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IMPACT OF GENETIC AND DEMOGRAPHIC FACTORS ON THE EXPANSION POTENTIAL OF ALIEN SPECIES *BUNIAS ORIENTALIS* IN THE SUBURBS OF VILNIUS

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Abstract

Šiukštaitė L., Patamsytė J., Žvingila D., 2013: Impact of genetic and demographic factors on the expansion potential of alien species *Bunias orientalis* in the suburbs of Vilnius [Genetinių ir demografinių veiksnių įtaka svetimkraštės rūšies *Bunias orientalis* vienos populiacijos plitimo galimybėms]. – Both. Lith., 19(2): 111–119.

Bunias orientalis L. (*Brassicaceae*) is an alien and already naturalized plant species in Lithuania. Our purpose was to assess genetic diversity and age structure within single Belmontas population of *B. orientalis*, using RAPD, ISSR assays and herbchronology analysis. Three selected RAPD primers produced 58 scorable DNA bands, 53.33% of which were polymorphic. RAPD analysis identified 31 DNA markers. Six ISSR primers produced 113 reliable bands, but the level of polymorphism was slightly lower (38.1%) than RAPD. The 43 ISSR markers were identified. DNA markers were used for analysis of population genetic structure. AMOVA revealed significant genetic differentiation ($\Phi_{PT} = 0.11$) among subpopulations of *B. orientalis* located on both riversides of the Vilnia River. UPGMA cluster analysis did not reveal identical individuals in the group of studied plants, which indicate that spreading by seeds prevails in Belmontas population. Based on herbchronology analysis, young individuals dominate and provide good perspectives for population spreading.

Keywords: *Bunias orientalis,* herbchronology analysis, genetic diversity, population structure, ISSR, RAPD.

INTRODUCTION

Invasive alien species play detrimental impact on indigenous biota changing natural and cultivated ecosystems. Alien species can also alter the structure and species composition of ecosystems by reducing or eliminating native species. Studies of biological invasions may help to reveal the causes and consequences of this process and find out effective control measures (Mack et al., 2000).

Successful species invasions often depend on rapid evolutionary changes, which can be caused by intraspecific or interspecific hybridization, drastic changes in selection regimes in new environments, genetic drift and inbreeding in founder population (Bossdorf et al., 2005). Moreover, phenotypic plasticity, coexisting of other aliens and increasingly warming climate also accelerates the invasion process (Thompson, 1988).

Ideally, it is advisable to carry out long-term studies on population dynamics. Due to lack of time and funds it is not so easy to carry out such studies. However, there are techniques that allow restoring the population history from the current population structure (Dietz & Ullmann, 1998). For instance, annual rings in tree stems allow determining the age of it in temperate zones (Schweingruber, 1996) and reconstructing population dynamics of tree species (Stoll et al., 1994). It has been suggested that counting annual rings in roots (method known as herbchronology) may help to determinate age of dicotyledonous herbaceous plants in temperate zones (Dietz & Ullmann, 1997). This herbchronology method helps to investigate biological invasions of perennial herbaceous plants as well as saves time in monitoring of selected populations or groups of individuals (Dietz & Ullmann, 1998). DNA markers are another

valuable tool for the monitoring of invasion process of alien species. Markers are widely used in biological studies since detailed analysis of the genetic diversity of invasive species populations is important from both a scientific and a practical point of view. Understanding, how this diversity is distributed, may help to predict the potential spread of invasive species and how to control it better (Sakai et al., 2001). High potential of DNA markers at the population level studies of invasive species was revealed (Clark et al., 2013; Barnaud et al., 2013). Therefore, the use of DNA markers in plant studies can help to identify genetic variation in populations at the establishment and expansion phases, and this can be particularly useful when reconstructing demographic processes.

The study object *Bunias orientalis* L. (*Brassicaceae*) is a perennial, cross-pollinated, sometimes self-pollinating and able to reproduce vegetatively, herbaceous plant, which diploid genome consists of 14 chromosomes (Dietz et al., 1999). This species belongs to a non-indigenous species list in Lithuania (Rašomavičius, 2012). *B. orientalis* originate from the Caucasus, Turkey and the southern west region of Russia. It has spread across Europe, by Russian army as they were using this plant to feed horses (Steinlein et al., 1996; Dietz et al., 1999). First time in Lithuania it was recorded in 1885, in Klaipėda district (Gudžinskas, 1997). This species continues to spread successfully through anthropogenically disturbed habitats, along the highways, railways and rivers.

The purpose of the present study was to investigate demographic structure and distribution of genetic diversity within the single population of *B. orientalis* and to assess the impact of these factors on the invasion potential of this population.

MATERIALS AND METHODS

Species

Warty cabbage (*Bunias orientalis*) is mostly perennial, but sometimes biennial, hemicryptophyte (Natkevičaitė-Ivanauskienė, 1961). This plant may live up to 12 years (Dietz, unpublished work) and grow up to 180 cm. The stems are vertical and thick; the taproot varies in width and sometimes can be extremely long, even 2 meters under soil (Natkevičaitė-Ivanauskienė, 1961), also it can generate seedlings vegetatively from tiny root fragments (Steinlein et al., 1996). The petals are rich in yellow, and the flower itself is highly fragrant. It flowers from late May till August, despite this fact there is a second generation in late summer or early autumn. *B. orientalis* produces large (up to 1000 seeds/m^2 soil) permanent seed bank (Dietz & Steinlein, 1998). A distinctive feature of this species is "warty bumps" on stems, leaves and fruits (Renz & Doll, 2008).

Study site

The studied *Bunias orientalis* population is located in Vilnius city, Belmontas purlieus (Vilnius, Lithuania, 54° 40' N, 25° 20' E). The area, where plants were sampled, is fragmented by the road and the Vilnia River. Whole plot was approximately 2700 m in length and 300 m in width. Individuals were collected from different types of habitats such as meadow, monoculture lawn, anthropogenically disturbed areas along the Belmontas road and the Vilnia River. Moreover, the territory of current population has an interesting historical background, which may be related with the establishment of warty cabbage plants in Belmontas. The water mill was established in the 19th century on the territory of the studied population. Later, in the middle of the 20th century, an equestrian centre was founded in Belmontas.

Plants for the analysis were sampled twice: first part of plants was collected in July, and another in early autumn after mowing. A total of 78 plants were sampled. At both collecting times, the plants were taken with parts above (leaves, stems and flowering structures) as well as below (10–15 cm of the main root) the ground for genetic polymorphism and age structural analysis. The minimal distance between harvested *B. orientalis* individuals was 0.9 m.

Establishment of age structure

The age of the collected plants was assessed by counting annual growth rings in the cross-section of secondary xylem of root. The tap roots of *Bunias orientalis* individuals were cut about 5–40 cm from the surface of the soil. Cross-sections of the root were stained with phloroglucinol/HCl and examined under binocular microscope (Motic SMZ–143). After the treatment, lignin turned to reddish colour. As a result, annual growth rings of secondary xylem be-

came visible. It is worth to note that the current year growth increment was included into the analysis.

Genomic DNA extraction

Genomic DNA was extracted from fresh *Bunias orientalis* leaves by using CTAB method (Doyle & Doyle, 1990). Only young, healthy and mechanically undamaged leaves were chosen. After that, leaves were rinsed with distilled water and drained. Both quantity and quality of DNA was measured by NanoDrop 1000 (Thermo Fisher Scientific) and electrophoretically in 1% agarose gel.

RAPD and ISSR analysis

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and Inter-simple sequence repeat PCR (ISSR-PCR) were carried out as described by Patamsyte et al. (2011). All reactions were performed in one type thermocyclers (Eppendorf, peqSTAR). Primer annealing temperatures were 35°C for RAPD primers and 51°C, 46°C or 39°C – for ISSR primers (Table 1). All of the samples were analysed at least twice in different RAPD-PCR and ISSR-PCR experiments.

Both RAPD and ISSR DNA amplification products were resolved on a 1.5% agarose gels stained with ethidium bromide. After the DNA fragment fractionation, agarose gels were analysed and photographed using BioDocAnalyse gel documentation system (Biometra).

Data analysis

Since both RAPD and ISSR markers are dominant, it was assumed that each band correspond the phenotype at a single biallelic locus. The presence of the DNA band was scored and represented with "1", whilst the absence was coded as "0" and two matrixes of the variant RAPD and ISSR haplotypes were assembled.

Genetic distances between individuals were determined by arithmetical complement of Nei's and Li's similarity coefficient (Nei & Li, 1979). It was calculated as follows GDxy = 1–2 Nxy/(Nx + Ny), where Nxy is the number of common bands in x and y, Nx is the number of total bands in individual x and Ny is the number of total bands in individual y. Two matrixes were subjected to cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA) by TREECON software for Windows (Van De Peer & Watcher, 1994).

To describe genetic structure of the studied population, the Analysis of Molecular Variance (AMOVA) was performed by using GenAlex v.6 programme (Peakall & Smouse, 2006). Pairwise genetic distances (Φ_{ST}) among subpopulations and their levels of significance were also acquired from the AMOVA. For every analysis, 1000 of permutations were run to obtain significance testing. Principal coordinate analysis (PCoA) was applied to assess relationships between individuals and among subpopulations.

Table 1. Sequences, annealing temperatures of both RAPD and ISSR primers and the number, percentage, size of RAPD and ISSR bands

Primer	Sequence 5'→ 3'	Annealing t °C	Number of DNA bands		Polymorphism, %	Size of DNA bands (bp)
			Scorable	Polymorphic		
RAPD						
Roth A 01	CAGGCCCTTC	35	24	12	50	240–1800
Roth A 02	TGCCGAGCTG	35	14	7	50	640–1480
Roth A 03	AGTCAGCCAC	35	20	12	60	410–1720
Average:			19.3 ± 5.0	10.3 ± 2.9	53.3 ± 5.8	
Total:			58	31		240–1800
ISSR						
ISSR-A	$CTC(GT)_8$	51	17	6	35.29	310–1200
ISSR-C	$(AG)_8TG$	51	18	7	38.88	360–1170
ISSR I-18	$GTG(CT)_7C$	51	22	13	59.09	500–1490
ISSR I-32	$(AGC)_4C$	39	16	5	31.25	360–1410
ISSR I-34	$(AGC)_4GG$	46	25	9	36	240–1800
ISSR 50a	$CCA(GCT)_4$	46	15	3	20	490–1500
Average:			18.8 ± 3.9	7.2 ± 3.5	36.8 ± 12.8	
Total:			113	43		240–1800

RESULTS

Testing of primers and markers identification

For DNA polymorphism detection in the studied population of *Bunias orientalis*, we applied RAPD and ISSR techniques. Two molecular markers were chosen to investigate various aspects of DNA variation (Hou et al., 2005; Žvingila et al., 2012). Firstly, 6 RAPD and 16 ISSR primers were tested for the generation of reproducible banding using genomic DNA of 12 *B. orientalis* individuals. After the initial screening, three RAPD (A 01, A 02, A 03) and six ISSR (A, C, I-18, I-32, I-34, 50a) primers were selected (Table 1). As the Vilnia River divides our population into two parts, plants from both riversides were analysed. The left side subpopulation was smaller and in this study represented by 13 individuals. The right side subpopulation was considerably larger and 65 plants were sampled from it.

Molecular diversity within population

RAPD-PCR with 3 selected RAPD primers resulted in 31 polymorphic RAPD bands of 58 scored (Table 1). Three selected RAPD primers produced with an average of 10.33 ± 2.89 bands per primer. The mean percentage of polymorphic bands was 53.3%. While, between 113 ISSR bands, only 43 were polymorphic in a range of molecular weight (240–1800 bp), they were revealed using six ISSR primers. In this case, 7.16 ± 3.48 bands per primer were detected. An average polymorphism for ISSR loci was 36.8 ± 12.8.

Only polymorphic bands were used for further analysis. In this case, the percentage of polymorphic RAPD bands revealed in the right side subpopulation was 97.1% and for ISSR – 97.7%. The genotypes of left side subpopulation possessed 80.9% of polymorphic RAPD loci and 79.1% of polymorphic ISSR loci. Based on these RAPD and ISSR markers, the estimates of Nei and Li genetic distances were calculated. To reveal genetic relationships among individuals, UPGMA cluster analysis was performed and two dendrograms were generated. The pattern of clustering of genotypes in RAPD and ISSR based dendrograms varied, but both dendrograms showed that all of the studied individuals differed between each other. The RAPD based dendrogram (Fig. 1, A) consisted of two main large clusters and few individuals (Bo6_D_I-36; Bo54_D_27; Bo57_D_63; Bo61_D_59; Bo17_D_II-47; Bo18_D_II-48; Bo14_D_II-4) that genetically differed from these two groups. The first (upper) cluster contained individuals only from the right side subpopulation, whereas the second cluster (bottom) grouped together all of the left coast subpopulation and some individuals from the right shore plants. Dendrogram also showed that smaller groups of plants (I, II, III, IV) did not form homogenous subclusters, but splited into small groups of 3–7 individuals. The ISSR-based UPGMA dendrogram (Fig. 1, B) indicated more specific grouping of individuals (at least five smaller clusters) than in the RAPD tree. However, genotypes from the left side of the river were located in the lower part of the dendrogram. Based on ISSR markers, there was also more pronounced grouping of individual genotypes into sub-clusters according to the site of sampling. In this dendrogram, it was clearly seen that individuals from sampling site I formed a separate subcluster and next to them the subclusters of individuals from sampling sites II and III were located.

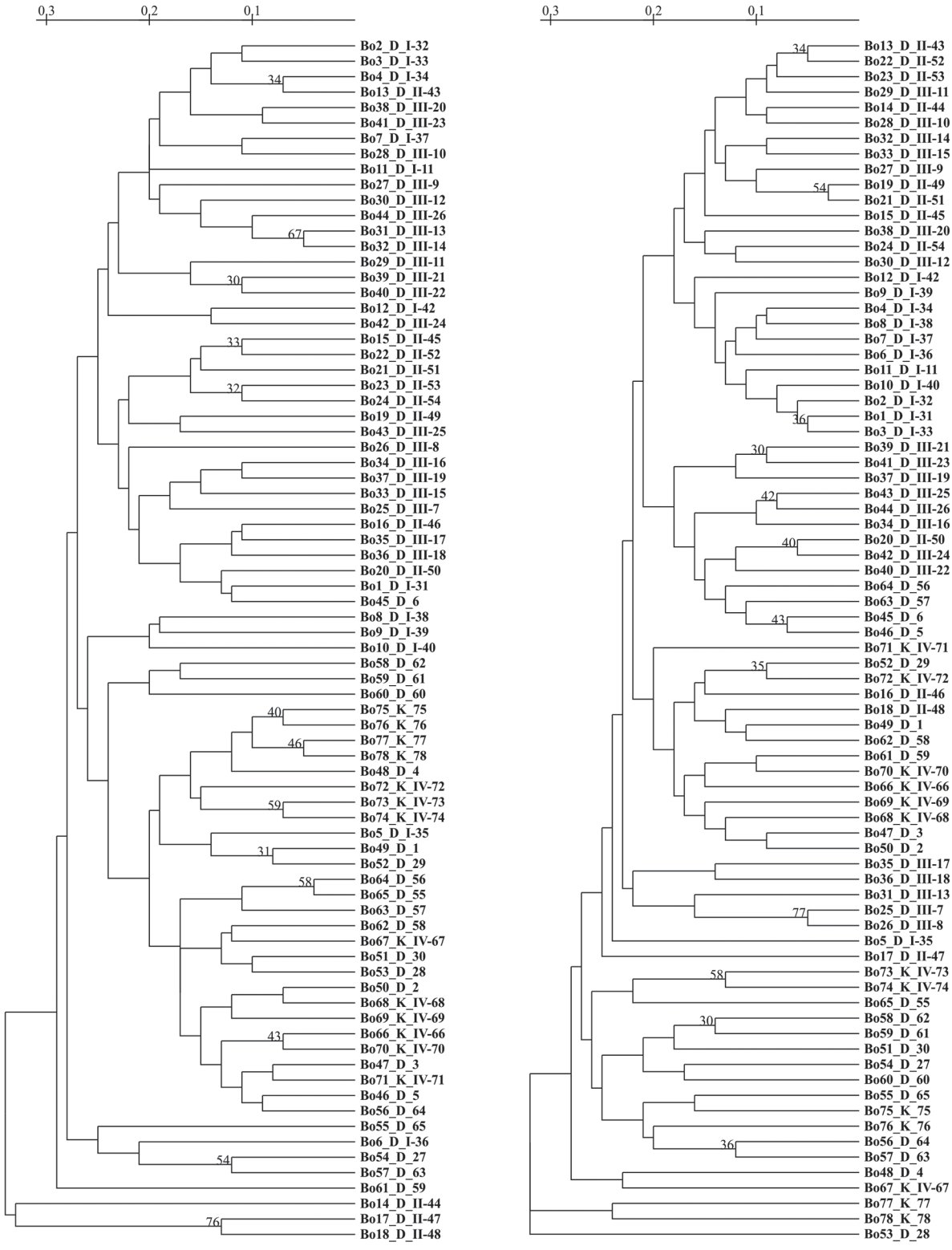
Principal Coordinate Analysis (PCoA) was used to ordinate distribution of RAPD and ISSR loci among individuals. PCoA showed that plants from the left side subpopulation were genetically distinctive (Fig. 2, A and B).

Genetic differentiation among subpopulations

The results of UPGMA cluster analysis and PCoA indicated some genetic specificity of plants growing on the left side of the Vilnia River. The existence of genetic differentiation among subpopulations from the left and the right side of the river was confirmed by the analysis of molecular variance (AMOVA). AMOVA indicated that about 11% of total genetic variance occurred among subpopulations. The analysis of both RAPD and ISSR data showed the same level of genetic differentiation ($\Phi_{PT} = 0.11$; $p < 0.5$).

Age structure of *Bunias orientalis* population

A total of 78 plants were collected. Due to core rot decay, 18 plants were excluded from examination. Thus, the age structure was determined to only 60 individuals. After the analysis of cross-sections of root cuts of all appropriate individuals, it was determined that plants in Belmontas population ranged in age from 1 to 9 years (Fig. 3). Most individuals from Belmontas population were 4 years old − there



A B

Fig. 1. UPGMA dendrogram of 78 plants of *Bunias orientalis* generated on the basis of Nei and Li genetic distances calculated using 31 RAPD (A) and 43 ISSR (B) markers

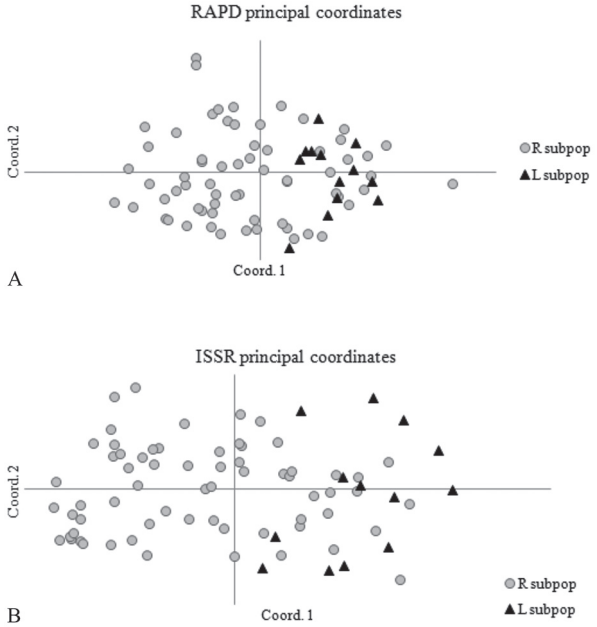


Fig. 2. Principal coordinate analysis of RAPD (A) and ISSR (B) data from genotypes of *Bunias orientalis* Belmontas population divided by the Vilnia River in two parts: the right riverside subpopulation (R Subpop) and the left riverside subpopulation (L Subpop)

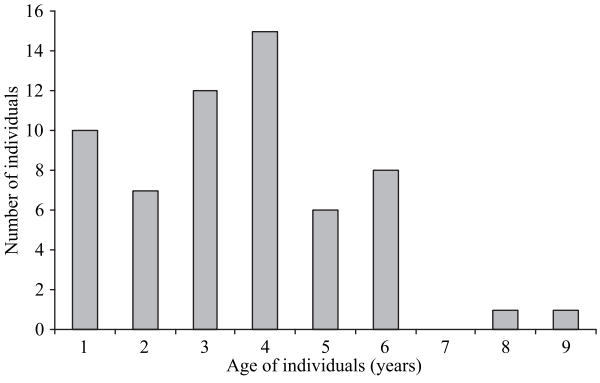


Fig. 3. Age structure of *Bunias orientalis* Belmontas population assessed by herbchronology method

were 15 plants of this age collected in 2012. However, none of the examined individuals were 7 years old. The average age of *B. orientalis* individuals was 3.58 ± 1.83 years.

In this work, we also examined whether genetic diversity differs among the groups of younger and older individuals. Individuals were divided into two age groups. The first age group (I) included young, 1–3-year-old plants, while the second group (II) formed individuals aged from 4 till 9 years. Both groups were almost equal in the number of plants. Neither AMOVA nor PCoA (based on both RAPD and ISSR) showed differentiation between these two groups.

DISCUSSION

In Lithuania, *Bunias orientalis* usually colonises two types of habitats: railways/roadsides and meadows on riversides (Patamsytė et al., 2013, in press). The studies on genetic micro-differentiation in a single population scale are used to assess the process of adaptation of species to microecological conditions (Owuor et al., 2003). It is very important in the background of biological invasions, where survival of dispersed individual populations can be followed by aggressive spread of invasive species. To evaluate the allocation of genetic diversity in the individual population of *Bunias orientalis*, we used RAPD and ISSR assays. The selected primers identified reliable polymorphic loci. For RAPD, the percentage of identified polymorphic loci was higher (53.33 %) than for ISSR −36.8%. Similar trend was mentioned in our previous study (Patamsytė et al., 2011) and can be explained by different molecular properties of these markers and their ability to reveal the unrelated features of genomic DNA. In previous study Dietz et al. using seven primers revealed that the level of RAPD variation in the single roadside population of *B. orientalis* (Middle Main valley, Germany) was also about 50% (Dietz et al., 1999). In roadside population study, the authors revealed 109 genotypes among 131 studied plants. In our study, based on UPGMA cluster analysis, no clonality was detected among sampled individuals. These results indicate that Belmontas population is mainly reproducing by seeds and the reproduction from root fragments is not typical for the spread of population. Moreover, UPGMA analysis shows certain genetic peculiarities of the subpopulations. The plants from the left side subpopulation tend to group into the lower dendrogram clusters, although there are inserts of a few individuals from the right coast subpopulation.

The Vilnia River the banks of which are overgrown with deciduous trees and shrubs divides the studied population into two subpopulations. The genetic distinctiveness of these subpopulations and the impact of small geographical barrier on genetic differentiation of subpopulation were assessed using AMOVA, PCoA and UPGMA cluster analysis. AMOVA indicated that

a rather significant proportion of variability (11%) is explained by genetic differences between the subpopulations. This same level of genetic differentiation was detected when both types of markers were analysed. Genetic differences between the individual subpopulations are not rare (Owuor et al., 2003; Patamsytė et al., 2010). Such differentiation in plant populations can be determined by variations in local ecological conditions (Owuor et al., 2003; Kupcinskiene et al., 2013). The population founding history also may have impact on the population genetic structure (Dlugosch & Parker, 2008) because of founder effect, increased selfing rates and geographical isolation (Barrett & Husband, 1990). PCoA also confirms results of UPGMA cluster analysis and AMOVA. RAPD-based and ISSR-based PCoA shows a certain grouping of genotypes from the left side subpopulation. As in UPGMA case, this tendency is more expressed when ISSR data are used for analysis. Two RAPD and five ISSR markers were identified as specific for the right side subpopulation. Taken into account the higher polymorphism of the right side population and the presence of subpopulation specific loci, it can be assumed that the left side subpopulation was founded from a limited number of propagules originated from plants growing on the right side of the river.

Our results confirm that herbchronology approach may provide useful information about the studies on plant populations and determination of population age structure. Sixty plants were tested during herbchronology analysis. Unfortunately, 18 individuals were not evaluated due to central root decay.

The obtained results in this research indicate that the largest number (15) of the tested individuals were 4 years old. The oldest individuals in Belmontas population were 8–9 years old in 2012. This result is similar to the data obtained by Dietz et al. (1999). The oldest plants of Middle Main valley population (Germany) were from 7 to 9 years old. In contrast to results of these authors, we did not reveal genetic differentiation between groups of young and older individuals. The data obtained in our study also indicate some tendencies of Belmontas population growth in 2009 and 2012. Our data show significant increase of the population in 2009, when the number of one-year-old individuals increased twice in comparison with previous year. The results also suggest that the number of new plants decreased in population significantly in 2006 as none of the 7-year-old individuals were detected to be alive till 2012. Despite of these fluctuations in some years, the age structure analysis of *Bunias orientalis* population shows the prevalence of young individuals, which indicates that population is in the phase of expanding.

Bunias orientalis spread is a linear process throughout all Europe (along rivers, railways, roads) (Woitke & Dietz, 2002). Transportation of cargo by various transport systems and industrial development is fostering the successful dissemination of this weed in new areas (Jehlik, 1988; Laviņš et al., 2006). It is both vegetative (by tiny root fragments) and generative (large seed bank and quantity) reproduction that gives a large potential for *B. orientalis* to be a widespread weed in Europe (Dietz et. al., 1996; Woitke & Dietz, 2002). In Lithuania, it usually colonises two types of habitats: railways/roadsides and meadows on riversides (Patamsytė et al., 2013). Unfortunately, we do not have any information about the time and the route of introduction of this species in Vilnius region. The existences of water mill and railway line in Belmontas environs for a long time possibly were the essential factors that promoted the settlement and spread of *B. orientalis*.

CONCLUSIONS

AMOVA revealed that there is a significant genetic differentiation (11%) between *Bunias orientalis* subpopulations from the right and the left side of the Vilnia River. Genetic differentiation was supported by PCoA and UPGMA cluster analysis and can be explained by founder effect. UPGMA cluster analysis showed that all individuals were genetically different, which indicates that sexual reproduction predominates in the studied population. Genetic differentiation between two age groups of *B. orientalis* plants (1–3 years and 4–9 years) was not detected.

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GENETINIŲ IR DEMOGRAFINIŲ VEIKSNIŲ ĮTAKA SVETIMKRAŠTĖS RŪŠIES *BUNIAS ORIENTALIS* VIENOS POPULIACIJOS PLITIMO GALIMYBĖMS

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Santrauka

Rytinė engra (*Bunias orientalis* L.) yra svetimkraštė, Lietuvoje natūralizavusis rūšis, plačiai paplitusi ne tik Baltijos jūros regiono šalyse, bet ir visame pasaulyje. Naudodami molekulinių žymenų metodus bei herbachronologinį amžiaus nustatymo metodą įvertinome Belmonto (Vilnius) populiacijos genetinę įvairovę ir amžiaus struktūrą. Atsitiktinai pagausintos polimorfinės DNR metodu (angl. *Random Amplified Polymorphic DNA* – RAPD) nustatytas vidutinis DNR polimorfizmo lygis (53,3 %), o paprastųjų pasikartojančiųjų sekų intarpų metodu (angl. *Inter-Simple Sequence Repeats* – ISSR) nustatytas mažesnis DNR polimorfizmas (38,1 %). Naudojant RAPD ir ISSR žymenis, UPGMA klasterių metodu sudaryti genetinio giminingumo medžiai atskleidė, kad visi tirti individai skiriasi tarpusavyje. Tai rodo, kad Belmonto populiacija dauginasi lytiniu būdu ir plinta sėklomis.

Naudojant molekulinės genetinės įvairovės analizės metodą (angl. *Analysis of Molecular Variance* – AMOVA), nustatyta reikšminga subpopuliacijų genetinė diferenciacija (Φ_{PT} = 0,11) populiacijos viduje. Herbochronologinė analizė atskleidė, kad Belmonto populiacijoje vyrauja jauni individai, todėl ji turi palankias sąlygas plisti.