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REGULAR PAPER

Habitat requirements and germination performance of some relict populations of *Ligularia sibirica* (Asteraceae) from Romania

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Background and aims – *Ligularia sibirica* is a glacial relict plant species in Europe. Its populations are rare and endangered in most of the European localities. Studies on glacial relics are insufficient; among them only a few focus on the population characteristics and their reproductive capacity. We aimed to determine the habitat requirements of *L. sibirica* populations; which factors affect the reproductive output of the populations and how the interaction of ecological and biological parameters influences their germination capacity.

Methods – We described habitat conditions in terms of the Ellenberg indicators (for nitrogen availability, moisture, light, soil reaction, and temperature) in each analysed population. To determine which factors affect the population viability we performed a series of regression analyses. Germination experiment was carried under laboratory-controlled conditions at a 14/10 h. photoperiod and 24/16°C temperature, for 32 days, with cold stored seeds (at 4°C), and seeds stored at room temperature on a different substrate (moist filter paper and oligotrophic soil). The parameters influencing population germination rate were determined with general linear models.

Key results – We found that the soil humidity, nitrogen availability, temperature, and lighting are the ecological factors influencing the morphological features of *L. sibirica* populations. The largest seeds are in the middle part of the inflorescence though this parameter has no influence on germination rate. The seeds germinated better on moist filter paper. Cold stored seeds did not show higher germination rate. Germination increased with the altitude of the populations and the seeds mass, whilst higher values of density had a negative influence on it.

Conclusions – Our results suggest that habitat conditions and population characteristics are highly related to the germination success of *L. sibirica*. The prosperity of a population (expressed by the number of individuals) is not a guarantee of reproductive success, the densest populations having the lowest rates of germination.

Key words – Glacial relicts, germination capacity, habitat characteristics, *Ligularia sibirica* populations.

INTRODUCTION

As a result of the climatic and geographic changes from the past 65 million years (Tertiary and Quaternary periods), many species have disappeared from large areas of their distribution range, whilst others have dispersed to new areas or survived in refugees (Hewitt 2000, Milne & Abbot 2002). Populations of those species persist in isolated enclaves and are considered climatic relicts. Of these, the glacial relicts had a wider spread in the cold period of the Quaternary and beginning with the LGM (Last Glacial Maximum) they suffered significant restraints (Hampe & Jump 2011). The habitat fragmentation processes during glaciations affected the characteristics of the glacial relict populations. Long-term isolation and low genetic diversity could have led to the reduction or even loss of the reproduction capacity of glacial relict populations (Vogler & Reisch 2013). Several studies though evidenced the natural isolation of the populations as not having a negative impact on their structure, reproductive capacity or genetic variation within the population (Putz et al. 2015). Research further evidenced the impact of habitat fragmentation on fecundity, but no certain conclusion was issued on their effect on population viability. Other factors, such as local habitat conditions, anthropic disturbances, intra- and interspecific competition, may be as important factors affecting viability of the populations as the biogeographic impact of fragmentation (Hobbs & Yates 2003).

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Populations can respond in three ways to climate change: migration, adaptation, or extinction. The migrating possibilities of the relict populations are limited. Many of them have undergone a strong lasting selection, compared to the conspecific populations from the main area of distribution. Research on the reproductive capacity of relict populations is important as they may provide the insight of the probability of survival in the current habitats or populate the favourable ones, due to their high capacity of stress resistance (Hampe & Jump 2011). Yet, studies on glacial relict species are insufficient, most of the existent ones focusing on their genetic variations, while only a few studies focus on the population characteristics and their reproductive capacity (Šmídová et al. 2011, Heinken-Šmídová 2012, Heinken-Šmídová & Münzbergová 2012, Vogler & Reisch 2013, Putz et al. 2015, Zahariev 2016).

Changes in habitat quality are considered the most important factors affecting the viability of the plant populations and their persistence over time (Brys et al. 2004, Schleuning & Matthies 2009). Thus, the quality of the habitat influences the germination rate, the plant survival, and the population growth rate. Most likely, the decline in population (both in the number of individuals and in the population area) leads to a decrease in the reproductive success of the plant species. Perennial clonal species do not depend only on a high rate of germination to maintain their populations. For these species, the survival and development of seedlings, as well as the survival of adult individuals, are more important factors for the long-term performance of populations (Hornemann et al. 2012, Heinken & Weber 2013). Identifying the ecological parameters that reflect the habitat conditions of populations (Boeye et al. 1997), and understanding the germination strategies of the endangered species are crucial to their proper management. One method used in the studies on the ecological preferences of plant (and even animal) species is describing the habitat condition with Ellenberg Indicator Values (EIVs) (Ellenberg et al. 1992, Diekmann 2003, Pielech et al. 2017). The latter are considered even more effective than the in situ surveys of the soil parameters (Heinken-Šmídová 2012). These indicators show the ecological preferences of phytocoenoses over time, both through the species combination and through the species quantitative contribution within the plant communities (Sürmen et al. 2014), unlike direct surveys which only show the habitat conditions at a certain time, while results may vary in time and space. To analyse the proficiency of the EIVs, several studies compared the data registered in the field and those of the Ellenberg Indices; the results provided close values (Kasprowicz 2010, Heinken-Šmídová 2012) in terms of the soil humidity, nitrogen availability and soil reaction (Schaffers & Sýkora 2000). Plants respond to environmental changes both at the population and individual level. Many studies tried to quantify the plant response using measurable characters, susceptible to change with habitat degradation. The terminology used and the extent of the analysed characters are diverse. Some authors relate these to plant traits (referring to seed mass, growth traits, and morphological traits) (Dyer et al. 2001), morphological and reproductive features (Yaqoob & Nawchoo 2017) or as population variability characters (Kostrakiewicz-Gierałt 2015, Kostrakiewicz-Gierałt et al. 2015, Kostrakiewicz-Gierałt & Stachurska-Sako 2017). Generally, these aspects refer to plant height, the number of flowering stems per individual, flowering stem height, the number of inflorescences per plant, length of inflorescence, the number of flowers per inflorescence, etc.

As with other glacial relicts, *Ligularia sibirica* (L.) Cass. has occupied larger areas in the past, its populations being fragmented about 10 000–7000 years ago (Šmídová et al. 2011). Both the communities of *L. sibirica* in Romania and those from the other European countries are not continuous but in the form of more or less isolated groups, with a distribution determined by habitat conditions. Open habitats and half-shaded areas, near trees and shrubs, seem to fit the habitat requirements of the species, whilst in shady habitats, the plant appears to suffer (Kukk 2003). There is no precise information on the soil acidity as a determining factor in the *L. sibirica* distribution in Central Europe, the species growing on both alkaline (Šegulja & Krga 1990) and acidic soils (Procházka & Pivničková 1999). The data on the soil types on which *L. sibirica* grows are poor. In Europe, it grows on temporarily or permanently flooded soils such as peat clay, hydromorphic soils, nutrient-rich soils, neutral to alkaline peat soils, and on soils rich in humus (Matei 2014). *Ligularia sibirica* was identified in Europe in a variety of wetland habitats such as mineralised marshes with *Scheuchzerio-Caricetea fuscae* (Poland, Estonia, Czech Republic, Slovakia, Romania, and France) (Bensettiti et al. 2002, Kukk 2003, Chifu et al. 2006, Heinken-Šmídová 2012, Nobis 2012), and mesophilic meadows and high grass communities of *Molinietalia* order (Poland, Estonia, Ukraine, France, Romania, Czech Republic, and Slovakia) (Bensettiti et al. 2002, Kukk 2003, Kobiv 2005, Chifu et al. 2006, Heinken-Šmídová 2012, Nobis 2012). Coenoses of *L. sibirica* were also found in the marginal areas of the alluvial communities of the *Salicion cinereae*, *Alnion glutinosae*, and *Alnion incanae* Alliances (Romania) (Chifu et al. 2006) or in phytocoenosis of the *Mulgedio-Aconitetea* Class (Neblea 2009). Even though the species distribution at the European level is well known, the species habitat preferences, population characteristics, and reproductive capacity data are insufficient (Sârbu et al. 2007). This is the reason *L. sibirica* is in Annex I of Bern Convention and Annex II and IV of Habitats Directive (http://eunis.eea.europa.eu/species/159920, accessed 24 Nov. 2017), listed as data deficient, with a decreasing population trend in the European Red List of vascular plants and also in the IUCN Red List of Threatened Species (Bernhardt et al. 2011, Bilz et al. 2011). Further studies from other areas of the distribution range are necessary to complete the data on the habitat requirements of *L. sibirica* and for highlighting the proper measures to preserve its populations (Stoicovici 1982).

Among various types of germination requirements, temperature, water balance, and light, alongside the population and individual characteristics (population density and size, the number of flowering individuals, length of inflorescence, seed size and mass) are regarded as very important (Zheng et al. 2004, Luzuriaga et al. 2006, He et al. 2007, Vandewoestijne et al. 2009, Heinken-Šmídová 2012, Hornemann et al. 2012, Grzyl et al. 2014, Chrzanowska et al. 2016, Brzosko et al. 2017, Jadwiszczak et al. 2017). The variation of seed size affects their dispersal and germination, as well as the characteristics of the seedling (He et al. 2007, Rewicz et al. 2016). Seed mass may affect plant fitness through the probability of dispersal, emergence, survival, and growth of the offspring (Harper et al. 1970, Westoby et al. 1992, Giménez-Benavides et al. 2005, Pérez-Harguindeguy et al. 2013). The effect of seed position within inflorescence of flowering plants is a common phenomenon considered as affecting the seed germination and seedling establishment through the different number, mass or dispersal strategies of the seeds from the different parts of the inflorescence (Menges 1990, Vandewoestijne et al. 2009, Xie et al. 2010, Heinken-Šmídová 2012). However, its exact effect on the plants reproductive capacity is not well known, sometimes varying with species from the same genus (Xie et al. 2010, Heinken-Šmídová 2012).

The existent studies cannot differentiate the main factors influencing the reproductive success of *L. sibirica*, though they support the existence of a complex interaction among genetic and ecological factors (Ilves et al. 2013). This statement is also sustained by other studies on rare plant species with isolated populations such as *Dictamnus albus*, *Dianthus gratianopolitanus* (Hensen & Oberprieler 2005, Putz et al. 2015). It is very difficult to individualise the factors affecting the reproductive success of low genetic variation, lack of pollinators or low habitat quality (Hensen & Oberprieler 2005, Putz et al. 2015). For *L. sibirica*, it is even more difficult to show which ecological parameters influence its reproductive success, because of the reduced number of studies on its genetic structure and population characteristics (in Europe, only the populations from Estonia, Czech Republic, and Slovakia were analysed) (Kukk 2003, Šmídová et al. 2011, Heinken-Šmídová 2012, Heinken-Šmídová & Münzbergová 2012, Ilves et al. 2013). Even at its main distribution area level, tests on the species' germination capacity were performed only on a few samples from Russia (Fomina 2016). Most such studies are performed in China, on other species (e.g. *L. virgaurea*, *L. przewalskii*, *L. sagitta*) (Ma et al. 2006, He et al. 2007, Wang et al. 2009, Wu et al. 2013). Therefore, the existent data on the germination capacity of *L. sibirica* are deficient.

The main focus of our study is to assess the reproductive success of *L. sibirica* populations from Romania. Considering that populations, through their reproductive output ensure their long-term persistence and since the habitat conditions affect performance of the populations (both their demographic and reproductive parameters), we first aimed to determine the habitat requirements of *L. sibirica* populations in its main distribution area in Romania and secondly the ecological factors affecting the population performance of *L. sibirica*. Furthermore, as we could not analyse the direct influence of the in situ conditions on the population germination capacity (since the germination tests were carried under laboratory-controlled conditions), we assumed that the interaction of ecological conditions and population characteristics influence the properties of the seeds, thus affecting the germination capacity. All these considered, the third aim is to evaluate the effect of the interaction of ecological and biological factors on the germination rate of *L. sibirica* populations.

MATERIAL AND METHODS

Study species

Ligularia sibirica (Asteraceae) is a perennial hemicryptophyte with ground rosettes of leaves and a short rhizome. In the flowering period (from mid-July to the end of August), it creates flowering stems with short pedunculate flower heads in lax, bracteate spikes (Chater 1976). The seeds are of an oval shape and have pappus which helps them disperse over short distances, by either wind or gravity (Šmídová et al. 2011). Due to a rhizome, the species also presents vegetative breeding, but sexual reproduction is predominant. *Ligularia sibirica* is a species of temperate-cold climates; its distribution range is considered being the continental region of Eurasia. Most likely, the species emerged in Europe through the Northeast during the Early Holocene, once the climatic conditions and vegetation in Europe became similar to those from Central Siberia (its distribution center) (Meusel & Jäger 1992, Hendrych 2003). From there, the species is considered having migrated towards other mountain areas of the boreal hemisphere, from the subarctic regions of Asia to the mountain range of the Pyrenees-Alps-Carpathians (Păun & Popescu 1971). As the climate cooled, the species descended to the South of the 52° N parallel, while in the North of the Carpathians the permafrost layer would pass the 47° N parallel (Hewitt 2004). During the glacial cycles, the cold-climate species have been able to withstand higher latitudes in areas such as the Carpathians which provide favourable conditions to survival (Provan & Bennett 2008). Thus, in the current distribution of the species, it turns out that the most concentrated populations are located along the Carpathian chain (Czech Republic, Slovakia, Ukraine, Romania) (Hendrych 2003), whereas the populations from the South, West, and Northwest Europe are disjunct, isolated (Meusel & Jäger 1992) (fig. 1B). In Romania, populations seem to be the most abundant of the European countries where the species occurs, having the EUNIS status, 'favourable' (http://eunis.eea.europa.eu/species/159920), unlike the other European countries where the species is 'critically endangered' (Bulgaria and Czech Republic), 'endangered' (Estonia, Poland) or otherwise, 'vulnerable' (Slovakia and France) (Bensettiti et al. 2002, Kukk 2003, Bancheva 2009, Heinken-Šmídová 2012, Nobis 2012). However, this status may be based only on the available distribution data from Romania, since in 2015 the populations of species were listed as not evaluated (Mihăilescu et al. 2015).

In Romania, the *L. sibirica* populations can be found in all the major subdivisions of the Eastern and Southern Carpathians (fig. 1A), being abundant in the Western depressions of Eastern Carpathians (Mânzu et al. 2013). Before the discovery of an enclave in the Rila Mountains (Bulgaria), the Southern Carpathians had been considered the Southern-most limit of the species distribution range (Stoicovici 1977).

Analysis of the characteristics of *L. sibirica* populations

Six *L. sibirica* populations from its main distribution area from Romania were selected following a North-South gradient in order to determine the habitat requirements of the studied species (fig. 1A). For evaluating the ecological parameters that feature the populations, one or two phytosociological relevés with a plot size of 100 m² were recorded in each locality such as to cover the sites heterogeneity. Species abundances were estimated using the Braun-Blanquet cover scale (Braun-Blanquet 1983). For each relevé, we calculated the weighted mean of Ellenberg Indicator Values for light (L), temperature (T), soil reaction (R), soil humidity (H) and nitrogen availability (N) (table 1). The altitude parameter was inferred from ArcGIS v.10.2. Other ecological parameters (such as slope, aspect, shading, and other climatic variables) from Worldclim (Hijmans et al. 2005) were initially considered for studying their influence on performance of the populations; however, due to their collinearity to the Ellenberg Indicators, they were eventually eliminated from the model. Considering that grazing could lead to a change in the floristic composition of phytocoenoses, in response to the increase of soil nitrogen content, we divided the sites into grazed/non-grazed. The effect of habitat conditions on population performance was analysed both in terms of the demographic and reproductive parameters. For the demographic study, each analysed population was mapped using a GPS. The resulting area was used to calculate the density of the individuals (as the number of individuals on a surface of one square meter). The recorded reproductive parameters from each locality were the number of flowering stems, the flower heads per flowering stem and the inflorescence length (table 2). A median value of the reproductive parameters was determined for each locality (table 2).

Germination experiment

The entire inflorescence of ten randomly selected individuals was collected from each population. Harvesting was made considering the position of the seeds in the inflorescence (lower, middle and upper part of the inflorescence), assuming that the seeds from different positions have a distinct germination rate. To this effect, all the flower heads were collected and stored in paper bags at room temperature, in the dark, for a few months prior to the experiment. To break dormancy (*L. sibirica* being known as a semi-dormant species; Puchalski et al. 2014) and to test the effect of storage conditions on germination capacity, the seeds from four out of ten individuals were stored at a constant temperature of 4°C for a couple of weeks. The fully matured and healthy-looking seeds out of the inflorescence regions were afterward selected from the material collected in the field. Based on the measurements of the seeds, using a Vernier calliper, we divided them into two classes, small (< 0.47 cm) and large seeds (> 0.47 cm). The seed mass is one parameter that indicates the seed viability, thus the samples comprising 100 seeds were weighed using an analytical balance. As the seed size is a parameter considered as influencing the seed dispersal and germination capac-

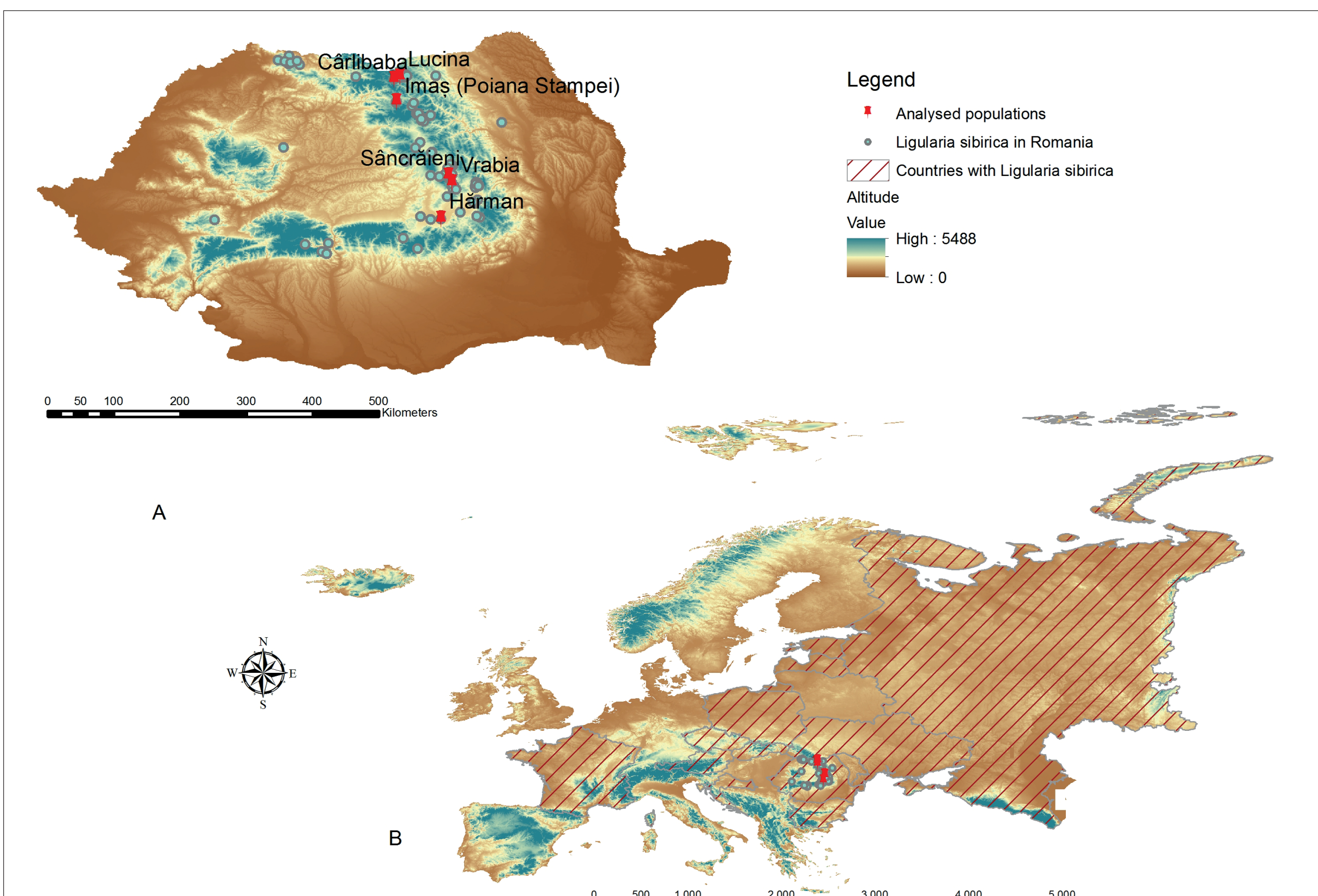

Figure 1 – A, distribution of *Ligularia sibirica* in Romania and the analysed populations; B, the European countries where *Ligularia sibirica* is present. Adapted after Bernhardt et al. (2011).

Table 1 – **Characteristics of the studied populations and localities of *Ligularia sibirica*.**
The values of the Ellenberg indicators represent the weighted mean area for each relevé.

Population	Location		Ellenberg Indicator values					Altitude (m)	Management
	Latitude	Longitude	Light (L)	Temperature (T)	Nitrogen availability (N)	Soil reaction (R)	Soil humidity (H)		
Lucina	47.65° N	25.18° E	7.11	3.9	5.6	5.34	7.86	1174	grazed
Cârlibaba	47.61° N	25.1° E	7.27	3.55	5.61	6.3	6.63	1064	grazed
Imaș	47.31° N	25.13° E	6.92	4.55	4.79	5.65	7.13	889	grazed
Sâncrăieni	46.31° N	25.83° E	7.45	4.49	4.09	6.75	7.28	649	non-grazed
Vrabia	46.22° N	25.88° E	6.89	4.12	2.6	6.25	7.36	639	non-grazed
Hărman	45.72° N	25.73° E	8.24	5.84	3.3	8.38	9.38	511	non-grazed

Table 2 – **Performance of *Ligularia sibirica* populations expressed through the demographic and reproductive parameters.**
In the table are listed the median values of the reproductive parameters of the *L. sibirica* populations.

Population	Demographic parameters			Reproductive parameters			Average length (cm) of 100 seeds	Weight of 100 seeds (g)
	Individuals density (inds/m²)	Total no. of individuals	Proportion of the flowering individuals (%)	No. of flowering stems	Inflorescence length (cm)	No. of flower heads per flowering stem		
Lucina	0.43	229	31	1	18	21	0.42	0.10
Cârlibaba	0.46	176	50	2	26	32	0.44	0.02
Imaș	0.31	291	84	2	21	28	0.48	0.11
Sâncrăieni	0.60	391	66	1	20	23	0.54	0.13
Vrabia	7.10	11000	46	1	17	19	0.44	0.08
Hărman	0.03	64	34	1	14.17	17.5	0.47	0.13

ity, as well as the characteristics of the seedlings, the Image J Software was used to determine the exact length and width of the selected seeds.

Before clustering the samples, the seeds were sterilised to avoid any contamination by using commercial bleach (Active Cl concentration less than 5%) for 5 minutes and then rinsed with sterile distilled water (4 times). Seeds were placed both in Petri sterile dishes with moist filter paper and in soil collected from one of the analysed *L. sibirica* populations (Poiana Stampei). In advance, the soil was sterilised in the autoclave to prevent any infestation. The assemblage of the samples was made considering the dimension of the seeds (class), their position in the inflorescence and the storage temperature of the seeds considering the substrate type of the experiment (Petri dishes with moist filter paper or soil). We therefore used a total number of 1200 seeds from each population (except for the Cârlibaba population of which only 460 seeds were healthy-looking) for the germination experiment, with 600 seeds for each substrate type divided according to temperature storage (300 seeds for each type of temperature storage) and to seeds dimensions from their position within the inflorescence (50 small and large seeds from each position of the inflorescence). A total number of 6460 seeds were used for this study.

The samples were placed in the growth chamber set to an alternate temperature 24/16°C corresponding to 14/10 hours photoperiod. Over a 32-day period of the experiment, the samples were regularly watered with sterile distilled water. The progress of the experiment was recorded daily, which allowed to assess the germination dynamics and the seedling mortality.

Data analyses

The influence of the habitat characteristics expressed by the Ellenberg Indicators Values on the parameters showing the performance of *L. sibirica* populations was evaluated by running a GLM (Generalised Linear Model) with stepwise backward elimination using all predictor covariate variables. We used the VIF (Variance Inflation Factors) function to check for multicollinearity. The variables with values higher than 4 were eliminated from the model. The best model selection was performed using the lowest AIC (Akaike's Information Criterion) value. To avoid potential errors, all the values of the variables were logarithmically transformed.

The linear regression of the dependent variables was performed by running a simple GLM with a Gaussian distribution. For testing the effect of the seed position within the in-

Table 3 – Analysis of the effect of the ecological parameters (Ellenberg Indicators – EI) on morphological features of the studied populations of *Ligularia sibirica*.
Only the significant values are in bold (P < 0.05).

EI	Density of inds. (inds./m²)		Total no. of inds.		Proportion of flowering inds. (%)		Number of flowering stems		Inflorescence length (cm)		Number of flower heads/ flowering stem		Seed length (mm)		Seed width (mm)		Seed weight (g)	
	t value	P	t value	P	t value	P	t value	P	t value	P	t value	P	t value	P	t value	P	t value	P
N	**-111.71**	**< 0.05**	**-266.14**	**< 0.05**	**19.48**	**< 0.05**	-1.56	0.11	**4.74**	**< 0.05**	**7.60**	**< 0.05**	**5.19**	**< 0.05**				
H	**-3.40**	**< 0.05**	1.64	0.10	**-119.57**	**< 0.05**	**-4.66**	**< 0.05**	**-6.69**	**< 0.05**	**-7.66**	**< 0.05**	**-30.33**	**< 0.05**	**-6.24**	**< 0.05**	**2.098**	**0.03**
T	**-51.02**	**< 0.05**	**-64.05**	**< 0.05**	**136.14**	**< 0.05**	1.75	0.08			**3.48**	**< 0.05**	**17.77**	**< 0.05**	**6.64**	**< 0.05**	**5.066**	**< 0.05**
L	**-18.24**	**< 0.05**	**-82.80**	**< 0.05**	**-6.77**	**< 0.05**			**2.40**	**0.01**			**8.62**	**< 0.05**	**3.14**	**< 0.05**	**-4.306**	**< 0.05**

florescence (upper, middle, and lower) on their length, width and weight, we also run a GLM with a Gaussian distribution.

The effect of the experimental (temperature storage conditions, substrate type, seeds position within the inflorescence) and morphological (length, width, and seed mass, individuals density) parameters on the germination capacity, was tested by using a GLM approach with stepwise backward elimination, with a Gaussian distribution. The best model was indicated by the lowest value of the AIC.

All the analyses were performed in R statistical software version 3.2 with stats package (R Core Team 2013).

RESULTS

After checking for multicollinearity of the variables, only four Ellenberg indicators showed a significant effect on the tested parameters of *L. sibirica* (table 3). The demographic and reproductive parameters of the populations are to a different extent affected by the habitat conditions (table 3). Soil humidity (Ellenberg H) furthest significantly affects the density of the individuals and the proportion of flowering individuals, among the demographic parameters considered, and all the analysed reproductive parameters (table 3). Light availability (Ellenberg L) and temperature (Ellenberg T) influence the morphological features of the seeds (table 3). The inflorescence length is affected by the light availability, as well as the demographic parameters, while the Ellenberg T significantly influences only the number of flower heads per flowering stem among the parameters showing the population reproductive output and all the parameters recording the viability of the *L. sibirica* populations (table 3). The results further indicate the high soil nitrogen availability (Ellenberg N) as influencing the number of flower heads and the inflorescence length (among the reproductive output of the *L. sibirica* populations) and as affecting the parameters indicating the population viability (density of the individuals and proportion of flowering individuals) (table 3).

While testing the relationship between the variables of the population performance, we determined the density of the individuals within the population as significantly affecting all the other variables (parameters), except for the number of flowering stems (electronic appendix 1). The latter, along with the seed mass are the parameters not significantly affected by either of the other indicators (electronic appendix 1). The results further evidenced the middle position of the inflorescence as significantly influencing the length and width of the seeds, thus considering that the largest sized seeds (both the longest and the widest) are located at the middle position of the inflorescence (electronic appendix 2).

The seeds collected at the same time showed different germination rates under different temperature storage conditions and on different substrate type (moist filter paper and oligotrophic soil) (table 4). Higher values of germination were recorded when the experiment was performed on Petri dishes with moist sterile filter paper (table 4). The population from Lucina recorded the highest germination values in any experimental variant, followed by the population from Imaş (table 4). Although the population from Vrabia has the high-

Table 4 – **Seed germination (%) under different temperature treatments and on different types of substrate.**
The values represent the percent of the total number of germinated seeds in each experimental form at the end of the experiment.

Temperature treatment and type of substrate	Population					
	Lucina	Cârlibaba	Imaș	Sâncrăieni	Vrabia	Hărman
Cold stored seeds on Petri dishes	39	7.8	32	13	5	15
Seeds under no temperature treatment on Petri dishes	38	19	36	17	6	23
Cold stored seeds on oligotrophic soil	29	2	15	7	1	2
Seeds under no temperature treatment on oligotrophic soil	17	12	12	13	0.3	16

Table 5 – **Analysis of the factors affecting the germination rate of studied populations of *Ligularia sibirica*.**
The variables that departed from the threshold value ($P < 0.05$) were eliminated from the model by the stepwise backward procedure. Only the significant values are in bold.

Variables	Germination (%)	
	t value	P
Temperature treatment (room temperature storage)	1.63	0.10
Substrate type (Petri)	**6.04**	**< 0.05**
Seed weight (g)	**2.17**	**0.03**
Altitude (m)	**5.96**	**< 0.05**
Individuals density within population	**-4.98**	**< 0.05**

est number of individuals, and the highest density values, it registered the lowest germination rate (table 4).

Our experiment showed the germination values as varying from 5.3% (6.6%) to 39% (38%) (cold stored seeds and seeds stored at room temperature), with a mean value of 18.9% (23.5%). Among the analysed parameters, a significant influence on the germination rate was exhibited by the substrate type (Petri dishes with moist filter paper), seed weight, the altitude, and the density of the individuals within populations (table 5). There was no evidence for a significant influence of the seeds vernalisation on the germination rate.

The cold-stored seeds first germinated on the 5th day of the experiment (in the Lucina and Sâncrăieni populations), while the seeds with no vernalisation showed germination starting with the 6th day (fig. 2A). Since the early days of the experiment, the Lucina population showed the highest germination (on the 8th day, already 48 germinated seeds, while the Vrabia population had only 2 germinated seeds) (fig. 2A). With the seeds stored at room temperature, all populations showed germination on the 8th day of the experiment (Lucina populations – 49 germinated seeds, the Vrabia population – one germinated seed) (fig. 2C). Both the cold-stored seeds and the seeds with no vernalisation, exhibited a germination maximum in the 11th–13th day of the experiment, followed by a germination flattening beginning with the 22nd–23rd day (fig. 2A & C). The seeds subjected to vernalisation showed higher seedling mortality than the seeds stored at room temperature. With vernalisation, the seedling recorded mortality on the 10th day of the experiment in the Sâncrăieni population (fig. 2B), while the seeds stored at room temperature recorded seedling mortality beginning with the 17th day (population from Vrabia) (except for the Cârlibaba population in which only one seedling was recorded on the 26th day) while the seeds stored at room temperature exhibited seedling mortality in all populations on the 22nd day (fig. 2D). A flattening of the seedling mortality registered on the 29th–30th day of the experiment in both types of temperature storage. An exception is in the Lucina population where the seedling continued to die until the last day of the experiment (seedling mortality of the Lucina population: 7.8% for the seeds stored at 4°C and 2.6% with seeds stored at room temperature). The tests run on the *L. sibirica* populations from Siberia, showed the seedling mortality as 2.3% (Fomina 2016).

DISCUSSION

The ecological characteristics (expressed by the Ellenberg indicators) vary in the analysed populations of *L. sibirica* from Romania, due to the altitude variations. Though a fluctuation was registered for the temperature values in the analysed sites, the cold-temperate climate is specific to all locations. Most of the phytocoenoses with *L. sibirica* include trees and bulrush, yet they leave enough space for good lighting (similar habitats were described from Estonia by Kukk 2003). In the Hărman site, though, the woody plant species are missing and the plant species that prefer rather intense light dominate. There were also recorded variations in the general nutrient supplies (Ellenberg N), from 2.6 (sites more or less poor in available nitrogen) to 5.6 (intermediate nitrogen availability). Giving that both the populations from Romania and those from the Czech Republic and Slovakia (Heinken-Šmídová & Münzbergová 2012) recorded intermediate values of the Ellenberg N, it appears that *L. sibirica* does not prefer high organic content in the substrate. Since there were sites that registered higher values of the Ellenberg N, we relate this effect to the anthropic-zoogenic influence (such as grazing) (in the Lucina, Cârlibaba, and Imaș populations). In most of the sites, both the analysed ones in this study and other European localities with *L. sibirica*, the habitats showed a low acid reaction of the soil. According to other analyses of European *L. sibirica* populations, the recordings of the water level confirm the mesophilic character of the species (Šegulja & Krga 1990, Kukk 2003, Slavík

2004). In the Hărman though, the Ellenberg R shows a low alkalinisation, and the specific phytocoenosis composition marks a water-saturated substrate. All these highlight the adaptive ability of the species to grow and persist in different ecological conditions.

The variation of ecological conditions of the sites with *L. sibirica* is an important factor significantly influencing the parameters that ensure the reproductive output of the populations. Thus, our results reached the sites having a higher nutrients content in the substrate as having a better reproduction success (due to the population features that provide better seed production). A similar effect was mentioned in the study from the Czech Republic and Slovakia (Heinken-Šmídová 2012). As other ecological studies on glacial relicts highlighted the restrictive role in the water and temperature regimes, our results uphold the influence of the substrate moisture on the species reproductive output. Thus, heavier seeds have a better germination capacity, this parameter (seed weight) being conditioned by soil moisture. Also, in laboratory tests, seeds that received more moisture had a higher germination rate. As germination capacity may vary depending on the annual variations in environmental conditions (especially temperature and humidity), further studies are needed to define their role in flowering and fructification periods and in germination. Other glacial relicts (such as *Betula humilis*) indicated similar effects (Chrzanowska et al. 2016).

Ligularia sibirica individuals are not only affected by the ecological parameters but also by the complex interactions within the populations. Thus, density plays an important role, as the populations with the highest values of this parameter have smaller inflorescences and seeds. And since the length of the inflorescence is related to the number of flower

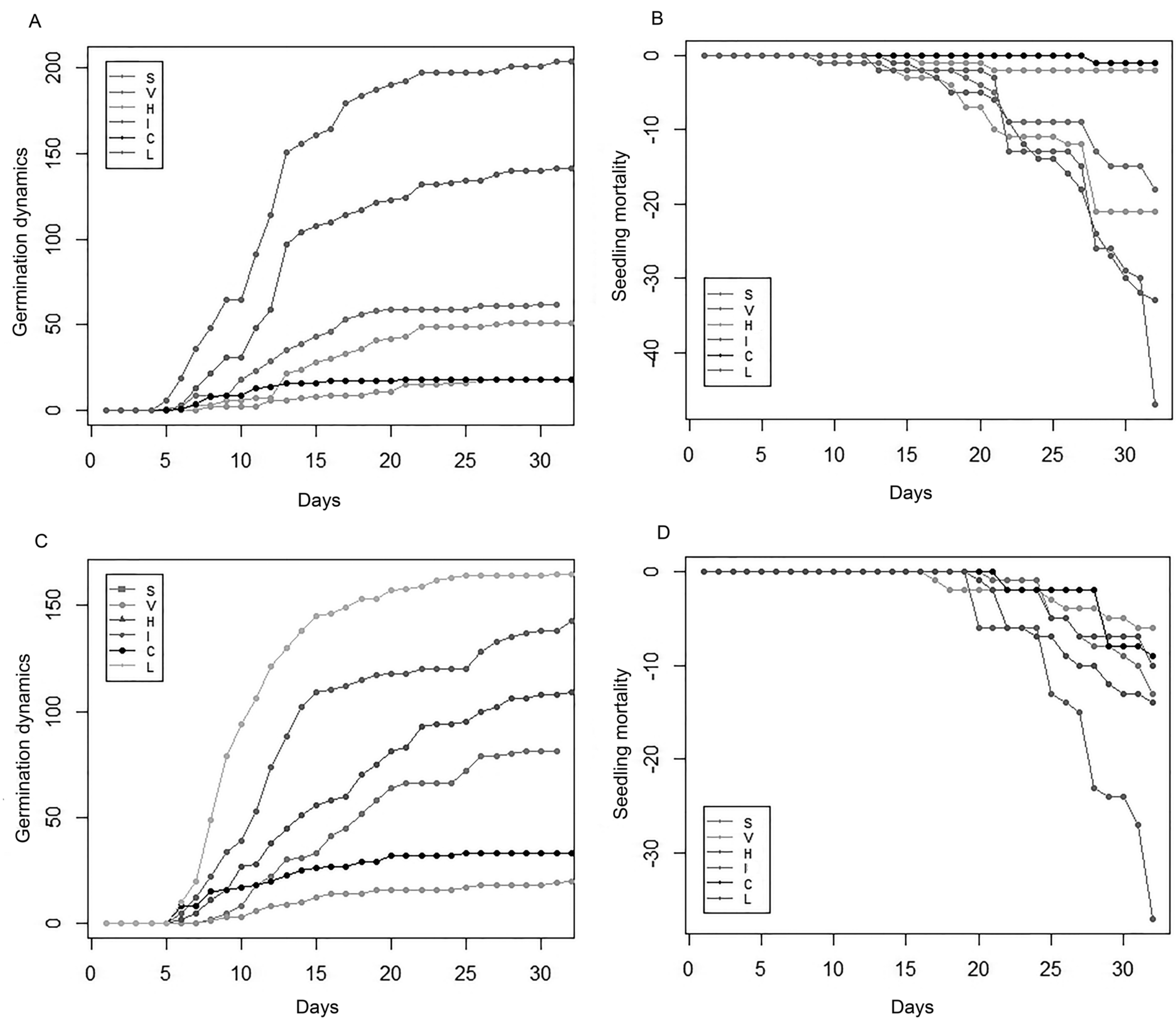

Figure 2 – Germination dynamics and seedling mortality: A & B, cold-stored seeds; C & D, seeds stored at room temperature (S – Sâncrăieni, V – Vrabia, H – Hărman, I – Imaș, C – Cârlibaba, L – Lucina).

heads (electronic appendix 1), we assume that the high-density populations provide a lower seed production.

Though in Europe the effect of the inflorescence position on the seed characteristics (dimensions, weight) has not been studied, we can relate our results to those obtained for other *Ligularia* species. Our study identified the largest seeds (length and width) in the middle inflorescence position. Furthermore, no significant interaction was registered between the seed position within the inflorescence and seed mass. Research on *L. virgaurea* populations found the heaviest seeds in the upper region of the inflorescence (Xie et al. 2010).

Measuring our results on the germination capacity of *L. sibirica* and those from Estonia (Kukk 2003 registered a germination value of 50%; Ilves et al. 2013 a proportion of 41–73% of germinated seeds and Kļaviņa et al. 2004 registered a germination rate of *L. sibirica* of 67%), we assign the germination capacity of the *L. sibirica* populations as low (18.96%). These values derived from tests run with cold-stored seeds. Without showing exact values, Kukk (2003) mentioned the *in situ* germination of *L. sibirica* populations from Estonia as low. According to Fomina (2016), the seeds from Siberia populations (reg. Trans Baikal) registered, under laboratory conditions, a germination rate of 45% (seeds with no vernalisation) up to 100% (cold-stored seeds). The latter shows high variability of the germination capacity of the *L. sibirica* populations in their Euro-Siberian distribution range. This situation is not singular but also encountered for other glacial relicts (Jacquemart 1998, for *Andromeda polifolia*), their germination rate ranging from 19% and 66.5%. Considering the biological particularities of the species (semi-dormant) and the germination values registered for other *L. sibirica* populations, we supposed the results of the germination capacity of the cold-stored seeds and the seeds stored at room temperature are contrasting. The differences were yet not the ones we expected, the germination rates being close, even exhibiting a slight advantage of the seeds stored at room temperature (regardless of the substrate). Although contradictory, such results have been registered for other species, such as *Andromeda polifolia* (Jacquemart 1998). Most of the studies that follow the reproductive behaviour of glacial relict plant species (Jacquemart 1998, Taylor et al. 2002, Chrzanowska et al. 2016, Jadwiszczak et al. 2017) were performed under laboratory-controlled conditions. In the particular case of *L. sibirica*, some studies analysed the germination capacity either in situ (Kukk 2003), or under laboratory conditions, from its main distribution area (Siberia) (Fomina 2016), to its western distribution limit (Estonia) (Kukk 2003, Kļaviņa et al. 2004, Ilves et al. 2013). Yet, due to the differences in the used methods, it is very challenging to compare the results and to draw a firm resolution on how successful the germination process is in different populations from the European range.

The higher germination recorded for the seeds mounted on Petri dishes (than those cultivated in soil) may be explained by the higher moisture provided by this substrate type. Our results confirm the heavier seeds as germinating better, similar to the results on *L. virgaurea* (He et al. 2007). As with other *Ligularia* species, the seed position within the inflorescence does not affect their germination capacity (Xie et al. 2010), yet it influences the reproductive success of the populations through the characteristics of the seeds (such as weight), which further influence their germination rate. The seeds from the populations at a higher altitude presented the highest values of germination. Without attempting to generalise this interaction, we can state, however, that, in terms of germination, the optimal altitude for the populations of *L. sibirica* studied is between 800 and 1200 m.

But how can we explain that the lowest values of the germination rate were registered in the apparent most prosperous of populations, in the Vrabia population? An explanation may be assigned to the density of the individuals whose high values negatively influence the germination, regardless the substrate and the storage condition of the seeds. Another answer to this question may lie in the low values of the organic matter content in the substrate (Ellenberg N) registered for the Vrabia site. It is probable that, due to the high density interacting with the low content of soil nutrients and the competition of other components of the phytocoenoses, resources are used more for plant nourishment and less for the development of the seeds, as a strategy for increasing the resilience of the plant. In contrast, in the populations with the best germination (Lucina and Imaș), the seeds are heavier, the N index has higher values, and the density is much lower. In addition, both populations occupy the half-shady, grazed habitats. Hence in the habitats with trees and bulrush, the survival of the seedlings is lower (Lanno & Sammul 2014); we can consider that a higher germination capacity is a strategy through which populations preserve their viability.

Research on the viability of *L. sibirica* populations from the Czech Republic and Slovakia reached the average lifespan of individuals as 61.3 years and the mortality of the adult individuals as low (Heinken-Šmídová 2012). In theory, the biological potential of these populations ensures their survival. But *L. sibirica* populations are endangered due to the anthropic impact, through the modifications in the traditional management of the marshland. Drainage (which emphasises the decrease of moisture through the climate warming), conversion of marshlands in pasture and competition with tall-herbs (such as *Filipendula ulmaria*) are the main factors affecting the populations. This situation is not representative only for Romania, but for other European countries (e.g. Estonia) (Ilves et al. 2013, Lanno & Sammul 2014). The importance of maintaining the natural habitats was highlighted in the experiments from Estonia (Lanno & Sammul 2014), being emphasised the negative effects of both overpopulations (due to competition) and vegetation removing, in which case transplants were damaged by animals. Also, the studies from the Czech Republic and Slovakia (Heinken-Šmídová 2012) provided an argument in favour of limiting grazing in *L. sibirica* habitats, giving that the populations from nitrogen-poor habitats have a lower probability of extinction than those from nitrogen-rich habitats. Mânzu et al. (2013) determined the favourable niche of *L. sibirica* as being covered in Romania only in a proportion of 33% by the Natura 2000 Network. The real efficiency of the Natura 2000 Network is in fact even lower, in the absence of conservative management practices. Habitat loss and degradation is a process faster than the ability to implement proper protection measures. According to Putz et al. (2015), upholding isolated populations contributes to preserving the entire evolutionary potential of a species. Accordingly, each *L. sibirica* population is important, regardless of its size. Therefore, it is required to assess all populations, since each of them may need different conservation approaches due to the particularities of the environmental conditions and their reproductive capacities.

CONCLUSIONS

According to our results, the habitat requirements of *L. sibirica* populations are well-lit places, submontane-temperate conditions, poor to intermediate nitrogen availability of the substrate, and constantly moist soil. Both the ecological and intra-population factors influence the population performance. Even though the latter fairly depends on the soil humidity, general nutrient supply, cold-temperate conditions, and sufficient light availability, extreme values of these parameters have a limitative effect. For example, increasing soil nutrient content, either drought or excessive soil humidity, and a warmer climate, will affect the viability and long-term persistence of *L. sibirica* populations, both through the effect on demographic and reproductive parameters and through increased competition of other plant species. The germination success of *L. sibirica* populations depends both on ecological and biological factors. The analysed populations registered different values of germination rate, the habitat characteristics being able to explain these variations. According to our results, population size is not a parameter reflecting the species reproductive success, since the largest population (Vrabia) registered the lowest germination capacity. Thus, we conclude that population size does not affect the *L. sibirica* germination; the population density, on the other hand, is one parameter affecting the species germination capacity. It has been proven that no matter how large the seeds are, they do not affect the germination capacity, but the seed mass does. The altitude and the soil moisture are the parameters the *L. sibirica* populations depend on for germination. It is expected that, under natural conditions, germination rate will be different, due to the complex interaction between ecological and biological factors. Regardless, laboratory tests are effective for species conservation if we consider the possibility of reinforcement of the threatened populations with individuals developed from seeds, under laboratory-controlled conditions.

Favourable habitat conditions for *L. sibirica* populations are very important parameters that ensure the survival of individuals (both young seedlings and adults), even under conditions of low germination rates. In this way, populations can overcome periods of unfavourable reproductive success. The demographic and reproductive parameters, along with the Ellenberg Indicators, are useful tools that reflect habitat conditions and the response of plant populations to their changes.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (https://www.ingentaconnect.com/content/botbel/plecevo/supp-data) and consist of the following: (1) relationship between the parameters showing the performance of the *Ligularia sibirica* populations; and (2) analysis of the effect of the position within the inflorescence (upper, middle, and lower part) on morphological features of the seeds.

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