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The assessment of the current status of *Gentiana lutea* L. populations of the Ukrainian Carpathians: Ecological and genetic approaches

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Abstract

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Based on the analysis of the genetic polymorphisms (RGAP- and CDDP-PCR) among populations and comparison of these results with ecological characteristics (density, area, spatial and vitality structure, age, ability of renewal and self-maintenance), the status of five natural (Lemska, Gutyn Tomnatyk, Sheshul-Pavlyk, Krachuneska, Troyaska-Tataruka) and one man-made (Pozhyzhevska) populations of *Gentiana lutea* from the Ukrainian Carpathians was assessed. The results of the complex ecological and genetic analysis have revealed that three populations are unstable (Krachuneska, Troyaska-Tataruka, Gutyn Tomnatyk), two are relatively stable (Lemska, Pozhyzhevska) and only one is stable (Sheshul-Pavlyk). The research results can be used for stabilizing the number of violations and restoring the endangered natural populations. Based on these data, the recommendations for the conservation and protection of *G. lutea* populations have been developed.

Keywords

CDDP- and RGAP-PCR, ecological characteristics of population, genetic diversity, *Gentiana lutea* L., population status, population strategies

Introduction

An endangered species *Gentiana lutea* L. (commonly known as yellow gentian), belonging to Gentianaceae family, is under protection in most European countries, including Ukraine. *Gentiana lutea* has been included in The Red book of Ukraine (2009) and has the category of threat – Vulnerable (VU). This species is at risk of extinction in natural habitats of the Ukrainian Carpathians because of the uncontrolled use of the plant material and long-lasting habitat disturbance.

Nowadays, *G. lutea* is intensively used for the needs of official and alternative medicine. The *G. lutea* plants possess antioxidant, immunomodulatory, stomachic, antifungal, anti-inflammatory, cardiotonic, sedative, haemostatic, antispasmodic, diuretic properties (Prakash et al., 2017). Yellow gentian has immense potential in preventing and treating several diseases (anaemia, malaria, stomachic disease, etc.). The medicinal value of *G. lutea* raw materials requires estimation of its resources in Ukraine and creation of the ways for their conservation and restoration.

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Current studies mostly include the analysis of the ecological parameters: age and vitality structure, the populations' ability to recover and self-sustain, the characteristics of phytocenoses etc. However, insufficient attention is paid to studying the genetic structure, genetic polymorphism within and between plant populations. The knowledge of the genome is necessary to determine the strategy of population's development.

Profound understanding of the level of genetic diversity and genetic structure of *G. lutea* populations helps to clarify their ability to adapt to the environment in response to its changes. Nowadays, polymerase chain reaction (PCR)-based methods of molecular-genetic analysis provide one of the most effective tools for studying genetic variation in plants and animals. Conserved DNA-derived polymorphism (CDDP) molecular markers are an innovative method based on conserved DNA sequences, developed by Collard B.C.Y and Mackill D.J. in 2009 (Poczai et al., 2013; Collard and Mackill, 2009). The single primers of CDDP method are designed and combined with the conserved regions of functional genes involved in responses to abiotic and biotic stress or plant development and can effectively produce markers related to the target traits (Liu et al., 2020; Jiang and Zang, 2018). Resistance gene analogue polymorphism (RGAP) is a unique molecular technique because it has a direct association with potentially functional genes for resistance to a large number of pathogens in plant species. RGAP has been used to identify tightly linked markers for disease resistance genes or link tightly to it (Dong et al., 2009; Mansureh et al., 2004). CDDP and RGAP techniques are advantageous because of the numerous fragments amplified by CDDP- and RGAP-primers and are undoubtedly cheap and useful genetic markers for evaluating the genetic structure of *G. lutea* population and also the outcome of *G. lutea* plants conservation.

Today, main populations of *G. lutea* have been established and described in the Ukrainian Carpathians (Mayorova et al., 2013; 2015). Several studies of genetic diversity and structure of *G. lutea* populations have already been conducted by different types of DNA-markers (RAPD-, ISSR-, IRAP) (Mosula et al., 2014a; 2014b; 2014c).

Therefore, the combined use of genetic and ecological data of *G. lutea* is urgently needed to create conservation activities for this species. Such a complex approach provides a basis for drawing scientifically valid guidelines on species conservation and rational management and creation of the effective protection strategies.

The aim was to evaluate the current state of *Gentiana lutea* L. populations from the Ukrainian Carpathian Mountains using the ecological indices and the parameters of genetic polymorphism.

Materials and methods

Analysis of ecological parameters of populations

Our studies were performed on five historically isolated natural *G. lutea* populations, located on the Krachuneska mountain valley, on the ridge slope between Troyaska and Tataruka Mountains (Svydivets ridge, Ukrainian Carpathians), on the Lemska mountain valley (Chornohora range, Ukrainian Carpathians), on Hutyn Tomnatek Mountain (Chornohora range, Ukrainian Carpathians) and on the ridge slope between Sheshul and Pavlyk Mountains. All these populations grow on the territory of the Carpathian Biosphere Reserve. In addition, we studied the artificially created (in the 1970s) agropopulation located on Pozhyzhevska Mountain (Chornogora ridge, Ukrainian Carpathians, Carpathian National Nature Park) (Mosula et al., 2015). The material for introduction of Pozhyzhevska population was taken from Sheshul-Pavlyk population (Bedej et al., 2010).

The natural factors of the abiotic environment (altitude, altitude zonation, relief, exposure, and slope steepness) were taken into account in the ecological analysis. Also, we studied the influence of phytocenotic environment (the biotic factor), sheep and cattle grazing, collection of medicinal raw materials, trampling (the anthropogenic factors). Altitude, exposure, location coordinates were determined by using a Garmin Oregon 450 GPS navigator.

The total density of populations (Dt) and their age and spatial structure were assessed in 20 test plots (1 × 1 m) stated by a method of random numbers (Rabotnov, 1950; Uranov, 1975; Uranov and Serebryakova, 1976). The number of individuals was counted, and their age and origin (vegetative or generative) were determined at each plot.

Number of indices (regeneration (RI), substitution (SI), aging (AI)) were used (Uranov, 1975; Zhivotovskiy, 2001; Kovalenko, 2005). These indices were calculated as follows:

$$RI = \frac{j + im + v}{g} \times 100\%,$$

$$SI = \frac{j + im + v}{g + s} \times 100\%,$$

$$AI = \frac{g_3 + s}{j + im + v + g + s} \times 100\%,$$

where j, im, v, g, g_3, s are the number of juvenile, immature, virgin, generative, old generative and senile plants per 1 m^2, respectively.

The type of an average population (young, maturing, mature, transitional, aging, old) was determined according to delta–omega criterion on the basis of the ageness index (Δ) and the efficiency index (ω) (Zhivotovskiy, 2001). Δ is the index of population ageness that estimates the age level of population at each point in time. ω is the efficiency index of population. Δ and ω indices were calculated using the formulas: $\Delta = \sum p_i m_i$; $\omega = \sum p_i \mathbf{e}_i$, where p_i is the proportion of number of plants in the i^{th} age state in the population, m_i is the ageness, defined as the ratio of energy consumed by the i^{th} age state to the total amount of energy available during ontogeny to an individual plant, e_i is the relative energy efficiency (or simply efficiency) of plants in the ith ontogenetic state (Uranov, 1975; Zhivotovskiy, 2001). The effective density of the population (De) was determined as follows: De = ωDt (Zhivotovskiy, 2001).

The mode of population self-maintenance was estimated from the numerical ratio between plants of generative and vegetative origin. Seed production was evaluated by using the coefficient of reproductive capacity (CRC) calculated as the ratio of the coefficient of variation in seed production to the arithmetic mean value of this parameter (Tsaryk and Holubets, 2001).

The analysis for the vitality status of populations was performed as it has been described (Zlobin, 1989). Interpopulation variation in morphometric parameters was assessed by comparing representative samples of 50 generative plants from each population. Since *G. lutea* is in the Red book of Ukraine (*Chervona knyha Ukrainy. Roslynnyi svit*, 2009), analysis was limited to parameters that could be determined without digging the plants out or damaging them by any other way. Biometric data were processed statistically with calculation of arithmetic mean (*X*) with standard deviation (*Sx*) and error (*Sx'*), coefficient of variation (*CV*), and limits of parameter values (min, max).

To determine the type of population strategy, we used an ecogenetic approach proposed by Tsaryk et al. (Tsaryk and Holubets, 2001), which combines methods used by Grime (1974, 1978, 1979) and Smirnova (1987) and takes into account differential characters at individual and group levels. Three types of primary strategies were distinguished: competitive (C), stress-tolerant (S), and ruderal (R). Secondary strategies were determined based on combinations of differential characters of primary strategy types (Tsaryk and Holubets, 2001).

DNA isolation, PCR amplification and statistical analysis of genetic data

Eighty six accessions of *G. lutea* were used in this study to evaluate genetic parameters. In molecular genetic analysis, six CDDP- and six pairs of RGAP-primers were used in PCR. Primer sequences are listed in Table 1. Total genomic DNA was isolated from fresh leaves using the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich, 1985). Amplifications were performed in Tertsyk MC2 thermocycler (Biotechnology, Russia). The 20 µl PCR mixture contained: 20–30 ng of genomic DNA, 0.2 mM each dNTP (Fermentas, Lithuania), 1.25 U Taq DNA-polymerase (AmpliSens, Russia), 0.5 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. At least three samples per marker were rerun to ensure results reliability.

Table 1. Nucleotide sequences of CDDP- and RGAP-primers

No.	Primer	Nucleotide sequence, (5 –3')
		CDDP-primers
1.	WRKY-A-R	5' GTGGTTGTGCTTGCC 3'
2.	WRKY-B	5' GCCCTCGTASGTSGT 3'
3.	MYB	5' GGCAAGGGCTGCCGC 3'
4.	ERF-F	5' CACTACCGCGGSCTSCG 3'
5.	MADS-A	5' ATGGGCCGSGGCAAGGTGC 3'
6.	ABP1-2	5' ACSCCSATCCACCGC 3'
		RGAP-primers
1.	RLRR for RLRR rev	5' CGCAACCACTAGAGTAAC 3' 5' ACACTGGTCCATGAGGTT 3'
2.	XLRR-INV1 XLRR-INV2	5' TTGTCAGGCCAGATACCC 3' 5' GAGGAAGGACAGGTTGCC 3'
3.	Pto kin3 Pto kin4	5' TACTTCGGACGTTTACAT 3' 5' AGTGTCTTGTAGGGTATC 3'
4.	XLRR for XLRR rev	5' CCGTTGGACAGGAAGGAG 3' 5' CCCATAGACCGGACTGTT 3'
5.	Cre3Ploop Cre3-k3	5' GCGGGTCTGGGAAATCTACC 3' 5' CTGCAGTAAGCAAAGCAACG 3'
6.	NLRR-INV1 NLRR-INV2	5' TGCTACGTTCTCCGGG 3' 5' TCAGGCCGTGAAAAATAT 3'

S = G/C.

The amplification conditions were the following: CDDP-PCR: 95 °C – 2 min, 35 cycles (94 °C – 30 sec, 53 °C – 60 sec, 72 °C – 1.5 min), 72 °C – 2.5 min; RGAP-PCR: 95 °C – 2 min, 40 cycles (94 °C – 30 sec, 53 °C – 45 sec, 72 °C – 1 min), 72 °C – 2.5 min. Amplified products were separated by electrophoresis on 1.3 % agarose gels in 1 × SB-buffer (5 mM $Na_2B_4O_7$, pH 8.5) and made visible using ethidium bromide staining. The amplified DNA fragments were documented by using image analysis software Total Lab 120 (TotaLab Ltd., Newcastle upon Tyne, UK).

Proportion of polymorphic bands (P), the expected heterozygosity (He), and Shannon's index (S) were calculated with GeneAlex 6.5 software (Peakall and Smouse, 2012). The nonparametric analysis of molecular variance (AMOVA) was conducted with GenAlEx to describe the genetic structure both between populations and between individuals in each sampled/investigated population.

Complex ecological and genetic analysis

The comprehensive analysis of the population states by their ecological and genetic parameters was performed similarly to the 5-point system of Beltyukova (2010). We created the scale with 13 parameters for the assessment of the current state of populations. In addition to the 12 indices (Dt, De, RI, SI, AI, Δ, ω, g/v, RC, P, He, S) listed in the Table 2, the degree of anthropogenic impact (ai) was also taken into account: insignificant (5 points), weak (4 points), medium (3 points), moderate (2 points), strong (1 point). The degree of anthropogenic impact (e.g. the plucked generative shoots and eaten or trampled plants by the cattle) has been determined visually by the proportion of damaged plants. Based on the results of complex ecological and genetic analysis of *G. lutea* populations, they were classified into three groups: stable (≥53), relatively stable (42–52), unstable (≤41).

Results and discussion

An integrated ecological and genetic approach to assess the current state of the six populations of *G. lutea* from the Ukrainian Carpathians was applied. Using molecular and ecological methods, we have divided *G. lutea* populations into three groups: stable, relatively stable and unstable (Figs 1, 2). Sheshul-Pavlyk population was stable (56 points by RGAP-PCR). Lemska population (50 points – CDDP-PCR, 44 points – RGAP-PCR) and man-made population on the Pozhyzhevska Mountain (47 points – CDDP-PCR, 47 points – RGAP-PCR) were classified as relatively stable. Hutyn Tomnatek (38 points – CDDP-PCR, 32 points – RGAP-PCR), Troyaska-Tataruka (27 points – CDDP-PCR, 26 points – RGAP-PCR) and Krachuneska (30 points – CDDP-PCR, 30 points – RGAP-PCR) populations were categorized to an unstable group.

In our opinion, the stability of Sheshul-Pavlyk population is defined by their firm environmental parameters, location in the conservation area and high genetic diversity. It was established that indices of genetic diversity (P, He, S) of this population were the highest of all investigated populations by RCAP-PCR.

Table 2. The scale for assessment of the current state of *G. lutea* populations

Indices	Points				
	1	2	3	4	5
Dt (ind. m^{-2})	<1.3	1.4–2.6	2.7–4.0	4.1–5.4	>5.5
De (ind. m^{-2})	<0.6	0.7–1.3	1.4–2.0	2.1–2.7	>2.8
RI (%)	<58	59–116	117–175	176–234	>235
SI (%)	<56	57–112	113–169	170–225	>226
AI (%)	>15.5	15.4–11.6	11.5–7.7	7.6–3.9	<3.8
Δ	>0.309	0.308–0.279	0.278–0.248	0.247–0.219	<0.218
Ω	>0.541	0.540–0.510	0.509–0.479	0.478–0.449	<0.448
g/v	<0.42	0.43–0.85	0.86–1.28	1.29–1.71	<1.72
CRC	<0.52	0.53–1.10	1.11–1.68	1.69–2.26	>2.27
P* (%)	<25.2	25.3–30.5	30.6–35.8	35.9–41.0	>41.1
He*	<0.064	0.065–0.090	0.091–0.115	0.116–0.141	>0.142
S*	<0.101	0.102–0.138	0.139–0.175	0.176–0.212	<0,213
P** (%)	<21.0	21.1–26.4	26.5–31.8	31.9–37.2	>37.3
He**	<0.068	0.069–0.092	0.093–0.116	0.117–0.140	>0.141
S**	<0.105	0.106–0.139	0.140–0.173	0.174–0.207	<0.208

Dt, total density; De, effective density; RI, regeneration index; SI, substitution index; AI, aging index; Δ, ageness index; ω, efficiency; g/v, ratio between plants of generative and vegetative origin; CRC, coefficient of reproductive capacity; P, proportion of polymorphic bands; He, the expected heterozygosity; S, Shannon's index. *data from RGAP-analysis; **data from CDDP-analysis.

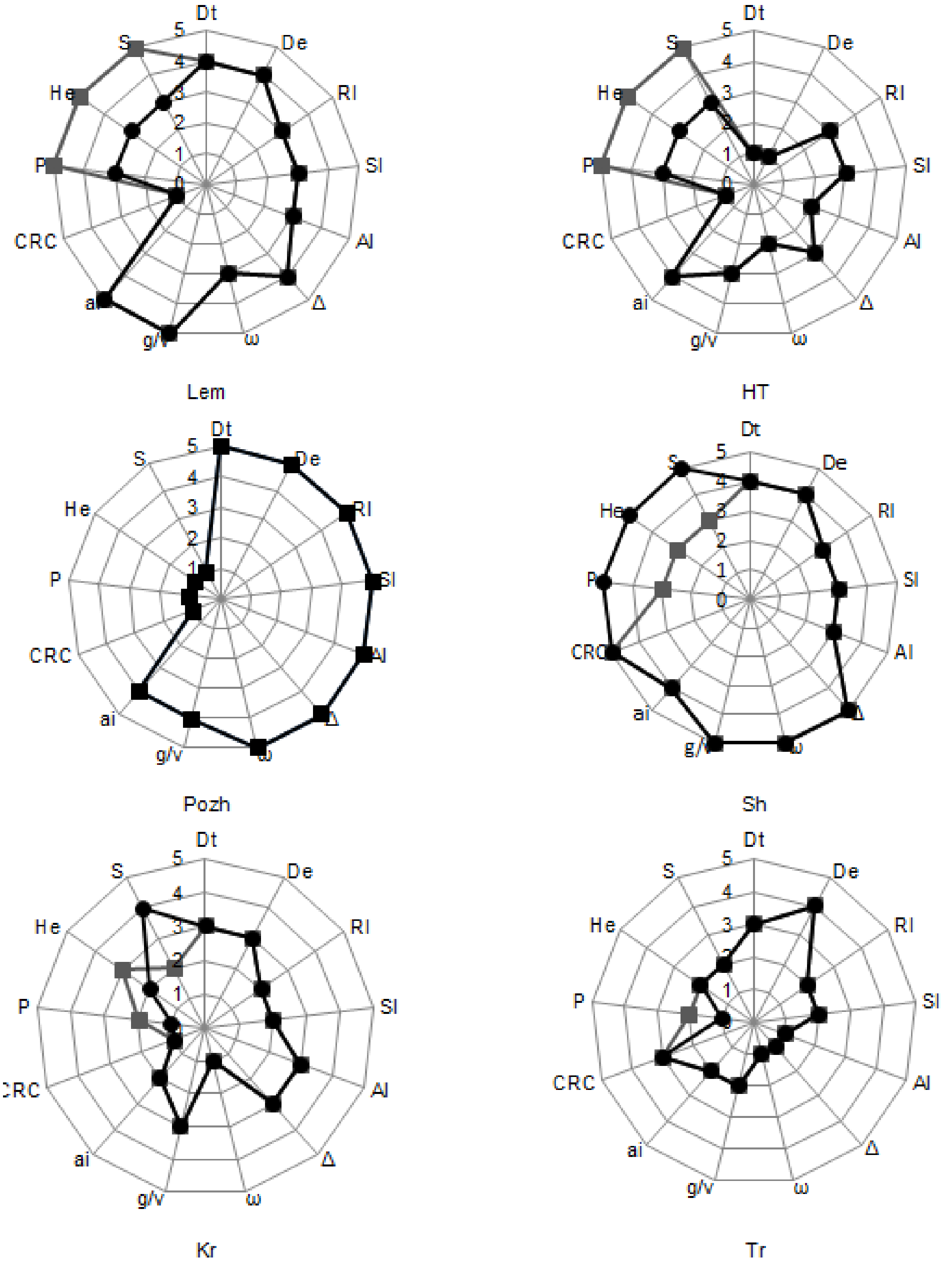

Fig. 1. Graphs based on ecological and genetic parameters illustrating stable (Sh, Sheshul-Pavlyk population), relatively stable (Lem, Lemska; Pozh, Pozhyzhevska populations) and unstable (HT, Hutyn Tomnatek; Kr, Krachuneska; Tr, Troyaska-Tataruka populations) states of *G. lutea* populations. -■- – CDDP-markers, -●- – RGAP-markers. The decryptions of indices are in Table 1.

According to the three coefficients of genetic polymorphism, the investigated populations were ranged as following: Sheshul-Pavlyk > Lemska ≈ Hutyn Tomnatek > Krachuneska ≈ Troyaska-Tataruka > Pozhyzhevska. The highest and the lowest values of all populations differed about 2–3 times (Fig. 2).

Sheshul-Pavlyk population was characterized by the large area (about 40 ha) of growing, high total and effective densities, diffuse distribution of plants, large proportion of juvenile individuals of generative origin, relatively high abundance of generative shoots within the population range (compared to other populations), high morphometric parameters, and high coefficient of reproductive capacity. The regeneration and substitution indices were high and the aging index was low (Table 3). As it is known, the large population sizes and high density provide the high level of genetic diversity of populations and can prevent the inbreeding and genetic drift (Zhang et al., 2007). According to delta–omega criterion, Sheshul-Pavlyk population was young. As a result of exposure to weak stress and strong disturbances, this population had secondary features indicative of competitive–ruderal strategy. Thus, this population is able to compete with the subalpine grassy dominant species *Nardus stricta* L. and

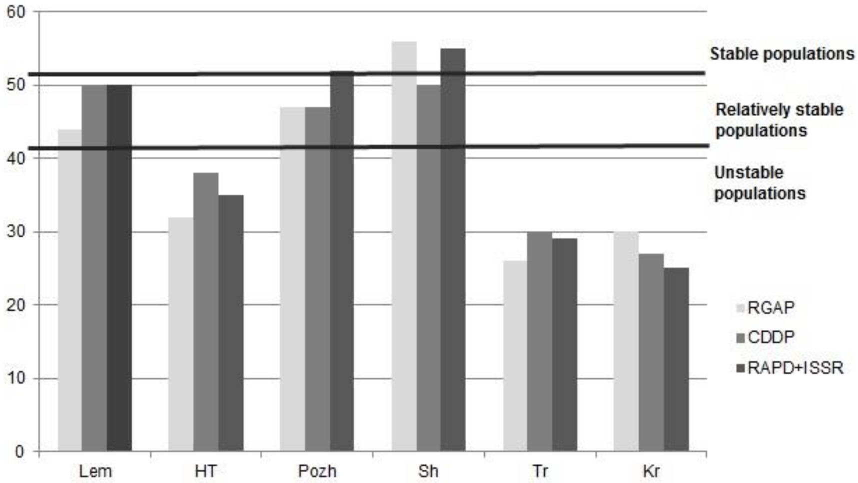

Fig. 2. Comparative characteristics of the state of *G. lutea* populations using ecological and genetic parameters (Mosula et al., 2014b). Sh, Sheshul-Pavlyk; Lem, Lemska; Pozh, Pozhyzhevska; HT, Hutyn Tomnatek; Kr, Krachuneska; Tr, Troyaska-Tataruka populations.

Poa chaixii Vill., to exist for a long time in the occupied territory, to create a continuous phytogenic field due to the diffuse structure, and to capture new territories. All these facts together enable the population to survive and adapt to changing environmental conditions.

These data have conformed to our previous studies of *G. lutea* populations (using RAPD- and ISSR-markers), where Sheshul-Pavlyk population was also evaluated as stable (Mosula et al., 2014b) (Fig. 2).

Lemska and Pozhyzhevska populations were grouped to relatively stable populations. The affiliation of the populations to relatively stable group proves their ability to survive and adapt to changing habitat conditions. The levels of genetic diversity by RGAP- and CDDP-markers of these populations were high (Table 3). Lemska population was characterized by stable age spectrum, diffuse distribution of individuals, high total and effective densities, low CRC index, prevalence of vegetative reproduction, slight rejuvenation of vegetative progeny, large phytomass volume, and wide leaf surface. The regeneration and substitution indices were high and the aging index was low (Table 3). According to delta–omega criterion, Lemska population was young. As a result of exposure to weak stress and slight disturbances this population has been characterized by competitive strategy. That is, the population is viable and has high ability to use environmental resources. The average annual growth of biomass and the efficiency of leaf surface of Lemska population were huge. The population elements can exist for a long time in the occupied territory. Lemska population has created a continuous phytogenic field due to the diffuse and compact-diffuse structure.

The lowest level of genetic polymorphism of all investigated populations was in the agro-population from the Pozhyzhevska Mountain by all indices (P, He, S) using both RGAP- and CDDP-PCR (Table 3, 4). Despite the low genetic polymorphism, this population has had high level of ecological indices conditioning the location of the population in favourable conditions. Pozhyzhevska population was characterized by the high total and effective densities, as follows from its compact–diffuse spatial pattern, left-sided age spectrum, with juvenile and immature individuals comprising the most abundant group. This population has had the highest regeneration and substitution indices and the lowest aging index compared to all investigated populations. The population is characterized by the competitive–stress–tolerant–ruderal strategy. Pozhyzhevska population is able to quickly capture new territories and compete with co-dominant species in groups (*Deschampsia caespitosa* (L.) Beauv, *Vaccinium myrtillus* L., *Poa nemoralis* (L.) subsp. *carpatica* Jirásek). This population has had the maximum intensity of life processes and the large biomass. The type according to vitality class of Pozhyzhevska population is flourishing.

The low rates of genetic polymorphism of this population, obviously, can be explained by its young age (~40 years), long ontogenesis of individuals (50–100 years) and long pregenerative period (12–15 years). The low intrapopulation polymorphism of plants of Pozhyzhevska population can illustrate the "founder effect". The loss of genetic variation of the population occurs when a new population is established by a very small number of individuals from a larger population. In addition, Pozhyzhevska population is located in tourist hotspots, where the habitat is heavily damaged, which may directly reduce genetic diversity.

Considering the results of ecological and genetic studies of the state of man-made Pozhyzhevska population, it is hypothesized that the artificial plantations in the highlands are effective in order to increase the number of habitats of this species.

Hutyn Tomnatek, Troyaska-Tataruka and Krachuneska populations were attributed to the unstable group. Troyaska-Tataruka and Krachuneska populations were characterized by the intensive livestock grazing, which

Table 3. Characteristics of *G. lutea* populations in the Ukrainian Carpathians

Characteristics	Population					
	Lemska	Hutyn Tomnatek	Pozhyzhevska	Sheshul and Pavlyk	Krachuneska	Troyaska and Tataruka
Location	1,600–1,750 SE; 20°–40°	1,800–1,850 SE; 20°–40° (>45°)	1,450–1,455 NE; 5°–10°	1,400–1,700 SW; 20°–40° (>45°)	1,500–1,730 SE; 20°–40° (>45°)	1,300–1,600 SE; 20°–40° (>45°)
Type of age structure	Complete, with left-sided age spectrum	Complete, with left-sided age spectrum	Complete, with left-sided age spectrum	Complete, with left-sided age spectrum	Complete, with left-sided age spectrum	Complete, with left-sided age spectrum
Dt (ind. m^{-2})	5.2	0.7	6.5	5.3	3.0	3.9
De (ind. m^{-2})	3.5	0.4	2.4	2.3	1.2	1.7
g/v (%)	65.8/34.2	42.5/57.5	58.3/41.7	73.8/26.2	46.7/53.3	41.1/58.9
CRC	0.44	0.32	0.23	3.10	0.43	1.64
RI	130.2	128.3	350.9	162.6	61.8	79.4
SI	116.9	114.1	338.5	146.9	58.2	57.5
AI	8.6	14.1	3.5	10.1	8.8	21.6
Δ	0.36	0.31	0.17	0.21	0.22	0.26
Ω	0.67	0.56	0.37	0.43	0.41	0.43
Delta–omega classification						
Vitality status	Flourishing	Balanced	Flourishing	Flourishing	Balanced	Flourishing
P* (%)	33.3	32.1	22.6	48.8	22.6	25.0
He*	0.106 ± 0.019	0.106 ± 0.019	0.051 ± 0.013	0.177 ± 0.023	0.075 ± 0.017	0.077 ± 0.017
S*	0.160 ± 0.028	0.159 ± 0.028	0.083 ± 0.019	0.263 ± 0.032	0.112 ± 0.025	0.118 ± 0.025
P** (%)	45.0	45.0	18.3	27.5	24.2	25.8
He**	0.152 ± 0.018	0.172 ± 0.019	0.056 ± 0.012	0.110 ± 0.017	0.095 ± 0.016	0.089 ± 0.015
S**	0.229 ± 0.025	0.253 ± 0.027	0.089 ± 0.018	0.160 ± 0.025	0.139 ± 0.023	0.134 ± 0.022
Types of strategies	Competitive	Competitive-stress-tolerant	Competitive-stress-tolerant-ruderal	Competitive-ruderal	Competitive-stress-tolerant	Competitive-stress-tolerant

Dt, total density; De, effective density; RI, regeneration index; SI, substitution index; AI, aging index; Δ, ageness index; ω, efficiency index; g/v, ratio between plants of generative and vegetative origin; CRC, coefficient of reproductive capacity; P, proportion of polymorphic bands; He, the expected heterozygosity; S, Shannon's index. *data from RGAP-analysis, **data from CDDP-analysis.

results not only in plant damages but also in the removal of phytomass, which provides conditions for expansion of *Dushekia viridis* (Chaix) Opiz. There were compact–diffuse distribution pattern, low density (Krachuneska), low morphometric parameters (Krachuneska), pauses in flowering (Troyaska-Tataruka, Krachuneska), small proportion of flowering plants (Krachuneska), high CRC (Troyaska-Tataruka), prevalence of vegetative reproduction (Troyaska-Tataruka, Krachuneska), and loss of continuous phytogenic field (Troyaska-Tataruka, Krachuneska) in these populations. The regeneration and substitution indices were low and the aging index was high (Troyaska-Tataruka) (Table 3). Slightly disturbed populations affected by strong stress acquire secondary features indicative of competitive–stress–tolerant strategy. They are characterized by long life in the occupied territory. Their individuals stay in pregenerative period longer. In addition, the low efficiency of leaves and a small surface area of leaves, low potential and actual productivity of seeds and vegetative rudiments of plants from these populations should be noted. A small stock of seeds in the population and unfavorable conditions for the survival of seedlings appear to be responsible for a shift from seed to vegetative reproduction.

The parameters of genetic diversities (P, He, S) of Troyaska-Tataruka and Krachuneska populations were the lowest of all natural investigated populations by CDDP- and RGAP-PCR (Table 3). The previous studies have shown that the gene flow between Troyaska-Tataruka and Krachuneska populations is restricted due to the large distance (around 8 km) and mechanical barriers (mountain ranges, exposition of slopes) (Tsaryk and Holubets, 2001). The populations from the Svydivets ridge are separated by the mountain from other populations of this species, and propagation efficiency is limited. A high level of such isolation may lead to inbreeding depression and consequent reduction in the level of heterozygosity and alterations in the overall genetic structure of the population, which can affect its stability (Malynovskyi, 1998). In additional, the populations from the Svydivets ridge suffer from the significant pastoral burden.

The results of the previous complex analysis by RAPD- and ISSR-markers further illustrated that Troyaska-Tataruka and Krachuneska populations were also unstable (Mosula et al., 2014b) (Fig. 2).

Hutyn Tomnatek population was characterized by the high genetic polymorphism (RGAP-, CDDP-, RAPD-, ISSR-PCR) and low population parameters (Table 3, Fig. 2). This population is located not far (about 2–3 km) from Lemska population and is separated from it only by a low spur of the mountain. Obviously it does not interfere with the free exchange of genetic information between them. Lemska population has had the lowest area, the lowest density and minor coefficient of reproductive ability. Therefore, despite the minor number of individuals, Hutyn Tomnatek population is characterized by high genetic polymorphism. The vitality class of this population is balanced. It has been experimentally proven that the viability of small populations is lower compared to large ones, in particular in terms of reproduction, viability of individuals and plasticity of reactions to changes in environmental conditions (Kahmen and Poschlod, 2000; Kery et al., 2000; Lienert et al., 2002).

Analysis of Molecular Variance (AMOVA) revealed 68% (RGAP-PCR) and 71% (CDDP-PCR) of total genetic variability of the differences between populations, while intrapopulation polymorphism accounted for 32% (RGAP-PCR) and 29% (CDDP-PCR). This is apparently due to the significant isolation of *G. lutea* populations, which is caused by significant geographical distances and physical barriers (mountain ranges).

Conclusions

Our results enable us to make the conclusion that, three studied populations of *G. lutea* from the Ukrainian Carpathians were unstable (Hutyn Tomnatek, Troyaska-Tataruka, Krachuneska), two of them were relatively stable (Lemska, Pozhyzhevska) and only one was stable (Sheshul-Pavlyk). Such distribution of populations is due to the anthropogenic impact (harvesting of raw materials, grazing, recreational pressing) and biotic influence (low plasticity of the species, overgrowing of shrub populations). Thus, the necessity of their renewal has been established. Therefore, we propose the following measures to be done to preserve and restore natural reserves of *G. lutea*:

- The regulation of impacts of human activities (limit cattle grazing) on the Svidovets ridge of the Ukrainian Carpathians is necessary in order to preserve the natural habitats of unstable Krachuneska and Troyaska-Tataruka populations.
- The monitoring of *G. lutea* populations in order to study the dynamics of populations and predict their development.
- The creation of artificial plantations of this species (by introduction or reintroduction) in natural habitats in order to maintain its genetic diversity and increase the number of habitats of this species.
- To create introduced intermediate populations in order to maintain genetic diversity due to the significant differentiation of *G. lutea* populations.

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