTATION

The indicators of genetic variation were close for individual populations, only Mygiia population had significantly higher values (Table 2). Perhaps this is attributable to its larger size of more than a thousand individuals, whereas the size of the rest of the studied populations ranged from 50 to 200 individuals. It is known that the level of genetic variation is positively correlated with the population size (Leimu et al., 2006). Small plant populations are more prone to extinction due to loss of genetic variation through random genetic drift, increased selfing, and mating between related individuals (Honnay and Jacquemyn, 2007, Aguilar et al., 2008). However, our results indicate that even small populations of *I. pumila* that are fragmented and forced into a restricted habitat still retain high levels of genetic variation. Some published studies demonstrate that plant species with a small fragmented habitat may have a relatively high level of genetic variation (Gitzendanner and Soltis, 2000). Therefore, it is obvious that the size of the habitat is not the only factor that affects the level of genetic variation. Life history traits, particularly breeding system and life form, also have a significant impact on genetic variation, because the loss of genetic variation in a fragmented habitat becomes more pronounced after several generations (Hamrick and Godt, 1996). Our findings may be explained by outcrossing of *I. pumila,* which results in a high variation due to recombination, and also by its ability to reproduce vegetatively by rhizomes ensuring the long-term survival of clones over periods unfavorable for propagation by seeds, increased generation time (the time between two consecutive generations), and potential protection against the loss of genetic variation due to significant changes in the environment. Furthermore, the high life span of the species promotes genetic exchange between generations (Wroblewska et al., 2003; Aguilar et al., 2008).

The ploidy level of plants can also affect the process of genetic depletion. As theory predicts, autotetraploids are less susceptible to the loss of

genetic diversity by genetic drift than diploids (Aguilar et al., 2008). *I. pumila* (2n = 32) is considered to be a natural amphidiploid hybrid between the two endemic Mediterranean species *I. attica* Tineo and *I. pseudopumila* Boissier & Heldreich, i.e., it is an allotetraploid (Sikura and Shisha, 2010). According to published data, polyploidy can influence the level of genetic variation, because polyploids are generally characterized by fixed heterozygosity (Adams, 2007). Polyploidy also counteracts genetic drift and can result in a slower rate of differentiation between populations (Artyukova et al., 2004).

Two populations involved in our study, namely Andriivka and Balaklava populations, are at the northern and southern limits of the species' range in Ukraine, respectively. Isolated marginal or peripheral populations may have lower levels of genetic variability due to the bottleneck effect, environmental fluctuations, small size, infrequent flowering, formation of seeds, and reproduction of the population. Such populations are often under stronger selection pressure than the core populations that leads to adaptation of peripheral populations to different local habitats (Kawecki, 2008). Habitat fragmentation may increase genetic differentiation of populations by limiting gene flow and increasing genetic drift in small populations (Slatkin, 1987). On the other hand, the opposite situation is possible to occur: higher genetic variation in peripheral populations living under marginal ecological conditions due to fluctuating selection caused by variable environment (Safriel et al., 1994). Despite this, we were unable to detect differences in the levels of genetic variation between similarly sized peripheral and central populations.

The results of our study show high genetic variation of *I. pumila* and low divergence between spatially distant isolated populations, including peripheral ones. Fragmentation and reduction of *I. pumila* habitat have occurred over one or two centuries, starting from the beginning of intensive economic development of the southern regions of Ukraine in the second half of the 18th century. It is quite possible that this period was not long enough for the loss of genetic heterogeneity and increase in divergence among remnant populations. In this case, a high level of genetic variation, which we observed in all populations, has been preserved from the time when the species habitat was not yet fragmented and modern isolated populations shared a common gene pool. Since we sampled mainly the plants from clonal colonies, the values obtained may partially reflect the higher level of genetic variation of the plants in the past (Curto et al., 2015). According to published data, many consequences of habitat fragmentation may take a long time to manifest themselves (Ewers and Didham, 2006; Haddad et al., 2015). A study performed on fragmented systems demonstrated that the species subjected to fragmentation conditions for more than 100 years have significantly stronger negative impact on genetic variation, compared to species evaluated in fragmented systems of less than 50 years and 50–100 years (Aguilar et al., 2008). As evidenced by our results and the data of other researchers (Collevatti et al., 2014; Severns et al., 2011), even the time period of about a century may not be long enough for manifestation of habitat fragmentation effects, particularly for species with long-lived individuals. Therefore, even without additional environmental measures, the negative effects of habitat fragmentation and reduction of the range on *I. pumila* genetic variation may become more pronounced in the future.

CONCLUSION

The key indices of population genetic variability of five *I. pumila* populations calculated from the ISSR markers data were on average as follows: Shannon diversity index (S) – 0.188; unbiased Nei's gene diversity (H_e) – 0.123; and the average measure of Jaccard's genetic distances between individuals within populations – 58.4%. Although the largest population had significantly higher values of S and He, the small and marginal populations also showed a comparable level of variation. Most of the genetic variation of *I. pumila* was distributed within the populations. A strong correlation was found between Nei's genetic distances and geographic distances between the populations. According to the Bayesian analysis, genetic structure of the populations was highly homogeneous; however, the presence of admixed genotypes indicated the possibility of gene flow between the populations at present.

AUTHORS' CONTRIBUTION

OB – molecular-genetic analysis, data analysis, interpretation of results and writing the manuscript; IA – sampling, data analysis, interpretation

of results and writing the manuscript; IP – sampling, interpretation of results and revising the manuscript; VK – interpretation of results and revising the manuscript. The authors have declared that there is no conflict of interest.

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