

Two populations of *Lunaria rediviva* L. (Cruciferae) at the eastern edge of its range

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Summary: *Lunaria rediviva* L. is a Tertiary relict species of European broad-leaved forests. This species is rare and endangered in Eastern Europe. Two of the most eastern *L. rediviva* populations are located in the Republic of Mordovia. The aim of our study was to evaluate the current status and endangerment of *L. rediviva* populations at the eastern edge of its range. We compared age structure, parameters of reproductive biology and morphology of some organs of individuals and composition of the accompanying flora of both researched populations. Our results show that *L. rediviva* individuals are very similar within each age group concerning the researched parameters. Both investigated *L. rediviva* populations are 'vegetatively-orientated'. The percentage of generative individuals in the age spectrum varies from 26.4% to 43.8%, and the fruit set usually makes up about 40% but may increase up to 55.4%. Declining share of generative individuals and reproductive ability in populations can be explained by unfavorable conditions of habitat (i.e. increase of shading under the forest canopy and sod-forming process due to invading grasses from the adjacent meadow communities). The best strategy for conservation of *L. rediviva* at the eastern edge of its range might be further monitoring of the populations and ex-situ conservation in botanical gardens by seeds or transplants.

Keywords: *Lunaria rediviva*, endangered species, population, reproductive biology, morphometrics, ex-situ conservation

Understanding of the consequences of isolation and decreasing of population size, the population persistence and ability to respond on changing conditions is increasingly important in the face of global change and habitat fragmentation (KAREIVA et al. 1993; LYNCH & LANDE 1993). While habitat loss and environmental stochasticity are of immediate conservation concern (LANDE 1988; SCHEMSKE et al. 1994), even species with protected status may face increased extinction risk (WILLI et al. 2006).

The most valuable knowledge about forest ecosystem functioning derives from observations and researches carried out in forests which remained untouched (PETERKEN 1996), where direct human impact can be excluded and where only natural disturbances occur. There are few natural or near-natural forests which exist primarily within national parks and nature reserves.

According to the abundant main hypothesis, the population abundance of a species is greatest at the centre of its geographical distribution and declines towards the edges of its range (BROWN et al. 1995; SAGARIN & GAINES 2002). Moreover, populations occurring at the edge of a range tend to be smaller and less dense than central populations (GASTON 2003; REED 2004), so they are thought to be less viable and more prone to extinction (GASTON 2003; CHANNELL 2004). When peripheral populations are exposed to harsh conditions, the environment may select individuals that are adapted to local conditions. This increases adaptive phenotypic plasticity (SEXTON et al. 2009). When this happens, peripheral populations may acquire an evolutionary potential for adaptation and speciation (LEVIN 1993). Therefore, some peripheral populations

may be prone to extinction, while in other cases they may be a source for speciation (LEVIN 1970) or cause range shifting in response to radical environmental changes (PARMESAN 2006). Long-term monitoring of population demography is essential (e.g. LESICA & MCCUNE 2004; ABELI et al. 2012). Furthermore, among all factors the mating system and available resources are the most important driving forces for adaptation and evolution or decline and extinction of peripheral plant populations (ABELI et al. 2014).

We investigated population structure, biology and ecology of the Tertiary relict species *Lunaria rediviva* L. (perennial honesty) at the eastern edge of its range. This European species of broad-leaved forest is rare and endangered in Eastern Europe (DOROFYEV 2002; MARKOV 2011). There are only few papers on the biology and ecology of *L. rediviva* and most of them are in Russian (ROMANOVA 1983; MARKOV 2011; SANAEVA 2011). The aim of our study was to evaluate the current status and endangerment of *L. rediviva* populations at the eastern edge of its range. We compared results of population-based studies in Mordovian State Nature Reserve (MR) in 2011–2012 and in National Park ‘Smolny’ (NP) in 2013–2014. We focussed on following questions: (I) Are there remarkable differences between the two investigated, spatially separated populations? (II) What is the status of these populations?

Materials & methods

Lunaria rediviva is a long-lived perennial up to 100–140 cm height with erect stems. Bottom leaves are opposite, but upper leaves are alternate. The bottom leaves are ovate with a pointed tip and dentate margins; intermediate leaves are cordate with spinulose-dentate margins on long petioles; upper leaves are ovate-acuminate on short petioles. Inflorescences are panicles and include apical and lateral racemes. Each raceme often has 3–18 (sometimes more) flowers which are regular, 15–17 mm in diameter. Petals up to 20 mm long, lilac to violet. Silicula 35–90 × 15–35 mm, elliptical, rare ovate-elliptical or elongate-elliptical. Each fruit produces 2–8 seeds (BALL 1993; ROMANOVA 1983; MARKOV 2011).

Lunaria rediviva is distributed from Italy and former Yugoslavia (in the south) to the Baltic Sea (in the north) and from Portugal (in the west) to the Volga River (in the east) (BALL 1993; DOROFYEV 2002). As alien species, *L. rediviva* is known in North America where it escaped from gardens and it is rarely found in long persisting populations outside of cultivated localities (ROLLINS 1993).

Some of the easternmost *L. rediviva* populations are located in the Republic of Mordovia where this species is present in forest communities of two protected areas: the Mordovian State Nature Reserve (MR) and the National Park ‘Smolny’ (NP). In MR *L. rediviva* is known since 1966 (TSINGER 1966). A population of this endangered species is located at its north-western part within lime-forests in the flood plain of Satis river. Investigations of this *L. rediviva* population were carried out in 1986–1990 (SANAeva 2011). *L. rediviva* was included in the Red Data Book of the Republic of Mordovia (SILAEVA 2003). The population in NP is known since 2006 (SILAEVA et al. 2006). A special study about this population was carried out in 2008. It was revealed that this population contains only juvenile and generative individuals (SHIGAEVA et al. 2009).

For the last few years we have been investigating other populations of *L. rediviva* in MR and NP (KHAPUGIN 2012; KHAPUGIN & ANDRYUSHECHKINA 2014; KHAPUGIN & CHUGUNOV 2014).

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The field investigations were carried out in 2011–2012 (Mordovian State Nature Reserve: 54.891290 N, 43.179322 E) and in 2013–2014 (National Park 'Smolny': 54.874840 N, 45.513740 E). We established one transect with 5 square plots (1 × 1 m) in each locality.

Assessment of *L. rediviva* populations was carried out based on individual parameters of plants (height and number of leaves per each individual and number of inflorescences, flowers, fruits per generative individual) and composition of accompanying flora. The density (number of individuals per m²) of *L. rediviva* per each plot was compiled.

Based on revealed morphometrical data, individuals of *L. rediviva* were divided into three age groups: juvenile (j), mature vegetative (v) and generative (g) according to ROMANOVA (1983) and MARKOV (2011). The generative group is divided into two subgroups: young generative individuals (if they form only one apical raceme) (g1) and mature generative individuals (if they form one apical and few lateral racemes) (g2). Comparison of individual parameters of *L. rediviva* populations was carried out for each age group.

We determined the population type of *L. rediviva* L. at the eastern edge of its range according to GORCHAKOVSKII & IGOSHEVA (2003).

Composition of accompanying flora was recorded within each plot. All species were sampled within three layers: tree layer (height > 3 m), shrub layer (1.3 < height ≤ 3 m) and herb layer (height ≤ 1.3 m). Accompanying flora of both studied localities was compared. We calculated a Jaccard's similarity index $JS = 100 \times C / (A + B - C)$, where *A* = number of species in locality *A*; *B* = number of species in locality *B*; *C* = number of species shared between *A* and *B* (JACCARD 1901).

Statistical analyses were carried out using R 3.1.1 (R CORE TEAM 2014) and Microsoft Excel.

Results

Accompanying flora of both investigated localities comprises 22 species of vascular plants (19 genera and 16 families) in MR and 22 species of vascular plants (20 genera and 17 families) in NP (Table 1). The Jaccard's similarity index calculated for investigated accompanying flora is 51.7%.

Lunaria rediviva occurs within the forest community. The tree layer consists of 4 species in MR and 3 species in NP, the shrub layer has 6 species in MR and 5 species in NP and the herb layer is presented by 15 species in MR and 17 species in NP. The Jaccard's similarity indexes calculated for tree, shrub and herb layers are 40.0%, 57.1% and 52.4%, respectively.

The population of *L. rediviva* from MR consisted of 127 individual plants in 2011 and 73 individuals in 2012; the population of *L. rediviva* from NP consisted of 77 individual plants in 2013 and 72 individuals in 2014. Density of plants varied from 21 (within MR in 2011) to 14 (within NP in 2014) individuals per m² with an average of 16.4 individuals per m².

Age spectrum of *L. rediviva* populations comprises three age groups with two subgroups within the generative group. Non-flowering individuals (juvenile and mature vegetative) predominate in both populations (Fig. 1). The largest percentage of generative individuals was marked in 2012 within MR (43.8%) and the smallest one in 2014 within NP (26.4%).

There are no significant differences between these two investigated populations regarding number of inflorescences, flowers and fruits per individual with one exception: In 2013 we recorded the highest numbers of flowers and inflorescences per individual (Table 2). However, the fruit set

Table 1. Species of accompanying flora divided into three layers. Species occurring in both investigated localities are indicated with an asterisk (*).

Mordovian State Nature Reserve	National Park 'Smolny'
Tree layer	
* <i>Acer platanoides</i> L.	* <i>Acer platanoides</i> L.
<i>Picea abies</i> L.	* <i>Fraxinus excelsior</i> L.
<i>Ulmus glabra</i> Huds.	* <i>Tilia cordata</i> Mill.
* <i>Tilia cordata</i> Mill.	
Shrub layer	
* <i>Acer platanoides</i> L.	* <i>Acer platanoides</i> L.
* <i>Euonymus verrucosa</i> Scop.	<i>Corylus avellana</i> L.
* <i>Fraxinus excelsior</i> L.	* <i>Euonymus verrucosa</i> Scop.
<i>Lonicera xylosteum</i> L.	* <i>Fraxinus excelsior</i> L.
* <i>Tilia cordata</i> Mill.	* <i>Tilia cordata</i> Mill.
<i>Ulmus glabra</i> Huds.	
Herb layer	
* <i>Aconitum septentrionale</i> Koelle	* <i>Aconitum septentrionale</i> Koelle
* <i>Anemone ranunculoides</i> L.	<i>Adoxa moschatellina</i> L.
* <i>Asarum europaeum</i> L.	<i>Aegopodium podagraria</i> L.
* <i>Corydalis intermedia</i> (L.) Merát	* <i>Anemone ranunculoides</i> L.
* <i>Corydalis marschalliana</i> (Pall. ex Willd.) Pers.	* <i>Asarum europaeum</i> L.
* <i>Corydalis solida</i> (L.) Clairv.	* <i>Corydalis intermedia</i> (L.) Merát
<i>Dryopteris filix-mas</i> (L.) Schott	* <i>Corydalis marschalliana</i> (Pall. ex Willd.) Pers.
* <i>Gagea lutea</i> (L.) Ker Gawl.	* <i>Corydalis solida</i> (L.) Clairv.
<i>Gagea minima</i> (L.) Ker Gawl.	<i>Ficaria verna</i> Huds.
* <i>Glechoma hederacea</i> L.	* <i>Gagea lutea</i> (L.) Ker Gawl.
* <i>Lamium maculatum</i> (L.) L.	* <i>Glechoma hederacea</i> L.
* <i>Mercurialis perennis</i> L.	* <i>Lamium maculatum</i> (L.) L.
<i>Rubus nessensis</i> W. Hall.	* <i>Mercurialis perennis</i> L.
<i>Stachys sylvatica</i> L.	<i>Pulmonaria obscura</i> Dumort.
* <i>Urtica dioica</i> L.	<i>Rubus idaeus</i> L.
	* <i>Urtica dioica</i> L.
	<i>Viola mirabilis</i> L.

was not significant in 2013 compared to the other years of investigation. This parameter was 37.7% and 55.4% in 2011 and 2012, respectively, for the population from MR, and 41.7% and 38.7% in 2013 and 2014, respectively, for the population from NP.

Height of individuals within each age group in the two *L. rediviva* populations varied slightly independent of the year of investigation or the origin of population (Fig. 2). Heights of young and mature generative individuals are similar. Mean heights are shown in Table 3.

Within each age group, the number of leaves per individual was stable independent of the population origin or the year of investigation (Fig. 3). Furthermore, this parameter is similar for adult plants (mature vegetative, young generative and mature generative) and it varied from 6 leaves per individual (mature vegetative and young generative plants) to 19 leaves (mature generative plants) (Fig. 4).

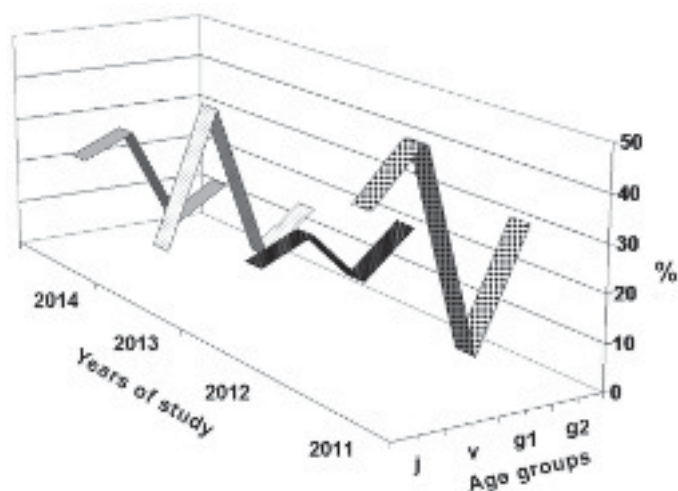
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Figure 1. Age groups of investigated *L. rediviva* populations: juvenile (j), mature vegetative (v), young generative (g1), mature generative (g2).

Discussion

The accompanying flora of the investigated localities is significantly similar. Both territories are located within the floodplain forests at the southern edge of the spruce range (YARUTKIN 1980). In addition, dominants (*L. rediviva* is a dominant (KHAPUGIN 2012; SHIGAEVA et al. 2009)) are very similar or even identical in both plant communities. For example, common species of both herb layers are *Anemone ranunculoides* and *Corydalis* spp. in spring, and *Mercurialis perennis* and *Asarum europaeum* in summer. Nevertheless, in the two herb layers, there are several differences. For example, the boreal species *Dryopteris filix-mas* and *Rubus nessensis* were noted only in MR, but the nemoral (broad-leaved) species *Aegopodium podagraria*, *Pulmonaria obscura* and *Viola mirabilis* were noted only in NP. This confirms data on greater similarity of Mordovian Reserve's

Table 2. Characteristics of generative reproduction.

		MR		NP	
		2011	2012	2013	2014
Number of inflorescences per individual	<i>M</i>	3.2	1.4	6.1	3.1
	<i>m</i>	0.2	0.1	0.6	0.4
	<i>min–max</i>	2–6	1–3	1–10	1–8
Number of flowers per individual	<i>M</i>	15.9	16.8	23.6	12.1
	<i>m</i>	0.9	1.6	3.0	1.4
	<i>min–max</i>	10–31	5–40	3–46	2–25
Number of flowers per inflorescence	<i>M</i>	5.1	10.8	3.9	5.0
	<i>m</i>	0.2	0.8	0.2	0.9
	<i>min–max</i>	2–7	4–21	3–5	2–15
Number of fruits per individual	<i>M</i>	6.3	8.8	9.2	4.8
	<i>m</i>	0.8	1.0	1.5	1.0
	<i>min–max</i>	1–21	2–21	3–25	1–12
FS [%]		37.7	55.4	41.7	38.7

M – mean value, *m* – error of the mean, *min* – minimal value, *max* – maximal value, FS – fruit set [(number of flowers / number of fruits) × 100%].

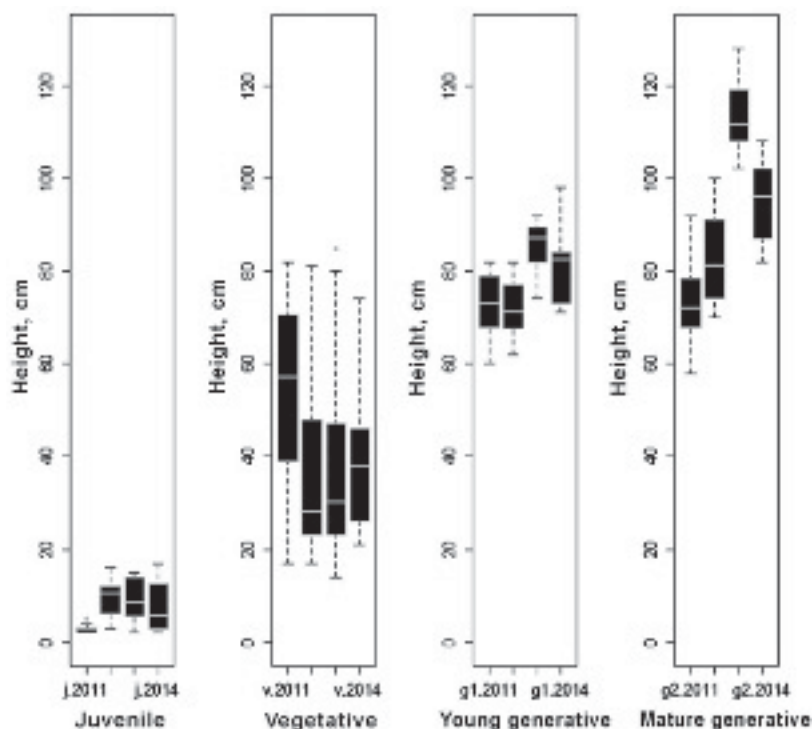


Figure 2. Box-plots for the height of *L. rediviva* individuals for each age group.

flora with the taiga flora than with the nemoral (broad-leaved) and forest-steppe flora of National Park ‘Smolny’ (SILAEVA et al. 2011; NIKITINA & VARGOT 2015).

Age structure of both *L. rediviva* populations is similar. It’s characterized by a high number of non-flowering plants (juvenile and mature vegetative individuals). Thus, these two populations could be called ‘vegetatively-orientated’ (in the sense of GORCHAKOVSKII & IGOSHEVA 2003) implying a stable population size. A high number of vegetative individuals can be interpreted positively as a high number of young plants which might later become generative individuals. But, it can also indicate a reduced percentage of generative plants due to unfavorable habitat conditions, what is typical for long-lived perennials (GRIME et al. 1986). SANAeva (2011) suggested that the most powerful unfavorable factors in MR are shading by maple-elm undergrowth and the sod-forming process due to invading grasses from the adjacent meadow communities. Nevertheless,

Table 3. Height of individuals for each age group (in cm).

Age group	Juvenile		Mature vegetative		Generative			
	MR	NP	MR	NP	Young generative		Mature generative	
Locality	MR	NP	MR	NP	MR	NP	MR	NP
Mean value per locality	4.9±0.5	7.9±0.9	48.9±2.3	37.7±2.0	72.1±1.6	83.6±2.2	77.1±1.3	105.4±2.3
Range of values	2–16	2–17	17–82	14–85	60–82	71–98	58–100	82–128
Mean generalized value	6.1±0.5		43.4±1.6		77.1±1.7		87.2±1.9	
Range of values	2–17		14–85		60–98		58–128	

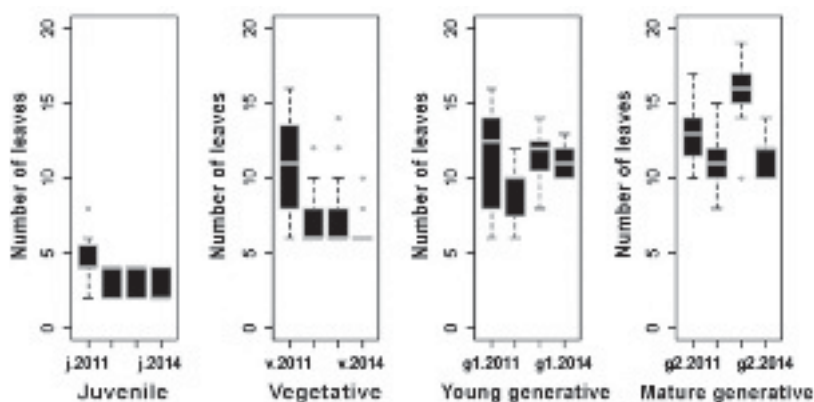
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Figure 3. Box-plots for the number of leaves per individual.

the large number of juvenile individuals indicates that a successful generative reproduction takes place in MR and NP. These data are consistent with MARKOV's observations (2011) of *L. rediviva* populations from the Central Forest Reserve. Additionally, regular vegetative reproduction of *L. rediviva* provides stability of its populations.

In 2013, the mean number of flowers per individual of NP's population was two times higher than in 2014 and markedly higher as all obtained values. Nevertheless, this difference decreases relatively the mean number of fruits per individual which only insignificantly varies among all obtained values. Our results agree with those of MARKOV (2011). He counted 1–20 fruits per flowering plant in the Central Forest Reserve. In contrast ROMANOVA (1983) counted 10–98 fruits per flowering plant in the Moscow region. This seems somewhat overstated to us. The low correlation between number of flowers and fruits in both *L. rediviva* populations is explained

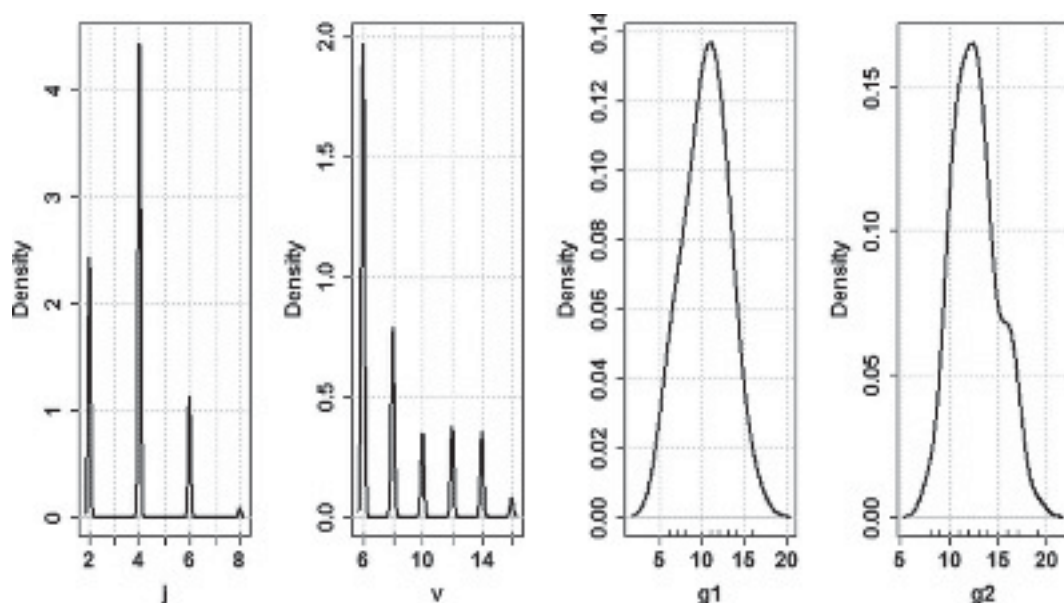


Figure 4. Density of the number of leaves per individual for each age group in *L. rediviva* populations: j = juvenile, v = mature vegetative, g1 = young generative, g2 = mature generative.

Height of individuals and number of leaves per individual varied insignificantly within each age group independent of the population's origin. In our opinion, mean height of *L. rediviva* individuals may be calculated as generalized value of height of all individuals from both populations, MR and NP. The number of leaves per individual is similar for adult plants which is in contrast to juvenile individuals. In general, values of several morphometric parameters of *L. rediviva* individuals are similar and often overlap. For example, we observed several individuals with more than four opposite leaves, but at the same time they still had traits of young plants. So they were classified as juvenile plants. Similar deviations from mean are not uncommon for many biological objects (e.g. JUNG et al. 2010; MESSIER et al. 2010; BOLSHAKOV et al. 2013). Expectedly, young and mature generative individuals have the greatest similarity in values of morphometrical parameters. These age groups were predominantly separated on the base of the number of inflorescences (a young individual has one inflorescence and a mature individual has more than one inflorescence) and of the height of individuals. Sometimes these age groups are difficult to be distinguished in nature. Nevertheless, a conjoint analysis of morphometrical parameters of plants allows to divide individuals into age groups and subgroups (Fig. 5):

Conclusion

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graph TD
    Root[ ] ---|flowers < 0.5| Left[ ]
    Root ---|flowers > 0.5| Right[ ]
    Left ---|leaves < 5.5| J1[j]
    Left ---|leaves > 5.5| Node1[ ]
    Node1 ---|height < 9| J2[j]
    Node1 ---|height > 9| Node2[ ]
    Node2 ---|height < 20.5| V1[v]
    Node2 ---|height > 20.5| V2[v]
    Right ---|leaves < 10.5| yg[ yg ]
    Right ---|leaves > 10.5| Node3[ ]
    Node3 ---|height < 22.5| mg[ mg ]
    Node3 ---|height > 22.5| mg2[ mg ]
  
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The decision tree structure is as follows:

- Root node splits on **flowers < 0.5**.
 - Left branch (flowers < 0.5) splits on **leaves < 5.5**.
 - Left child (leaves < 5.5) is leaf node **j**.
 - Right child (leaves > 5.5) splits on **height < 9**.
 - Left child (height < 9) is leaf node **j**.
 - Right child (height > 9) splits on **height < 20.5**.
 - Left child (height < 20.5) is leaf node **v**.
 - Right child (height > 20.5) is leaf node **v**.
 - Right branch (flowers > 0.5) splits on **leaves < 10.5**.
 - Left child (leaves < 10.5) is leaf node **yg**.
 - Right child (leaves > 10.5) splits on **height < 22.5**.
 - Left child (height < 22.5) is leaf node **mg**.
 - Right child (height > 22.5) is leaf node **mg**.

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The age structure analyses of both *L. rediviva* populations show that they are ‘vegetative-orientated’ with 26.4%–43.8% generative individuals. Usually the fruit set is about 40% (both populations) and does not exceed 55.4% (MR).

In our opinion, the low percentage of generative plants, connected with a high percentage of mature vegetative individuals can be explained by unfavorable habitat conditions. The most significant factor is heavy shading under the forest canopy. Another important factor for the *L. rediviva* population in MR is the sod-forming process due to invading grasses from adjacent meadow communities. Special protection regulations in MR and NP prevent human interventions of the processes of natural successions. The best strategies for conservation of *L. rediviva* might be continuing the monitoring of these populations and conserving seeds or transplants in botanical gardens, what has already been realized in other parts of the world (e.g. KAYE et al. 2005; CHAU et al. 2013; CIEŚLAK et al. 2014).

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