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Short Title: Criteria for Evaluating the State of Rare Plant Species Populations

RESEARCH ARTICLE

Criteria for evaluating the state of rare plant species populations

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Abstract

Assessments of the state of rare endangered plant species are based on the results of the analysis of their individuals and populations. The article is devoted to the assessment of the state of populations of rare plant species based on a complex population analysis. The most informative is the complex population analysis, which involves the combination of several methods of population assessment and is based on the accounting of not one, but a set of significant biological and ecological features characterizing the population. But the methodology and techniques of such analysis are poorly developed so far. Research in this direction is relevant. We are tasked with identifying the main components of a complex population analysis and showing their informativeness for the comparative assessment of phytopopulations. Using the example of five populations of L. martagon and P. patens, it is shown that populations of one plant species are similar in structure of the individuals forming them, but can differ significantly in terms of population characteristics, and vice versa. Morphological variability of vegetative and generative organs is pronounced in this species. It was established that the scale of this variability varies in different ecological and phytocenotic conditions.

Keywords: Complex population analysis, Rare plant species, Environment protection, Population structure, *Lilium martagon, Pulsatilla patens*.

Introduction

Assessments of the state of rare plant species populations that are threatened with extinction are based on the results of analyzing their individuals and populations. The materials of such analysis allow conclusions to be drawn about the stability of a specific local population and to predict its dynamics. Additionally, in cases where a rare species is represented by several independent local populations, their comparison allows those that are most threatened with degradation to be identified.

Currently, the most widely practiced approach is the assessment of either the ontogenetic structure (Rabotnov, 1950) or the vitality (Zlobin, 1989) of populations. The former is based on data on the ratio of individuals of different ontogenetic stages in the population, while the latter is based on the ratio of individuals with different viability. However, these approaches, taken separately, do not always provide accurate results.

A more informative approach is a complex population analysis that involves the combination of several population assessment methods and is based on the consideration of not one, but a set of biologically and ecologically significant characteristics that characterize populations. However, the methodology and methodology of such analysis are poorly developed. Research in this direction is relevant (Andriyenko, 2008; Peregrim et al., 2010). Our task is to identify the main components of a complex population analysis and to demonstrate their informativeness for the comparative evaluation of phytopopulations.

When developing criteria for assessing the condition of populations of rare plant species, it is necessary to take into account the fundamental features of the organization of plant cover. One of the central and practically important such features is the hierarchy of vegetation (Zlobin 2015; Jarić et al., 2015), which Rosenberg (2018) rightly considers a "universal principle of organization" of all biosystems in general.

In this hierarchy, the basic units are individual plants. More complex biosystems are formed sequentially based on them, forming a hierarchical sequence: individuals-populations-phytocenoses-biosphere. The higher the position of the biosystem in the hierarchy, the more complex it is organized and the greater the set of features that characterize the specificity of this organization.

The condition of individual plants, as the primary link in the hierarchy of biosystems, may vary. It is determined by the features of morphogenesis, the level of vitality, and the characteristics of the stages of ontogenesis. In addition, as Zhukova (2001) wrote: "each ontogenetic group is heterogeneous and can include diverse subgroups: vitality, biomorphological, rhythmic, developmental rates, modes of reproduction, and in some cases-by physiological and biochemical characteristics, and even less frequently in plants - by sexual characteristics".

At the second level of the hierarchy, the structural features and properties of populations that determine their ecosystem status have two sources. Firstly, the properties and corresponding characteristics of the population are determined by the characteristics of the individual plants that make up the population. Secondly, these are the properties of the population as a biological entity, which give rise to important characteristics such as the number of individuals in the population, population density, and the type of distribution of individuals in the population field, ontogenetic composition, vitality structure, level of generativity, and others. Therefore, the properties of the population cannot be reduced to the properties and characteristics of its individual components.

The current state and development trend of the population as a second-level biosystem can be most adequately assessed only by taking into account

- The characteristics of its individual components and
- The properties of the population as a biosystem. Unfortunately, this aspect of the problem is often overlooked by most researchers.

Another feature of individual plants and populations as biosystems is their multi-attribute nature. This is an integral feature of these biosystems. However, in studies devoted to the study of rare plant species, methods based on assessing the population by a single characteristic are often used. Although methods have been developed and exist for assessing individuals and populations based on a complex of their characteristics. Below is a scheme of possible options for assessing the state of individual plants and plant populations based on the concept of hierarchical organization of biosystems and their multi-attribute nature.

Evaluation of plant individuals:

- univariate evaluation
- Multivariate evaluation

Evaluation of plant populations

Evaluation based on traits of individuals in the population

- univariate
- multivariate

Evaluation based on traits of the population

- univariate
- Multivariate

An example of univariate methods is the analysis of gender structure in populations, which for dioecious plant species is based only on one trait - the structure of the androecium and gynoecium. In this case, such an approach is valid and informative. Another example is the method of evaluating individuals based on one trait - size, and then using this trait to evaluate the size structure of the population. It has been shown that this method is not very effective, since the term "size" does not have a clear understanding among specialists and does not fully reflect the life or ontogenetic state of the individual (Zlobin, 2009; Rahman, 2018). An example of multivariate methods is the vitality analysis, in which the life state of individuals is evaluated by a complex of quantitative morphological traits.

Thus, the most accurate assessment of the state of populations of rare plant species can be provided by a combination of three multi-criteria methods:

- Vitality analysis based on a multi-criteria evaluation of the condition of plant individuals forming the population.
- Multi-criteria assessment of population structure as an independent biosystem.
- Assessment of ontogenetic state of the population using the Uranov-Zhivotovsky method, which takes into account two features: relative energy efficiency (Uranov's delta) and efficiency index (Zhivotovsky's omega) of individuals that make up the population.

We set the task of analyzing the state of ten populations of two rare protected plant species using a complex of multi-criteria methods for assessing individuals and populations.

Materials and Methods

Two rare protected species of plants were taken as objects for testing the method of assessing individuals and populations of plants as multi-feature biological systems: *Lilium martagon* L. (Liliaceae) and *Pulsatilla patens* (L.) Mill. s.l. (Ranunculaceae), which grow in the territory of Desnyansko-Starogutsky National Nature Park in Ukraine.

Five populations of *Lilium martagon* were analyzed:

- Ecotone between Betuletum corylosa and Pinetum corylosa-convallariosum,
- From Pinetum corylosa-convallariosum communities,
- From Pinetum corylosa-sparsiherbosum,
- From Pinetum coryloso-maianthemosum, and
- From Fraxinetum corylosa-convallariosum.

Five populations of *Pulsatilla patens* were also evaluated:

- From the Querceto-Pinetum corylosa-luzulosum community,
- From the Querceto-Pinetum frangulosa-festucosum,
- From Pinetum callunosa-hylocomiosum in the Starogutsky forest area,
- From Pinetum callunoso-hylocomiosum in the Desnyansky part of the national park, and
- Also from Pinetum callunosa-hylocomiosum in the Desnyansky part of the park, but the conditions of growth for *P. patens* in this population are significantly different from all the others and from population 4 because it is located in a recently cut area that is just starting to grow back into a forest.

A morphometric analysis of more than 700 plant specimens was conducted within these populations. The traits of individuals and populations were recorded over three consecutive growing seasons, which allowed for the characterization of their annual variability.

The following morphometric parameters were used to assess the condition of the individuals: plant height, number of leaves, number of leaflets, length and width of leaves, average area of individual leaves, total leaf surface, number of generative structures, weight of flowers and fruits, as well as ontogenetic stage (from "r" to "ss").

To assess populations as biological entities, the following parameters were taken into account: number of individuals in the population, population density, renewability index, generativity index, age index (Kovalenko, 2015), population vitality index Q (Zlobin, 1989), delta (Uranov, 1975), and omega (Zivtovsky, 2001).

Classification of plant individuals and populations as multivariate biosystems was performed using the method of discriminant analysis (Zlobin et al., 2022), which allows the degree of similarity between objects in a multidimensional space to be evaluated. Distances between groups of individuals or between populations were determined by the values of Mahalanobis distance squared. The Wilks lambda served as a metric of classification reliability, with values ranging from 0 to 1, where smaller values indicate more accurate classification and more reliable groups of similar objects - individuals or populations. The found value of Wilks lambda was evaluated by the Fisher criterion with a significance level of p. In combination with canonical analysis, the results of classification are presented in the form of two-dimensional graphs.

Results and Discussion

Lilium martagon. The results of assessing the status of five populations of this plant based on the condition of the individuals forming them are presented in Fig 1. It can be seen that all five populations, evaluated by the characteristics of plant individuals, do not overlap, differing in the morphological structure of individuals.

The main difference is in the number of leaves, leaf surface area, and the width and length of leaf blades. Obviously, the source of variation in the structure of plants is mainly the phytocenotic environment, since all five populations were located in different forest syntaxa. The greatest difference is observed between populations:

- From Pinetum corylosa-convallariosum and
- From Pinetum corylosa-sparsiherbosum, while populations
- From the ecotone between Betuletum corylosa and Pinetum corylosa-convallariosum, and
- From Pinetum coryloso-maianthemosum are most similar to each other in terms of the characteristics of plant individuals.

The dissimilarity of different populations of L. martagon in terms of morphological structure of plants is statistically significant. The classification accuracy is high: Wilks' lambda is only 0.019, with a statistical significance of less than 0.0082 (Tab 1).

The data for three consecutive vegetation periods were included in the analysis. These data (Fig. 1) show that within one population, the morphostructure of L. martagon plants was not identical over the years. Undoubtedly, such fluctuations in the morphology of plants are related to weather and climatic conditions during the three years of studying the populations. According to data from a local weather station, in 2009, 225.7 mm of precipitation fell in May, June, and July, while in the following two years, it did not exceed 180 mm for this period. Intra-population seasonal fluctuations in the morphology of individuals affected all five populations to approximately the same extent.

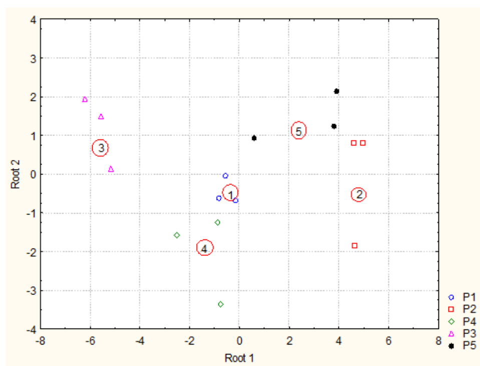

Figure 1. Discrimination of *L. martagon* populations based on a set of morphometric traits of individuals in the space of the first and second canonical roots. 1-5 population numbers

The comparison of five populations of L. martagon based on seven population parameters (such as population density, vitality structure, etc.) showed that this method significantly complements and refines the conclusion about the presence of individual specificity of each population. According to Mahalanobis distances based on population parameters, populations 1 and 4, 3 and 4, 3 and 5 differ most from each other (Fig. 2) (Tab 1). The two parameters that contributed most to the similarity or dissimilarity of populations were vitality structure and population density. At the same time, the distances between populations in the space of the first and second canonical roots were significantly larger when evaluated by population parameters than by the traits that form these populations.

Table 1. . **Results of discriminant analysis for populations of L. martagon over three consecutive growing seasons**

	Based on morphometric parameters of individuals	Based on population parameters
Wilks' lambda	0.019	0.0000193
Fisher's criterion and significance level	2.38/ p<0.0082	5.95 p<0.0041
	P1-P2=33.18/p<0.20	P1-P2=171.79/p<0.23
	P1-P3=36.63/p<0.20	P1-P3=5.25/p<0.15
	P1-P4=8.75/p<0.20	P1-P4=409.17/p<0.23
	P1-P5=20.62/p<0.20	P1-P5=372.04/p<0.23
Values of Mahalanobis distance squares between groups of individuals and populations and their statistical significance.	P2-P3=118.11/p<0.30	P2-P3=193.08/p<0.15
	P2-P4=44.61/p<0.30	P2-P4=90.61/p<0.23
	P2-P5=24.96/p<0.30	P2-P5=176.33/p<0.23
	P3-P4=38.66/p<0.15	P3-P4=408.20/p<0.23
	P3-P5=80.95/p<0.15	P3-P5=380.89/p<0.23
	P4-P5=40.22/p<0.15	P4-P5=99.53/p<0.23

Populations of *L. martagon* either increase their population size and density (populations 4 and 5) or maintain it steadily. This phenomenon is explained by the analysis of the ontogenetic structure of the populations. According to the Uranov-Zhivotovsky criterion, all five populations of *L. martagon* are considered young (Fig. 3). The value of the Uranov delta does not exceed 0.25, and the Zhivotovsky omega -0.55.

Thus, the comprehensive multi-feature analysis of *L. martagon* populations allowed for the most complete, comprehensive, and accurate assessment of their status.

The comparison of five *Pulsatilla patens* populations by the discriminant analysis method based on the traits of the plants comprising them showed (Fig. 4) that individual populations were almost identical in their complex of these traits, while others were quite distinctive. Populations 1, 2, and 3 were similar to each other. Populations 4 and 5 (both from the Pinetum callunosa-hylocomiosum association but from different parts of the National Park) differed significantly from them and from each other. The classification accuracy was quite high, with a Wilks' lambda of 0.5. All these differences were statistically significant (p<0.000) (Tab. 2).

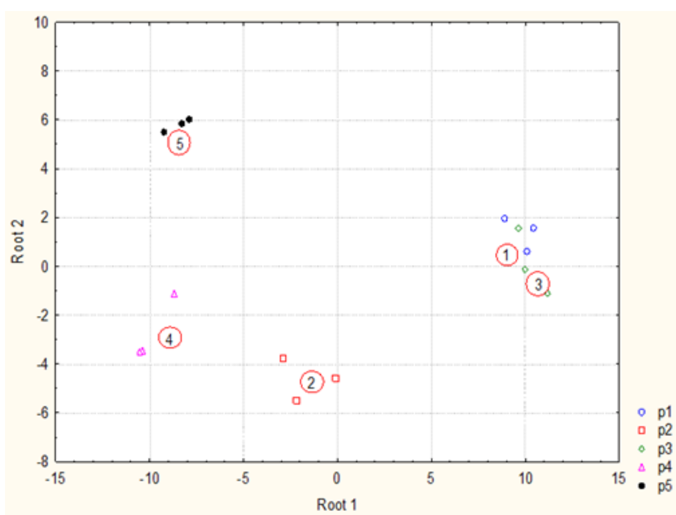

Figure 2. **Discrimination of *L. martagon* populations based on a set of population characteristics in the space of the first and second canonical roots during three consecutive vegetation periods. Populations 1-5 are numbered**

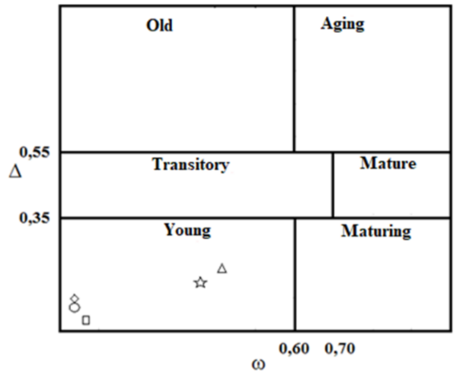

Figure 3. **Position of *Lilium martagon* populations in the Δ/ω plane. P1-circle, P2-diamond, P3-square, P4-star, P5-triangle**

The isolated position of population 5 is related to the fact that, unlike other forest populations, this population is located on a clearcut area that is only being overgrown by the forest. The age of the forest stand here is 50 years-60 years, while populations 1-4 are located in phytocenoses with a forest stand age of 80 years-100 years. It has been previously shown (Panchenko & Klimenko, 2013) that the ecological discomfort index for *P. patens* is the highest here. Population 5 stands out in terms of its ontogenetic status, as it is transitional, while the other populations belong to the categories of young, maturing, or mature (Fig. 6). It should be noted that, in combination, this indicates the high effectiveness of discriminant and canonical analysis in the study of plant populations.

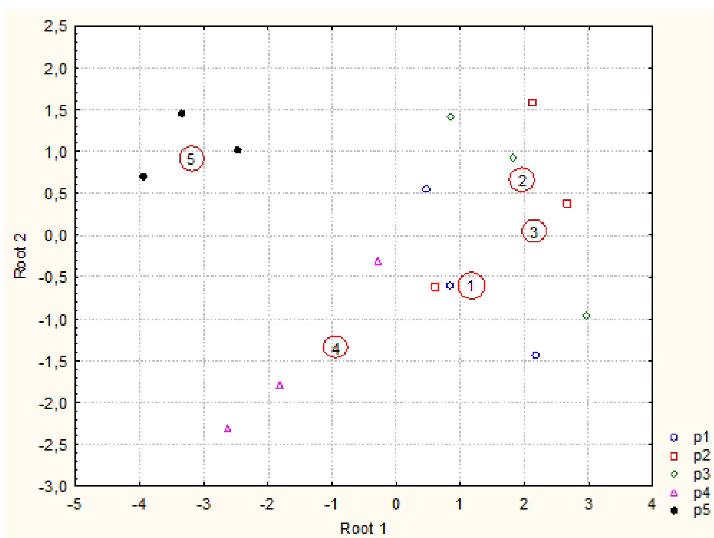

Figure 4. **Discrimination of *P. patens* populations based on a set of morphometric characteristics of individuals in the space of the first and second canonical roots. 1-5 -population numbers**

Like with *L. martagon*, a displacement of population centroids over the years was detected in the space of canonical roots for *P. patens* populations (Fig 4). The magnitude of this displacement is even greater than that observed for *L. martagon*, reflecting significant changes in morphostructural parameters of individuals depending on changes in weather conditions during the vegetation period, primarily precipitation and temperature, as noted above. Changes in the morphostructure of individuals depending on weather conditions during the vegetation period were most pronounced in population 4 of Pinetum callunoso-hylocomiosum in the foothills of the national park.

Table 2. Discriminant analysis results for *P. patens* populations over three consecutive vegetation periods

	Based on morphometric parameters of individuals	On the basis of population parameters
Wilks' lambda	0.5	0.00017
Fisher's criterion and its significance	3.62/p<0.000	5.73/p<0.0003
	P1-P2=9.15/p<0.08	P1-P2=30.06/p<0.14
	P1-P3=7.67/p<0.08	P1-P3=524.73/p<0.001
	P1-P4=16.45/p<0.00	P1-P4=303.98/p<0.004
	P1-P5=19.55/p<0.04	P1-P5=36.60/p<0.13
Values of Mahalanobis distance squares between groups of individuals or populations and their statistical significance	P2-P3=6.96/p<0.00	P2-P3=828.31/p<0.0005
	P2-P4=25.46/p<0.00	P2-P4=517.27/p<0.001
	P2-P5=36.01/p<0.01	P2-P5=141.81/p<0.02
	P3-P4=22.11/p<0.00	P3-P4=62.98/p<0.08
	P3-P5=35.20/p<0.05	P3-P5=356.40/p<0.002
	P4-P5=16.94/p<0.18	P4-P5=215.16/p<0.009

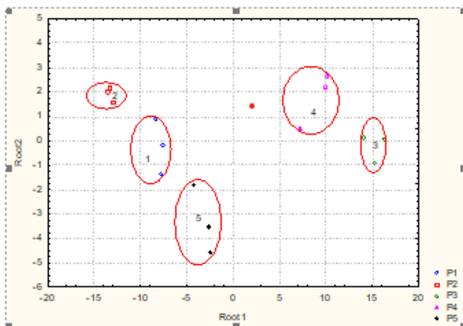

Figure 5. Discrimination of ***Pulsatilla patens*** populations based on a set of population characteristics in the space of the first and second canonical roots for three consecutive vegetation periods. 1-5 -population numbers

A comparative analysis of *P. patens* populations based on their population characteristics revealed significant differences between the five studied populations (Fig. 5). The classification accuracy in this case was very high, with Wilks' lambda equal to 0.00017. The differences between populations are statistically significant, with p<0.0003 (Table 2).

Based on the population characteristics, populations 3 and 4 were found to be the most similar to each other, while the largest differences were observed between populations 2 (from Querceto-Pinetum frangulosa-festucosum) and 3 (from Pinetum callunosa-hylocomiosum of the Starogutsky forest area). These differences reflect the different population densities and ontogenetic states of the populations. Among the five *P. patens* populations, one is young, two are maturing, one is mature, and one is transitional (Fig. 6). This fact largely explains the differences between *P. patens* populations, primarily in the state of their forming plant individuals, which in turn affects the population density and level of generativity.

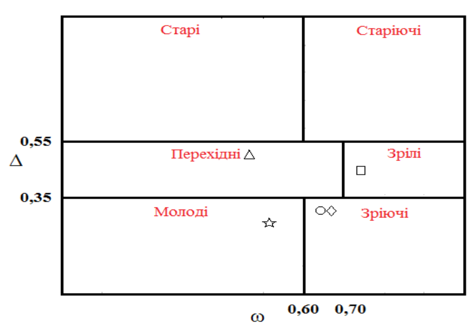

Figure 6. The position of ***Pulsatilla patens*** populations in the Δ/ω plane. P1 -circle, P2 -diamond, P3 -square, P4 -star, P5 –triangle

Using the example of five populations of *L. martagon* and five populations of *P. patens*, it was shown that populations of the same plant species with similar structures of their forming individuals can significantly differ in their own population characteristics, and vice versa. Both studied rare plant species exhibit morphological variability in vegetative and generative organs (Klymenko, 2013). It was found that the scales of this variability in different ecologically-phytocenotic conditions are not the same.

Based on individual characteristics, populations of *L. martagon* were found to be more distinctive compared to populations of *P. patens*. The average Mahalanobis distance between populations of *L. martagon* is 44.66, while between populations of *P. patens* it is only 19.55. In the conditions of the National Park, individuals of *L. martagon* were found to be more morphologically variable than individuals of *P. patens*.

Data on the state of plant populations obtained on the basis of multi-feature assessments allow for predicting the dynamics of phytocenoses of rare plant species, which we emphasized earlier (Klymenko & Zlobin, 2014).

Conclusions

Based on the principle of hierarchical organization of biosystems, a classification of methods for assessing the state of plant populations was developed, with a subdivision into methods based on accounting for a single feature of individuals or the population as a whole, or on accounting for a complex of features of both individuals and populations. The use of discriminant analysis showed that the most informative methods are two main multi-feature methods: the first is the assessment of the population based on the characteristics of the individuals that form it, and the second is the assessment of the population based on its own population parameters. The combination of these two methods multi-feature assessment of individuals and multi-feature assessment of populations -characterizes them most fully and comprehensively.

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