VILNIUS UNIVERSITY

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GENETIC STRUCTURE, ADAPTATION AND INVASIVENESS OF DAISY FLEABANE (*ERIGERON ANNUUS* (L.) PERS.) POPULATIONS

Summary of Doctoral Dissertation

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VILNIAUS UNIVERSITETAS

Virginija Tunaitienė

VIENMETĖS ŠIUŠELĖS (*ERIGERON ANNUUS* (L.) Pers.) POPULIACIJŲ GENETINĖ STRUKTŪRA, ADAPTYVUMAS IR INVAZYVUMAS

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1. INTRODUCTION

Biological invasion by alien species is recognized as a significant component of global environmental change in our planet (Bleeker et al., 2007). Regulation no. 1143/2014 of the European Parliament and of the Council defines that invasive species are alien species whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services. The rate of spread of invasive species is growing due to increasing migration and international trade (Mack, 2000; Ward et al., 2008). For example, there are over 12000 registered plant species alien to Europe (Hulme et al., 2009). The number of invasive species in Europe is growing almost exponentially. During the last 40 years, the number of invasive species has increased by 76 percent (Butchart et al., 2010). Invasive plant species cause not only serious environmental, economic, but also human health impacts. Increase of such species and economic losses worry scientists in the entire world. Worldwide losses due to biological invasions are estimated to near 300 billion USA dollars per year (Luque et al., 2014).

Why do some species become invasive but others do not is one of the most urgent questions of biological invasion studies. The actual consequences of nowadays processes will be seen after a few or more decades, because sometimes non-native plants spend many years in the new environment before they become invasive. Catford et al. (2009) suggest that 26 of 29 invasion hypothesis are ecological. However, it has become clear that ecological factors alone, at least in some cases, cannot explain why some species become invasive but others do not. Considering that invasion consists of several stages, it is supposed that invasion often depends also on genomic changes during the lag phase (Clements and Ditommaso, 2010). During establishment in the new environment, genomic and genetic changes arise, which are determined by gene drift, mutations, hybridization with native species and other reasons (Bossdorf et al., 2005; Ainouche et al., 2009; Nagy and Korpelainen, 2015). These genetic changes can generate various biological modifications of species, one of which is increased invasiveness (Ellstrand and Schierenbeck, 2000; Ellstrand, 2009).

The inventory of alien plant species of Lithuania shows that there are about 500 such species, and a major part of them are herbaceous plants (Gudžinskas 1994, 1995). One of them, daisy fleabane (*Erigeron annuus* (L.) Pers.), is the subject of this dissertation. This alien forb originated from the eastern part of North America (Stratton, 1992). In the 17th century it was introduced to botanical gardens of Europe as an ornamental plant (Rothmaler, 1995). Although *E. annuus* is cosmopolitan species, in Europe it is especially widespread and during the past 30 years its spreading has been rapidly increasing (Frey, 2003). Though *E. annuus* is an apomictic plant, but sexual reproduction does occur occasionally and there are genotypes in which asexual reproduction is rare (Edwards et al., 2006).

It seems likely it was introduced to Lithuania from Western Europe at the end of the 19th century as an ornamental plant. Firstly, planted in gardens and cemeteries, *E. annuus* later started to spread out to natural ecosystems. The plants of this species usually grow on roadsides, spread as weeds in towns and disturbed habitats. *E. annuus* also penetrates into stable semi-natural communities, such as dry meadows and pastures (Motiekaitytė, 2001). Although there are no studies, showing the environmental impact of *E. annuus,* there is no doubt that its ability to compete with native species, tolerate moving, produce large amount of seeds and allelopathic compounds can promote the spread of this species (Stratton, 1992; Trtikova, 2009; Nazaruk, 2010).

Although the problem of biological invasions in Lithuania is becoming increasingly relevant, few genetic studies of invasive plants are done in Lithuania (Patamsytė et al., 2011, Vyšniauskienė et al., 2011; Zybartaite et al., 2011; Danusevičius et al., 2013; Kupcinskiene et al., 2013; Vyšniauskienė et al., 2013). Understanding the tendencies of invasive species' population dynamics and development is important in order to predict their impact on native ecosystems, also to look for the most suitable control ways. *E. annuus* was chosen as a fast spreading species which possesses remarkable individual biological features (high phenotypic plasticity, dominant agamospermy and occasional sexual reproduction) and belongs to a large *Asteraceae* family rich in invasive species.

AIM OF THE STUDY was to assess genetic structure, invasiveness and adaptation of *E. annuus* populations.

MAIN TASKS OF THE STUDY

1. To study the spreading pattern, genetic and genotypic structure of Lithuanian *E. annuus* populations using RAPD and ISSR markers;
2. To assess genetic diversity of *E. annuus* populations from different parts of the invasive range in Europe, to learn if the spreading history and time since the establishment of this exotic species in a specific territory had impact on genetic variability of local populations;
3. To compare genetic and genotypic diversity and the allelopathic properties of *E. annuus* populations collected from stable and disturbed habitats;
4. Using a common garden experiment to evaluate whether some morphological and phenological differences among *E. annuus* populations are the result of phenotypic plasticity or caused by local adaptation.

STATEMENTS TO BE DEFENDED

1. High genetic differentiation, which is possibly caused by founding events, is characteristic of Lithuanian populations of *E. annuus*;
2. Genotypes with asexual reproduction predominate in Lithuanian populations of *E. annuus*;
3. There is a tendency of decreasing of genetic diversity in *E. annuus* populations in transect from Switzerland to Latvia;

4. *E. annuus* populations from anthropogenic and semi-natural habitats show genetic differentiation and some difference in allelopathic properties;
5. Though *E. annuus* shows high phenotypic plasticity, which helps to colonize new habitats, in some populations genetically determined local adaptation occurs.

SCIENTIFIC NOVELTY AND PRACTICAL VALUE OF THE STUDY

1. The genetic and genotypic structure of Lithuanian invasive *E. annuus* populations was evaluated using two types of DNA markers (RAPD and ISSR);
2. Our study revealed the existence of *E. annuus* genetic diversity gradient in the part of invasive European range;
3. We revealed statistically significant genetic differentiation between *E. annuus* populations in different phases of invasion occurring in disturbed and stable habitats.
4. Differences in the alelopathic potential of *E. annuus* populations from stable and disturbed habitats were detected.

2. MATERIALS AND METHODS

1. Genetic and genotypic diversity studies of *E. annuus* populations

Plant material used in the study of genetic and genotypic structure and the spreading pattern of Lithuanian daisy fleabane populations. For this purpose, 29 populations from different regions of Lithuania were collected (Table 1). The number of collected individuals depended on the population size. Typically, twelve plants were arbitrarily collected from each population, with the exception of four populations (PN16,18,22,23) where fewer plants (5-10) were collected. A total of 328 individuals were sampled for DNA analysis (Table 1).

Plant material used to assess genetic diversity of *E. annuus* populations from different parts of the invasive range in Europe with the different time of settlement. Sixteen invasive populations of *E. annuus* from Switzerland (4 populations), Poland (6), Lithuania (4), Latvia (2) were evaluated (Table 2). One native population from Canada was also included in this study. The number of plants per population ranged from 12 to 25. A total of 253 plants were analysed.

Material for study of genetic and allelopathic differences between populations of *E. annuus* during the primary and secondary phase of invasion. Plants for this study were collected in disturbed and stable habitats where daisy fleabane populations in primary and secondary invasion phases occur (Table 3). This study was conducted with populations mainly located in South and East Lithuania with a focus on Vilnius city and its surroundings. This location was among the first where samples of *E. annuus* in natural ecosystems were found in the territory of contemporary Lithuania (Mowszowicz 1938).

TABLE 1. ***E. annuus*** **populations examined in this study with description of coordinates of collection sites and habitats**

PN	Population	N	Coordinates (long. E; lat. N)	Habitat
1	Užutrakis	12	54°39'50", 24°56'40"	Ungrazed/unmowed meadow
2	Giedraičiai	12	55°02'46", 25°15'20"	Ungrazed/unmowed meadow
3	Vilnius A	12	55°02'53", 26°13'26"	Artificial slope by road with intensive traffic
4	N. Janavas	12	54°02'53", 26°13'26"	Ungrazed/unmowed meadow
5	Vilnius B	12	54°42'06", 25°14'06"	By pathway going through pinewood
6	Pagiriai A	12	54°34'11", 25°12'14"	Area of abandoned farms
7	Roduka	12	54°06'53", 24°14'51"	Unmowed meadow, young pinewood
8	Daniliškės	12	54°35'48", 25°08'45"	Ungrazed/unmowed meadow
9	Marijampolė	12	54°33'47", 23°18'57"	Garden area
10	Pagiriai B	12	54°34'59", 25°10'36"	Residential area
11	N. Vilnia	12	54°41'40", 25°25'44"	Territory of former factory
12	Bezdonys	12	54°47'07", 25°33'13"	Grazed/mowed meadow
13	Gelgaudiškis	12	55°04'37", 22°51'30"	Young pinewood
14	Kėdainiai	12	55°18'36", 23°58'35"	Area near railroad
15	Jurbarkas	12	55°04'55", 22°46'51"	New residential area
16	Vilnius C	10	54°43'41", 25°15'01"	Meadow by high-tension line
17	Lielius	12	54°34'28", 24°22'27"	Slope by road
18	Babtai	6	55°04'58", 23°48'03"	Ungrazed/unmowed meadow
19	Vilnius D	12	54°39'44", 25°14'57"	On trackbed
20	Kulautuva	12	54°56'42", 23°37'59"	City park
21	Kalvarija	12	54°24'22", 2l°13'27"	Meadow by pathway
22	Mikytai	5	54°07'09", 21°54'56"	Unmowed meadow by road
23	Betygala	7	55°21'03", 23°22'40"	Cemetery
24	Kavarskas	12	55°26'08", 24°55'54"	Cemetery
25	Svėdasai	12	55°40'43", 25°21'31"	Cemetery
26	Kamajai	12	55°49'09", 25°30'54"	Cemetery
27	Kena	12	54°38'45", 25°38'31"	Partially mowed meadow
28	Mažeikiai	12	56°17'11", 22°21'44"	Near sand pit
29	Pervalka	12	55°24'54", 21°05'46"	Near railroad

PN – population number (code); N – number of individuals collected from population;

Population samples from stable habitats were collected in dry meadows and pastures. These habitats were not polluted directly by transportation and various sources of waste. Some of these meadows were mowed episodically. The vegetation cover was almost continuous. On the other hand, anthropogenic disturbance of ecosystems during urbanization and building generates the majority of habitats suitable for establishment of *E. annnus* populations. Populations of these disturbed (anthropogenised) habitats are located in road sides, landfills, closed waste disposals, building sites. The vegetation cover in disturbed sample collection sites was not completely formed and in addition to daisy fleabane, other ruderal and pioneer species were found, such as *Conyza canadensis*, *Arctium lappa*, *Medicago lupulina*, *M. falcata*, *Cichorium intybus*, *Cir-*

TABLE 2. ***E. annuus*** **populations used in genetic diversity gradient studies with description of coordinates of collection sites and habitats**

PN	Population	Code	N	Coordinates (long. E; lat. N)	Habitat
1	Locarno	LOC	24	46°09'51", 8°47'16"	Mowed meadow
2	Sigirino	SIG	12	46°04'41", 8°55'07"	Mowed meadow
3	San Antonio	SAN	12	46°10'07", 9°03'15"	Mowed meadow
4	Zürich	ZÜR	12	47°23'14", 8°31'59"	Unmowed meadow
5	Lublin A	LUA	12	51°11'21", 22°31'32"	Mowed meadow near lake
6	Szczuczyn	SZC	12	53°34'18, 22°18'05"	Unmowed meadow by road
7	Mrągowo	MRA	12	53°51'55", 21°17'26"	Mowed meadow by road
8	Suwalki	SUW	12	54°06'15", 22°56'50"	Mowed meadow near railroad
9	Lublin B	LUB	12	51°11'43", 22°32'17"	Unmowed meadow by road
10	Swieta Lipka	SWI	12	54°01'30", 21°13'00"	Mowed meadow near church
11	Daugavpils A	DAA	25	55°51'56", 26°31'42"	Mowed meadow near factory
12	Daugavpils B	DAB	12	55°52'23", 26°29'42"	Unmowed meadow
13	New Brunswick	NEW	12	46°13'15", 64°32'20"	Unmowed meadow
14	Patašinė	PAT	12	54°32'30", 23° 24'52"	Unmowed meadow
15	Prienai	PRI	24	54° 37' 38", 23° 57'31"	Mowed meadow
16	Sudervė	SUD	24	54° 46' 51", 25° 4'15"	Unmowed meadow
17	Kernavė	KER	12	54° 53' 3", 24° 52' 29"	Meadow on the slope

PN – population number; N – number of individuals collected from population;

TABLE 3. **Genetic and genotypic diversity parameters at 100 ISSR loci for** ***E. annuus*** **populations from semi-natural and anthropogenic habitats**

PN	Code	N	Coordinates (long. E; lat. N)	Habitat
			Semi-natural	
1	NJA	12	54°02'53", 26°13'26"	Abandoned meadow
2	MRA	12	54°33'47", 23°18'57"	Mowed meadow
3	BEZ	12	54°47'07", 25°33'13"	Grazed meadow
4	GEL	12	55°04'37", 22°51'30"	Forest meadow
5	VLNA	12	54°43'41", 25°15'01"	Mowed meadow
6	KEN	12	54°38'45", 25°38'31"	Partially mowed meadow
7	TAR	13	54°27'24" 23°07'03"	Abandoned meadow
8	SVA	15	54°15'08" 24°33'31"	Mowed meadow
9	DRU	12	54°13'50" 24°26'51"	Mowed meadow
10	PAT	24	54°32'30" 23°24'52"	Abandoned meadow
11	PRI	24	54°37'38" 23°57'31"	Mowed meadow
12	TRA	24	54°36'25" 24°46'50"	Mowed meadow
13	CEK	24	54°44'26" 25°05'37"	Abandoned meadow
14	DUK	24	54°49'28" 24°58'25"	Abandoned meadow
15	KERA	12	54°54'07" 24°50'44"	Mowed meadow
16	SUD	24	54°46'51" 25°04'15"	Abandoned meadow
17	AUK	24	54°34'26" 24°30'40"	Abandoned meadow
18	NEM	12	54°38'49" 25°21'28"	Abandoned meadow

TABLE 3 (continuation). **Genetic and genotypic diversity parameters at 100 ISSR loci for *E. annuus* populations from semi-natural and anthropogenic habitats**

PN	Code	N	Coordinates (long. E; lat. N)	Habitat
19	KLK	12	54°50‘58“ 25°09‘17“	Mowed meadow
20	VLNF	14	54°42‘36“ 25°15‘23“	Abandoned meadow
21	JURA	12	55°04‘52“ 22°45‘40“	River valley meadow
			Anthropogenic	
22	PGA	12	54°34’11”, 25°12’14”	Territory of abandoned farm
23	VLNB	30	54°39‘57“ 25°15‘01“	Urbanized territory
24	KAR	24	54°42‘56“ 24°57‘45“	Closed landfill
25	PGS	24	54°34‘56“ 25°11‘18“	Territory of abandoned greenhouses
26	GAR	12	54°39‘58“ 25°09‘13“	Territory near power-station
27	VLNC	24	54°40‘19“ 25°11‘56“	Roadside
28	MIC	24	54°42‘31“ 25°33‘19“	Closed landfill
29	BEL	24	54°41‘25“ 25°20‘54“	Closed landfill
30	VLND	12	54°42‘37“ 25°17‘10“	Roadside
31	GRI	24	54°40‘22“ 25°05‘46“	Territory of factory/ roadside
32	UZK	24	54°37‘08“ 25°04‘24“	Roadside
33	VLNE	24	54°40‘09“ 25°13‘26“	Territory of abandoned greenhouses
34	MIN	24	54°38‘46“ 25°20‘01“	Roadside
35	VLNG	24	54°44‘41“ 25°13‘59“	Closed landfill
36	JURB	24	55°04‘52“ 22°45‘40“	Garage territory
37	VLNH	24	54°40‘23“ 25°17‘36“	Roadside

PN – population number; N – number of individuals collected from population;

sium arvense, *Artemisia vulgaris,* etc. Samples were collected in the summer of 2012. The least distance among the collected plants was at least 3 meters.

Material for study of the allelopathic properties of populations was collected from 10 populations (NJA, PAT, AUK, TRA, PRI, KERA, SUD, NEM, DUK, CEK), which were established in stable habitats, and from 9 populations (KAR, BEL, VLNB, VLND, VLNE, VLNH, UZK, MIC, GRI) from disturbed habitats (Table 3.). The samples were collected in the summer of 2013 (June – July).

2. Methods

Genomic DNA extraction. Total genomic DNA was isolated from ground fresh leaves using the modified CTAB method (Doyle and Doyle, 1990).

DNA concentration and quality evaluation. DNA concentration and the quality were measured using “BioPhotometer” (Eppendorf, Germany). The working concentration of DNA was 5 ng/µl.

ISSR-PCR analyses. ISSR analyses were performed as described by Patamsytė et al. (2011). Each 20 µL ISSR polymerase chain reaction contained 2 µL 10×PCR

buffer (Thermo Scientific), 200 μM dNTPs, 1 unit Taq polymerase (Thermo Scientific Scientific Baltics), 300 μM MgCl2, 0.4 μM of the primer and approximately 20 ng of DNA. The ISSR-PCR was performed in PeqSTAR thermocycler. Conditions were as follows: 1 cycle of 7 min at 94°C, 32 cycles of 30 s at 94°C, 45 s at 55°C, 2 min at 72°C, and 1 cycle of 7 min at 72°C. All reactions were run at least twice. A negative control PCR without DNA template was performed in parallel with each amplification. The selection of primers for reproducibility and DNA band polymorphism was performed before the main analysis using 10 samples from the different populations. Primers generating complex or weak banding profiles were discarded. For the ISSR analysis, five primers were used: ISSR-O – $GAG(CAA)_5$, ISSR-B – $(AG)_8CG$, ISSR-C – $(AG)_8TG$, ISSR-D – $(AG)_8$, and ISSR-G – $(GCC)_5$.

RAPD-PCR analysis. Each 20 μL RAPD polymerase chain reaction contained 2 μL 10×PCR buffer (Thermo Scientific), 200 μM dNTPs, 1 unit Taq polymerase (Thermo Scientific), 300 μM MgCl2, 0.4 μM of the primer and approximately 20 ng of DNA. The RAPD-PCR was conducted for 4 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C, followed by a final extension step of 5 min at 72°C. For RAPD analysis, we used four selected primers (in parentheses – percent of polymorphic fragments): Roth A-03 – AGTCAGCCAC, Roth A-04 – AATCGGGCTG, Roth A-05 – AGGGGTCTTG, and Roth A-07 – GAAACGGGTG.

Electrophoresis of ISSR-PCR and RAPD-PCR products on an agarose gel. PCR products were resolved on a 1.2% agarose gel (4 h, 4 V/cm), stained with ethidium bromide and photographed using a BioDocAnalyse system (Biometra, Germany). The size of scored DNA fragments was estimated with DNA size standard (GeneRuler DNA Ladder Mix, Thermo Fisher Scientific Baltics).

Data analysis. All reactions were run at least twice including positive and negative controls. Only well-defined and reproducible bands were included in the binary data matrix. In this situation only 000 genotype was scored as 0, whereas the indistinguishable genotypes 111, 110 and 100 were considered as 1. Because allele frequencies of dominant markers cannot be unambiguously estimated in polyploids, band based analysis of ISSR data was carried out (Kloss et al. 2011). The percentage of polymorphic loci (P), Shannon's information index (I, Nei's gene diversity (h) and the population genetic differentiation coefficient (G_{ST}) (Nei, 1972) were calculated using POPGENE v. 1.31 software (version 1.31; www.ualberta.ca/~fyeh/ popgene). PCoA was conducted using GenAlEx v. 6.4 (Peakall and Smouse, 2006). An unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on pairwise Nei's (Nei, 1978) unbiased genetic distances (GD) was used to assess genetic relationships among populations and to present the results as a dendrogram. Nei and Li's (1979) genetic distances (GDxy) among the individual plants in the separate populations were calculated using the TREECON program v. 1.3b (Van de Peer and De Wachter, 1994). Based on molecular data, expected heterozygosity (Hj) according to Lynch and Milligan (1994)

was estimated using AFLP-SURV v. 1.0 (Vekemans et al., 2002). AFLP-SURV v. 1.0 was also used to evaluate genetic differentiation (Fst) among populations, among populations within habitats and among habitats and to estimate pairwise F_{ST} values between populations following the approach of Lynch and Milligan (1994). The number of permutations for Fst testing was 1000. The band richness (Br) and polymorphic band proportion (PLP; at the 5 % level) for standardized sample sizes, according to Petit et al. (1998), were calculated using AFLPdiv v. 1.1 (Coart et al., 2005). The variation in ISSR profiles within and among habitats was estimated by analysis of molecular variance (AMOVA) (Excoffier et al., 1992) using GenAlEx 6.5 (Peakall and Smouse, 2006) and the genetic differentiation among habitats (Phi_{RT}) and among populations (Phi_{PT}) was calculated. Phi_{PT} corresponds to Wright's (1965) fixation index (F_{ST}). For each analysis, 1000 permutations were performed to obtain significance levels. For estimation of genotypic diversity, proportion of distinguishable genotypes G/N ($t = G/n$) was calculated according to Ellstrand and Roose (1987), where G is the number of genotypes and N is the number of analyzed plants. To estimate the evenness of genotypic diversity, the evenness index (E) based on Simpson index was applied (Williams et al., 1964; Solé et al., 2004). Mann-Whitney U-test in IBM® SPSS® Statistics v.23 was used for a standardized sample size and population number (12 individuals from each population. For populations represented by larger sample size 12 individuals were randomly chosen. The analysis included 16 populations from stable habitats and 16 populations from disturbed habitats). It was also used to assess statistical significance of differences of genotypic diversity and genetic differentiation (F_{ST}) among the habitats. To assess the number of genetically different clusters in *E. annuus* and to evaluate if grouping of populations into clusters corresponds to habitat assignments, Bayesian clustering algorithm STRUCTURE v. 2.3.4 was used (Pritchard et al., 2000; Falush et al., 2007). Ten independent runs (K = 1-20) were carried out with 200 000 burn-in repetitions and 200 000 MCMC. The results were processed with STRUCTURE HARVESTER (Earl and von Holdt 2012), and the STRUCTURE output was displayed using DISTRUCT v1.1 (Rosenberg, 2004). Mantel test, carried out using the GenAlEx v. 6.5 program, was used to evaluate significant correlations between the genetic and geographic distance among all populations and among populations from different habitats.

Evaluation of allelopathic properties of studied populations. Germination test was used to examine potential allelopathic properties of plants from different habitats. This test was carried out according to Csiszár (2009) to evaluate juglone index (Szabó 2000). The juglone index (Ij/x) of a substance with unknown allelopathic effect was calculated using the formula

$$Ij/x = (Hj + Rj + Gj) / (Hx + Rx + Gx),$$

where Hj is the average of shoot lengths of white mustard seeds treated with 1 mM juglone, Rj – the average of root lengths white mustard seeds treated with 1 mM juglone,

Gj – the average of germination rates of white mustard seeds treated with 1 mM juglone, Hx – the average of shoot lengths of white mustard seeds treated with substance from *E. annuus*, Rx – the average of root lengths of white mustard seeds treated with substance from *E. annuus*, Gx – the average of germination rates of white mustard seeds treated with substance from *E. annuus*.

For this examination, plant material was collected and dried at room temperature. Aqueous extracts were prepared at two different concentrations by soaking 1 g and 5 g dried plant materials (leaves and stems) of the donor species in 100 ml distilled water at 20 °C for an hour, shaken every 10 minutes and filtered through filter paper. White mustard seeds were germinated between two filter paper sheets wetted with 5 ml extract, in darkness, placed in a biological thermostat at 20 °C. 100 white mustard seeds were placed in each Petri dish, the experiment with each concentration and population was repeated three times. Germination rate, shoot and root length were registered on the 6th day counted from the beginning of imbibition. The effects of the different plant species extracts on the germination rate, shoot and root length of white mustard were compared to the treatment with distilled water (control).

Common garden experiment. The seeds collected in 2012 from Lithuanian (Narbutas street, Pagiriai, Marijampolė, Grigiškės, Akropolis), Polish (Mragowo, Zeglarska, Szczuczyn), Swiss (Sigirino, Locarno, Zürich) and Canadian (New Brunswick) populations were sown in a greenhouse. Common gardens were set up at the VU Botanical garden (Vingis) in 2013 and 2014. Individuals from populations were planted every 25 cm. Fifty plants were planted from each population. A total of 600 individuals were planted in the common garden. Plant phenology changes (formation of flower buds, flowering) and height were observed every ten days. Three months after planting, the plants were harvested. Plant final height, weight, number of stems, number of extra branches, stem diameter at the 40 cm high, length and width of leaves, diameter of flower were measured. Measurements were done on 50 plants of each population. Mean and standard deviation were calculated and histograms were drown using Microsoft Excel. PAST program was used to assess statistical differences between populations.

Chemical elements analysis of topsoil samples. Topsoil samples were also collected and the concentrations of 13 elements (As, Ba, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Sn, V, Zn) were determined in the soils from the studied population location sites at the Laboratory of Geochemistry of Natural Research Center (Vilnius, Lithuania) via radio-fluorescence analyses with the EDXRT equipment SPECTRO XEPOS (METEKGmbH, Germany) as described in Čėsnienė et al. (2014). A cluster analysis was used to group the sites according to the amount of elements established at these sites. Ward's method using Euclidean distance was chosen from IBM® SPSS® Statistics v.23 for Windows to plot the dendrogram.

3. RESULTS AND DISCUSSION

3.1. Study of genetic structure of invasive *E. annuus* populations in Lithuania

3.1.1. Genetic variation within populations

A total of 328 individuals from 29 Lithuanian populations were investigated in the study of genetic structure (Figure 1).

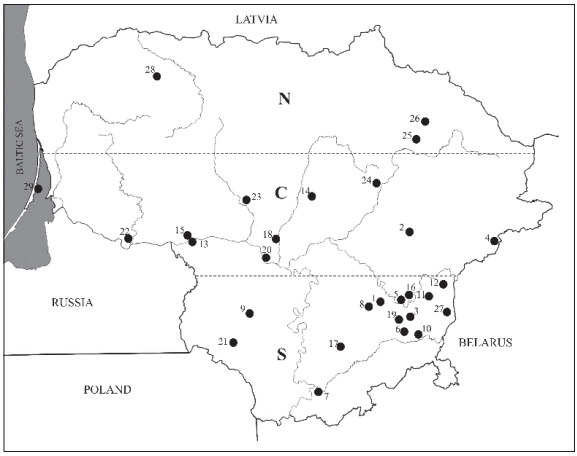


FIGURE 1. **Geographic distribution of the studied populations (PN 1-29) of *E. annuus* in Lithuania.** Regions: S – southern; C – central; N – northern. The sampling site characteristics and population numbers (PN) are presented in Table 1.

A total of 113 reproducible RAPD and 156 ISSR bands were identified in DNA samples with 4 RAPD (A-03, A-04, A-05 ir A-07) and 5 ISSR (ISSR-O, ISSR-B, ISSR-C, ISSR-D, ISSR-G) primers. Two types of DNA markers, RAPD and ISSR, were used in our study not only to generate more precise and reliable data but also to obtain additional information about the genotypes of modern and possibly old *Erigeron annuus* populations and the peculiarities of their distribution across Lithuania. The use of a two-marker system is especially important for polyploids such as *E. annuus* (triploid) because part of the genotype diversity in polyploid species may be masked by the dominant character of RAPD and ISSR markers (Chapman et al., 2004). Only a coincidence of results between two or more marker systems can eliminate arguments about the suitability of such markers for invasive plant studies (Kliber and Eckert, 2005; Wang et al., 2008; Fitzpatrick et al., 2012). Both types of markers were highly correlated in the identification of monomorphic populations. The RAPD assay revealed 14 polymorphic populations out of the 29 studied, while the ISSR analysis indicated genotype diversity among 15 of the populations out of the 29 studied (Table 4).

TABLE 4. Intra-population variation of polymorphic populations of *E. annuus*

Population	PN	H		I		P (%)		Gdxy	
		RAPD	ISSR	RAPD	ISSR	RAPD	ISSR	RAPD	ISSR
Southern region									
Užutrakis	1	0.25	0.16	0.36	0.23	61.43	39.13	0.38	0.18
Vilnius B	5	0.25	0.25	0.37	0.36	61.43	59.13	0.23	0.3
Daniliškės	8	0.25	0.25	0.37	0.37	70	69.57	0.19	0.2
Marijampolė	9	0.24	0.24	0.36	0.34	60	56.52	0.20	0.21
Bezdonys	12	0.09	0.07	0.15	0.11	34.29	24.35	0.04	0.05
Vilnius C	16	0	0.03	0	0.04	0	10.43	0	0.01
Lielius	17	0.1	0.16	0.15	0.23	25.71	42.61	0.04	0.11
Kalvarija	21	0.25	0.23	0.37	0.34	61.43	55.65	0.32	0.26
Kena	27	0.29	0.29	0.43	0.33	78.57	62.61	0.26	0.19
	Average	0.19 ± 0.19	0.18 ± 0.06	0.28 ± 0.12	0.26 ± 0.09	50.32 ± 20.21	46.67 ± 14.03	0.18 ± 0.11	0.17 ± 0.07
Central region									
Gelgaudiškis	2	0.11	0.15	0.17	0.23	35.71	52.17	0.04	0.09
Giedraičiai	13	0.24	0.21	0.35	0.31	60	57.39	0.20	0.18
Kėdainiai	14	0.25	0.14	0.36	0.21	62.86	36.52	0.24	0.11
Jurbarkas	15	0.13	0.09	0.19	0.15	44.29	33.91	0.07	0.05
Mikytai	22	0.21	0.15	0.3	0.22	52.86	38.26	0.21	0.15
Pervalka	29	0.19	0.20	0.28	0.29	45.71	46.96	0.20	0.26
	Average	0.19 ± 0.05	0.16 ± 0.03	0.28 ± 0.06	0.23 ± 0.04	50.24 ± 8.34	44.20 ± 7.97	0.16 ± 0.07	0.14 ± 0.05
All polymorphic populations									
	Total average	0.19 ± 0.07	0.17 ± 0.07	0.28 ± 0.10	0.25 ± 0.08	50.29 ± 15.47	45.68 ± 12.61	0.18 ± 0.09	0.16 ± 0.07

PN – population number; H – Nei's genetic diversity (1973); I – Shannon Index; P – percent of polymorphic DNA fragments; GDxy – Nei and Li's (1979) genetic distance

So, only approximately half (48.3 % according to RAPDs, 51.7 % according to ISSRs) of the 29 investigated populations were polymorphic. The number of genotypes per polymorphic population established using both assays ranged from 2 to 4, with the exception of Vilnius C population for RAPD markers, where only one genotype was detected by the RAPD assay. The highest number (4) of genotypes detected by both assays was found in Daniliškės and Kena from the southern region (Table 5).

TABLE 5. **Number of individuals per phenotype found using RAPD and ISSR assays among 328 individuals of *E. annuus***

Population	Total samples	Number of genotypes	
		RAPD	ISSR
Southern region			
Užutrakis	12	2	3
Vilnius B	12	2	2
Daniliškės	12	4	4
Marijampolė	12	2	2
Bezdonys	12	2	2
Vilnius C	12	1	2
Lielius	12	2	2
Kalvarija	12	2	2
Kena	12	4	4
Central region			
Giedraičiai	12	2	2
Gelgaudiškis	12	4	3
Kėdainiai	12	2	2
Jurbarkas	12	2	2
Mikytai	5	2	2
Pervalka	12	2	2

Among the polymorphic populations, the highest differences in percentage of polymorphic loci were observed in the southern region. Relatively low DNA polymorphism was found in Bezdonys (P_{ISSR} = 24.35 %; P_{RAPD} = 34.29 %) and Lielius (P_{RAPD} = 25.71 %; P_{ISSR} = 42.61 %) populations. The highest polymorphism for both molecular markers was detected in Daniliškės (P_{RAPD} = 70.00 %; P_{ISSR} = 69.57 %) and Kena (P_{RAPD} = 78.57 %; P_{ISSR} = 62.61 %) populations. Almost half of the polymorphic populations (Vilnius B, Daniliškės Marijampolė, Kalvarija in the southern region and Giedraičiai, Pervalka in the central region) presented a full or almost full coincidence of genetic diversity parameters as indicated by RAPD and ISSR assays. However, there are some populations (Užutrakis, Lielius, Vilnius C, Gelgaudiškis, Kėdainiai and Mikytai) where differences between RAPD and ISSR data were observed. Moreover, in several of them (Užutrakis, Kėdainiai, Mikytai) RAPD polymorphism prevailed, while in the other three populations (Vilnius C, Lielius, Gelgaudiškis), variation was higher for ISSR markers (Table 4).

Only in one population (PN16, Vilnius C) all plants were monomorphic according to RAPD markers (h = 0, I = 0, P = 0%, Gd_{xy} = 0), but slight variation was revealed by the ISSR (h = 0.03, I = 0.04, P = 10.43 %, Gd_{xy} = 0.01). The small differences observed between the two types of markers can possibly be explained by stochastic variation in allele assortment in connection with occasional recombination events (Kjølner et al., 2004).

Out of 328 fleabane individuals only 18 plants presented unique molecular phenotypes (Table 5). The pairwise genetic distances between populations established using RAPD markers significantly correlated with genetic distances based on ISSR markers ($r = 0.91$, $p < 0.05$).

The absence of genetic polymorphism in half of the populations and the small number of genotypes in the polymorphic populations indicate predominating apomyctic reproduction of *E. annuus* in Lithuania. Edwards et al. (2006) established that most of the populations of this species from Northern America and Western Europe were polymorphic and possessed rather high levels of intrapopulation genotypic diversity. In another study, Trtikova et al. (2011) found that 83 % of the studied populations from Switzerland were polymorphic. A detailed comparison of Lithuanian *E. annuus* populations with the western and central European populations studied by the aforementioned authors is complicated because of the differences in assays used and number of loci studied. In a study conducted by Edwards et al. (Edwards et al., 2006), 39 RAPD loci were analyzed, and in the last study, Trtikova et al. (Trtikova et al., 2011) identified 94 AFLP markers. Nevertheless, at the population level a trend towards reduced genetic variability in Lithuanian populations is evident. Possible causes of low genetic diversity are the shorter history of *E. annuus* in Lithuania that began only at the end of the 19th century (Gudžinskas, 1997) and expressed founder effect (Husband and Barrett, 1991; Ren and Zhang, 2007; Budde et al., 2011).

E. annuus is rare in the northern region of Lithuania. In our study, this region was represented by only three populations – Svėdasai (No. 25), Kamajai (No. 26), Mažeikiai (No. 28). All these populations are monomorphic. Two (Svėdasai and Kamajai) of them were situated in old cemeteries. Two other populations from old cemeteries (Betygala (No. 23) and Kavarskas (No. 24)) though sampled in the central region, were also monomorphic. Three monomorphic cemetery populations (PN24-26) are closely related according to the UPGMA analysis (Figure 2).

E. annuus is more frequent in the central and especially the southern region of the country (Figure 1). Polymorphic and monomorphic populations were found in both regions. All populations in the western direction: Gelgaudiškis (No. 13), Jurbarkas (No. 15), Mikytai (No. 22), Pervalka (No. 29) were polymorphic. A similar level of genetic diversity parameters was observed in both of these regions (Table 4). The average DNA polymorphism in polymorphic populations from the central region was 50.24 % for RAPD and 44.20 % for ISSR markers. In the southern region, DNA polymorphism was 50.32 % for

RAPD and 46.67 % for ISSR. Nei gene diversity was almost identical in both regions. In the central region, the diversity was 0.19 for RAPD and 0.16 for ISSR, and in the southern region, the diversity was 0.19 for RAPD and 0.18 for ISSR. Shannon's index presented the same tendency: central region values were 0.28 (RAPD) and 0.23 (ISSR) and southern region values were 0.28 (RAPD) and 0.26 (ISSR) (Table 4).

The western regions of Lithuania, according to Gudžinskas (Gudžinskas, 1997), were previously free from *E. annuus*. An *E. annuus* population was found only in one site in Pervalka (a large village and health resort) in a small patch in a meadow near a bus station. It can be hypothesized that this population is a new site of *E. annuus* invasion. Therefore, it was surprising that a variation was found in this population, as revealed by RAPD (P = 45.71 %, h = 0.19, I = 0.28) and ISSR (P = 46.96 %, h = 0.20, I = 0.29) markers (Table 4). The UPGMA analysis of the RAPD data indicated a genetic relationship between Pervalka (No. 29) and Marijampolė (No. 9) populations (Figure 2).

The monomorphic population group includes four cemetery populations (Betygala (No. 23), Kavarskas (No. 24), Svėdasai (No. 25), Kamajai (No.26) populations), Vilnius A (No. 3), Pagiriai A (No. 6), Roduka (No. 7), Pagiriai B (No. 10), Naujoji Vilnia (No. 11), Vilnius D (No. 19), Naujasis Janavas (No. 4), Babtai (No. 18), Kulautuva (No. 20) and Mažeikiai (No. 28) populations (Table 1). The monomorphic population group included significant portions of the samples from Vilnius and adjacent regions.

3.1.2. Genetic relationships among the populations

Dendrograms based on UPGMA analyses of RAPD and ISSR data revealed that one clone of *E. annuus* is shared among nine populations (Vilnius A (No. 3), Naujasis Janavas (No. 4), Pagiriai A (No. 6), Pagiriai B (No. 10), Roduka (No. 7), Naujoji Vilnia (No. 11), Vilnius C (No. 16), Babtai (No. 18), Vilnius D (No. 19) (Figure 2). It has expanded into Vilnius city and adjacent territories. Several populations of this main clone were found 90 and 130 km from Vilnius, in rural areas. A comparison of UPGMA dendrograms generated on the basis of RAPD and ISSR data indicates almost full coincidence among the main clone populations.

A two-dimensional plot diagram of the PCoA analysis of the RAPD data indicates the relatedness of the main clone with the cemetery (Betygala (No. 23), Kavarskas (No. 24), Svėdasai (No. 25), Kamajai (Nr. 26)) and some other populations (Giedraičiai (No. 2), Daniliškės (No. 8), Bezdonys (No. 12), Gelgaudiškis (No. 13), Kėdainiai (No. 14), Jurbarkas (No. 15), Lielius (No. 17), Kulautuva (No. 20), Mikytai (No. 22) (Figure 3, a).

The other observation is the more dispersed character of the tested populations on the PCoA diagram generated from the ISSR data (Figure 3, b). Another interesting property of the PCoA analysis is evident on both PCoA plots – a group of seven populations (Užutrakis (No.1), Vilnius B (No. 5), Marijampolė (No. 9), Kalvarija (No. 21), Kena (No. 27), Mažeikiai (No. 28), Pervalka (No. 29)) that are genetically distant from the

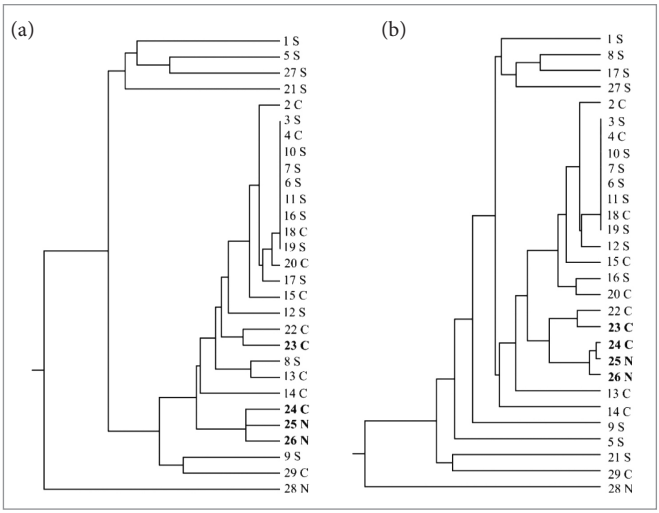


FIGURE 2. Dendrograms of the UPGMA cluster analysis based on Nei's (1978) unbiased measure of genetic distances estimated using RAPD (a) and ISSR (b) markers. Regions: S – southern; C – central; N – northern. Cemeteries populations are indicated in bold

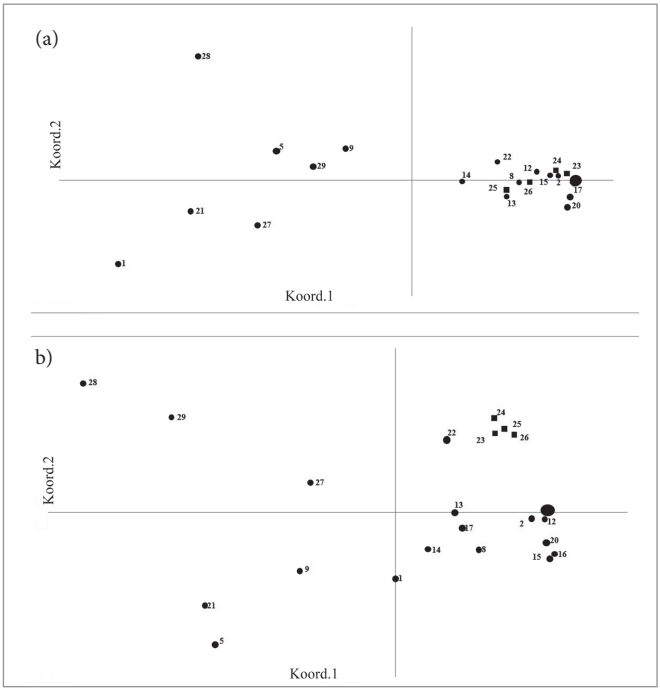


FIGURE 3. **Principal coordinate analysis (PCoA) plot based on RAPD (a) and ISSR (b) markers showing similarity and genetic variation among 29 populations of *E. annuus*.** Black squares indicate cemetery populations. Black large circle indicates populations of the main clone.

group of populations more or less related to the main clone. All these populations, with the exception of Mažeikiai, were polymorphic (Table 4).

According to our data, the studied *E. annuus* populations can be divided into two groups on the basis of results of DNA polymorphism study. The first population group is represented by a widely spread main genotype (clone) and some related monomorphic populations. The second population group is represented by polymorphic populations. A similar situation was described among *Saxifraga cernua* populations (Kjølner et al., 2004). In a study of *S. cernua*, 26 AFLP and 32 RAPD multilocus phenotypes (putative clones) were identified, of which 21 were identical and each of the remaining five AFLP clones were split into two to three very similar RAPD clones.

3.1.3. Partitioning of genetic diversity

An AMOVA revealed that 45 % of the total variation established using the RAPD analyses occurred among populations within regions and 47 % occurred within populations. When an analysis was conducted on the basis of the ISSR data, 55 % of the total variation was found among populations within regions and 40 % occurred within populations (Table 6).

TABLE 6. **Summary of the analysis of molecular variance (AMOVA) for 328 *E. annuus* plants from 29 populations revealed on the basis of RAPD and ISSR data**

Level of variation	[1]df	[2]SS	[3]MS	Est. var	[4]%	[5]Φ stat.
On the basis of RAPD markers						
Among regions	2	203.57	101.78	0.62	8	Φ_{RT} 0.08
Among populations	26	1108.90	42.65	3.46	45	Φ_{PR} 0.49
Within populations	299	1093.68	3.66	3.66	47	Φ_{PT} 0.53
Total	327	2406.15		7.74	100	
On the basis of ISSR markers						
Among regions	2	318.03	159.02	0.73	5	Φ_{RT} 0.06
Among populations	26	2278.43	87.63	7.31	55	Φ_{PR} 0.58
Within populations	299	1574.62	5.26	5.27	40	Φ_{PT} 0.60
Total	327	4171.07		13.31	100	

$p < 0.01$; [1]df – degrees of freedom; [2]SS – sum of squares; [3]MS – mean squares; [4]% – the percentage of the total variance contributed by each component; [5]Φ stat. – Φ statistics; $p < 0.01$ (Wright, 1950)

The coefficient of genetic differentiation between populations (G_{ST}) was 0.58 based on the RAPD marker analysis and 0.64 based on ISSR marker analysis (Table 7).

Trtikova et al. (2011) also identified a high level of genetic differentiation among populations from Switzerland. Nearly half of the total genetic diversity was established among populations ($G_{ST} = 0.46$). However, there was a significant difference among the populations from the lowland and high altitudes. Lowland populations were less differentiated

(G_{ST} = 0.33) than those from high altitudes (G_{ST} = 0.55). A higher differentiation among Lithuanian populations (G_{ST} = 0.58 for RAPD markers; G_{ST} = 0.64 for ISSR markers) was noted in comparison to Swiss lowland populations (Table 7). This difference can possibly be explained by the shorter history of existence of these populations in Lithuania, reduced gene flow among them and a more pronounced founder effect. The pairwise genetic distances between populations (Nei, 1978) established using the RAPD and ISSR markers were rather similar (Table 7). The average genetic distance between populations based on RAPD markers was 0.18 and 0.21 based on ISSR markers. The largest genetic distance according to both types of data was estimated between the Mažeikiai (PN28) and Užutrakis (PN1) populations (0.62 for RAPD and 0.56 for ISSR). A Mantel test indicated weak correlation between the genetic distance and the geographic distance (km) of *E. annuus* populations (RAPD markers: $r = 0.276$, $p < 0.05$; ISSR markers: $r = 0.344$, $p < 0.05$).

TABLE 7. **Among-population diversity parameters established using RAPD and ISSR markers**

Diversity parameter	RAPD	ISSR
G_{ST}	0.58	0.64
N_m	0.36	0.28
GD_{pop}	0.18 ± 0.13	0.21 ± 0.12
$GD_{pop\ max}$	0.62	0.56
$GD_{pop\ min}$	0	0

G_{ST} – mean genetic differentiation between populations; N_m – estimate of gene flow between population; GDpop – mean genetic distance (GD) among populations; GDpop max – maximum GD Mažeikiai (PN28) and Užutrakis (PN1); GDpop min – minimum GD between populations

The expansion of *E. annuus*, as with other invasive species, might be promoted by global warming (prolonged vegetation season, rise of minimal temperatures) and anthropogenic factors, such as changes in agricultural practices in the past few decades, increasing international trade, and the extension of urban areas. *E. annuus* seedlings are able to photosynthesize even at low temperatures in early spring or late autumn. Consequently, under climate warming conditions, plants that regrow after mowing are able to produce dormant seeds. Thus, they can occupy territories where the vegetation period was previously too short for *E. annuus*. This is important in establishing the northern boundary of *E. annuus* spread in some European countries, including Lithuania.

Although precise information concerning the date and type of introduction of *E. annuus* into Lithuania does not exist, it seems likely that it has been distributed in the country as an ornamental plant or as an admixture of the seeds of other ornamental plants. The only place we could expect to find the original or older genotypes would be old cemeteries and Vilnius surroundings. Two opposing assumptions concerning the origin of the initial genotype(s) may be proposed. First, the initial genotype arose or was introduced in the southeastern region and later partially diverged into several clones and spread into the

north and northwest directions. The other possibility is that the initial genotype was similar to those currently found in old cemeteries and some genotypes (including the main clone) diverged from the initial genotype and proceeded to invade natural ecosystems. The wide spread of this species in old Lithuanian cemeteries in modern times supports this point of view. We can find *E. annuus* in old cemeteries even in the regions where it is not observed in natural ecosystems (e.g. PN25, 26). As *E. annuus* is still grown as an ornamental plant in some gardens and cemeteries, uncontrolled and invasive introduction into natural ecosystems is possible from such specific "genetic preserves" when favourable environmental conditions arise, i.e., global warming or a disruption of natural ecosystems by human activity. Edwards et al. (2006) when considering patterns of genetic variation in American and European populations of *E. annuus*, noted that much of the observed variation in RAPD phenotypes in Europe has been locally generated. As mentioned earlier, our work demonstrates the possible genetic relationships among cemetery populations of *E. annuus* composed of several widely spread genotypes, including the main clone.

The apomictic nature of *E. annuus* and other invasive species with restricted outcrossing is favourable for the settlement and maintenance of individual genotypes (Parker et al., 2006). Occasional hybridization events may be favourable for the emergence of new genotypes with high invasive potential (Ellstrand and Schierenbeck, 2000; Noyes, 2006; Lavergne and Molofsky, 2007). Multiple introductions can also cause genotype diversity among and within *E. annuus* populations and other invasive plant populations (Lavergne and Molofsky, 2007). Previously, some authors have noted the importance of somatic mutations as a cause of variation in *E. annuus* (Ellstrand and Rose, 1987). It is becoming recognized that rapid evolution in introduced species may also be driven by punctual changes in genome structure and organization (Prentis, 2008; Lavergne et al., 2010). Molecular marker assays that are used for genomic DNA fingerprinting are able to detect such rearrangements (Kalendar et al., 2000). It has been shown that environmental stress can cause variation in RAPD phenotypes (Čėsnienė et al., 2010) and the pattern of DNA methylation (Rančelis et al., 2012). Chapman et al. (2004) noted that both recombination and mutation contributed to the intrapopulation genotypic diversity of the triploid weed *Hieracium lepidum*. *E. annuus* and some other invasive plants are distributed and make adventive groups on roadsides, near waste disposal areas and along railways. These conditions are extremely genotoxic because mutagenic substances are brought to the soil by the emissions associated with different enterprises and forms of transportation. Therefore, we cannot exclude the possibility that the current pattern of genotypic diversity of *E. annuus* may be caused not only by genotypically distinct founding events (Ward et al., 2006) and occasional sexual recombination (Edwards et al., 2006), but also by mutations induced by environmental stress. Molecular marker assays give us a unique possibility of identifying the genotypes that are spread in many populations. These genotypes possibly retained or acquired during invasion some properties that are most suitable for expansion in a given environment. We hope that common garden experiments and gene expression studies us-

ing the most widespread clone of *E. annuus* will provide additional information on what genetic properties of this particular genotype facilitate intensive spreading and the ability to rapidly adapt to new environments. On the other hand, we must be alert for the appearance of invasive plants such as *E. annuus* in unique natural ecosystems such as the Curonian Spit. This possible hazard to natural vegetation preserves necessitates further observations and management.

3.2. Study of the gradient of genetic diversity of *Erigeron annuus* in the part of invasive European range

A total of 161 ISSR bands were detected in ISSR-PCRs analyses of genomic DNA of 253 individuals. Mean number of bands per individual was 72.4. Mean DNA band size was 781 bp. The number of bands per population differed beween the countries of origin (Table 8).

TABLE 8. **Genetic characteristics of 17 populations of *E. annuus* based on 161 ISSR markers**

Population code	No. of bands	No. of genotypes	No. of unique genotypes	Br [12]	h	I
SWITZERLAND						
LOC	113	11	8	1.512	0.185	0.279
SIG	119	6	3	1.553	0.184	0.280
SAN	117	7	3	1.565	0.187	0.283
ZÜR	115	5	2	1.460	0.155	0.233
Average ± SE	116.0 ± 1.3	7	4	1.523 ± 0.024	0.178 ± 0.008	0.269 ± 0.012
POLAND						
LUA	134	4	0	1.683	0.229	0.349
SZC	79	1	0	1.000	0	0
MRA	116	4	0	1.522	0.174	0.264
SUW	102	4	2	1.416	0.151	0.223
LUB	110	4	0	1.422	0.149	0.224
SWI	88	2	0	1.174	0.059	0.089
Average ± SE	104.8 ± 8.1	3	0	1.370 ± 0.100	0.127 ± 0.034	0.192 ± 0.051
LATVIA						
DAA	82	1	0	1.000	0	0
DAB	82	1	0	1.000	0	0
Average	82	1	0	1.000	0	0
CANADA						
NEW	95	4	1	1.391	0.132	0.199
Average	95	4	1	1.391	0.132	0.199
LITHUANIA						
PAT	76	1	0	1.000	0	0
PRI	90	2	0	1.118	0.037	0.059
SUD	100	5	2	1.318	0.100	0.157
KER	94	2	1	1.360	0.098	0.153
Average ± SE	90.0 ± 5.1	3	1	1.199 ± 0.085	0.059 ± 0.024	0.092 ± 0.038

Br [12] – band richness after rarefaction to 12 individuals per population; h – Nei's gene diversity (expected heterozygosity); I – Shannon's index of diversity

The highest average number of ISSR bands (116 ± 1.3) was revealed in Swiss populations. It ranged from 113 (Locarno) to 119 (Sigirino). Very high differences in the number of detected ISSR bands were revealed among six Polish populations. It ranged from 79 (Szczuczyn) to 134 (Lublin A). The average band number detected in Polish populations was 104.8 ± 8.1. In each of the two monomorphic Latvian populations, 82 bands were revealed. The average number of bands identified in the four studied Lithuanian populations was 90 ± 5.1. One population (New Brunswick) from Canada possessed 95 polymorphic bands.

Various numbers of genotypes were identified in the studied populations (Table 8). Four populations (Szczuczyn, Daugavpils A, Daugavpils B and Patašinė) were monomorphic. The largest number of genotypes were found in Swiss populations. It varied between five (Zürich) and 11 (Locarno) per population. The most numerous clone was found in Daugavpils populations, where all 37 plants were of the same genotype. The highest proportion (16) of unique genotypes also was found in Swiss populations.

Parameters of genetic diversity (Br, h and I) in polymorphic populations varied between 1.118–1.683, 0.037–0.229 and 0.059–0.349, respectively (Table 8).

The mean band richness (Br) was highest in Swiss populations (1.523 ± 0.024) and the lowest in Lithuanian populations (1.199 ± 0.085) (Figure 4).

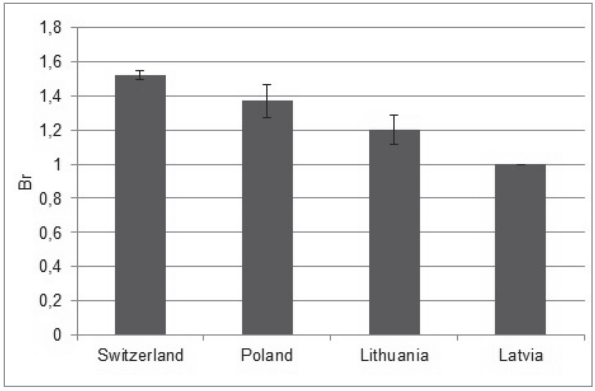


FIGURE 4. **Differences in mean band richness in populations of *Erigeron annuus* from Switzerland, Poland, Lithuania and Latvia**

The highest mean Nei's gene diversity and Shannon's indices were also in Swiss populations (0.178 ± 0.008 and 0.269 ± 0.012, respectively), the lowest – in Lithuanian populations (0.059 ± 0.024 and 0.092 ± 0.038, respectively). The monomorphic Latvian populations had only one allele per locus (Br = 1.0; h = 0; I = 0).

The Mantel test of isolation by distance (IBD) in all studied daisy fleabane populations showed correlation between the genetic and geographic distances (r = 0.261; p = 0.03), when native Canadian and monomorphic Latvian populations were excluded from

calculation. These results indicate that populations within regions are not experiencing high rates of gene flow (Baker and Dyer, 2011). Thus, there is a tendency of decreasing of the genetic diversity towards northern direction: the highest mean values of all genetic diversity parameters were revealed in Swiss populations, the least – in Latvian Daugavpils populations that were monoclonal. Populations of different countries differ significantly in band richness (ANOVA, p = 0.044). Our results show the tendency of decreasing of genetic diversity in south-north direction, which in general correlates with the time of settlement of this species in a corresponding country. For example, in Lithuania, the introduction of *E. annuus* occurred more than two hundred years later than the introduction of the plant to Switzerland. Very limited spreading of this species in Latvia and monoclonal structure of very rare populations found in this country reflects recent founder event. It is known that expansion of invasive species creates a gradient of genetic diversity. Many authors revealed decreasing of genetic diversity towards front of an expansion of invasive species (Austerlitz et al., 1997; Klopfstein et al., 2006; Baker and Dyer, 2011). Baker and Dyer (2011) noted that patterns of spatial genetic structure produced following the expansion of an invasive species into novel habitats reflect demographic processes that have shaped the genetic structure we see today.

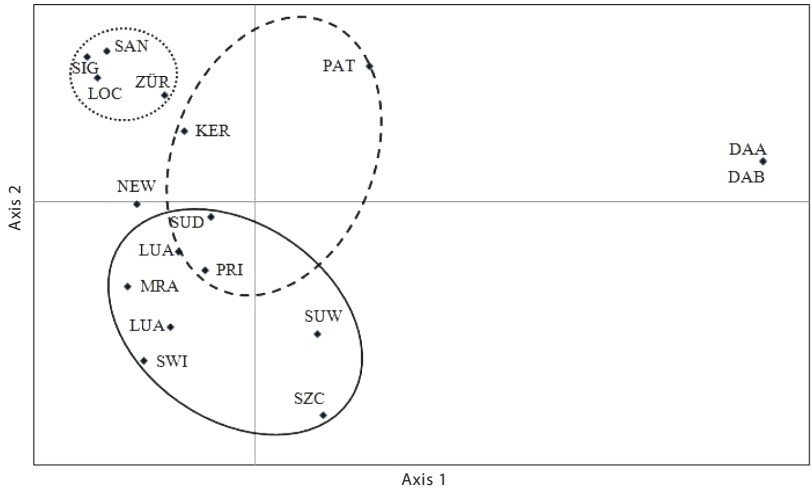


FIGURE 5. **Principal coordinate analysis of the 17 populations of *Erigeron annuus*.** Population names are coded as in Table 2

In our study, we used Bayesian cluster analysis and PCoA to study genetic structure of *E. annuus*. The first three PCoA axes explained 24.34%, 21.70% and 17.53% of the total genetic variation among populations of this species, respectively (Figure 5). PCoA

analysis showed the grouping of populations from Switzerland (LOC, SIG, SAN, ZÜR) and Poland (LUA, SZC, MRA, SUW, LUB, SWI). Two Lithuanian populations (PRI and SUD) were closely related to the Polish populations, while the other two Lithuanian populations (especially KER population) had tendency to group with Swiss populations. Monomorphic populations from Latvia lay separately from all other populations.

In the STRUCTURE analysis ΔK reached its maximum at K = 3 (Figure 6) suggesting three clusters indicated by white, black and grey colour (Figure 7). The distribution of populations among clusters did not show clear geographic pattern, however, there are some regional differences. Most individuals Locarno (LOC), Sigirino (SIG), San Antonio (SAN), Zürich (ZÜR), Lublin A (LUA), Mragowo (MRA), Lublin B (LUB), New Brunswick (NEW), Kernavė (KER) populations were assigned to grey cluster. All individuals of Latvian populations were allocated to white cluster. Most individuals of Prienai (PRI) and Sudervė (SUD) populations were grouped into black cluster. Szczcucyn (SZC), Suwalki (SUW), Swieta Lipka (SWI) and Patašinė (PAT) populations showed a high degree of admixture and were not assigned to any of the three clusters. Single native population New Brunswick (NEW) from Canada was allocated in gray cluster. Populations from Switzerland were genetically similar (all belonged to grey cluster). In contrast, Polish and Lithuanian populations were rather genetically heterogeneous. The monoclonal nature of Latvian populations and grouping into small white cluster implies the founder effect (Barrett et al., 2008).

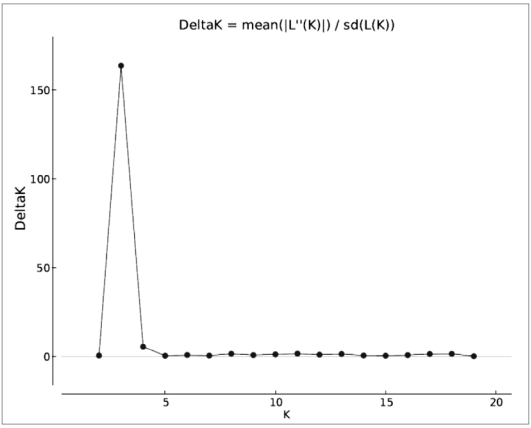


FIGURE 6. **Graph from the admixture model of STRUCTURE analyses of ΔK for *E. annuus* populations.** ΔK showed one peak at K = 3

This result corresponds with the previous considerations of Klopfstein et al. (2006) that populations exhibiting founder effect would be at the front and that more diverse populations would be at the core of the expansion (Baker and Dyer, 2011). In general, the STRUCTURE and PCoA results are congruent. Both analyses revealed relatedness

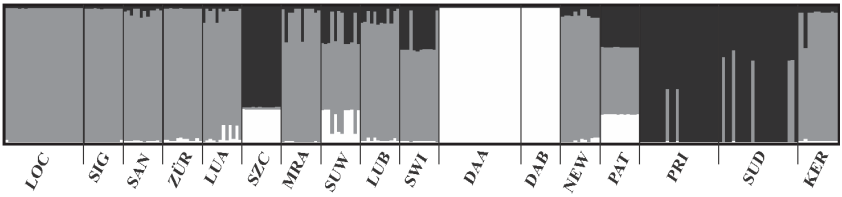


FIGURE 7. **Visual output of the Bayesian analysis of seventeen populations of *Erigeron annuus* using STRUCTURE software for K = 3.** Populations from Switzerland, Poland, Lithuania, Latvia and Canada indicated as in Table 2. Each individual is represented by thin vertical bar. Populations are separated by black lines

among the populations from Switzerland and Poland, which implies that related genotypes are spread in both countries. On the other hand, the analyses indicate some divergence of these populations and higher admixture in the populations from Poland. An exception of this is the SZC population that is genetically very specific and is similar to PRI and SUD populations from Lithuania. The different level of clonality revealed in populations from different regions could be explained by mixed reproductive strategy of species. Edwards et al. (2006), using RAPD markers, showed that sexual reproduction exists in *E. annuus* and is sufficient to maintain a high genetic variation in some populations. These authors also assumed that some genotypes possibly have a stronger tendency towards agamospermy than others. The longer invasion history implies higher probability of genetic recombination and origin of new clones.

3.3. Comparison of genetic, genotypic, diversity and allelopathic potential of *Erigeron annuus* populations from stable and disturbed habitats

The interactions between non-native plants and new habitats and the factors driving this process are of primary importance in invasion biology (Dietz and Edwards, 2006; Riis et al., 2010; Erfmeier et al., 2011; Pahl et al., 2013). Relatively little is known about the dynamics of genotypic diversity of invasive plants associated with habitat changes during the invasion process. Alien plant species may experience fluctuations at the population level, and these variations may cause major and complex genomic modifications (Novak and Mack, 1993; Bossdorf et al., 2005; Prentis et al., 2008). Pyšek et al. (1999) proposed that invasion into more closed communities may be associated with selection for increased competitive ability, and certain traits, such as clonal propagation, may be advantageous. To describe the invasion phase more precisely, Dietz and Edwards (2006) suggested dividing it into primary and secondary phases. This extended model of plant invasion proposes that a life-history strategy of a species is likely to change during the invasion process (Erfmeier et al., 2011). According to Dietz and Edwards (2006), certain physiological traits, such as the ability to produce allochemicals, may change at different

stages of invasion or among contrasting habitats (Dietz and Edwards, 2006). Trtikova et al. (2011) found a significant decline in genotypic diversity within invasive populations of daisy fleabane (*Erigeron annuus*) with increasing altitude. Highland populations of this weed in the Alps were more differentiated than lowland populations; however, the observed pattern of genetic variation did not show an adaptive character. However, Erfmeier et al. (2011) observed a change in life history strategy during a secondary invasion of *Acer negundo*, which indicated local adaptation. In our study, we assessed the impact of habitat and its stability on the genetic and genotypic variation of *E. annuus* populations. This species belongs to the syntaxa of the *Dauco-Melilotion* alliance, which includes progressively spreading ruderal communities. Most of these communities are located in urbanized territories. This species usually colonizes disturbed and potentially polluted habitats, such as along roadsides, landfills, vacant plots, waste disposals, wastelands, building sites and other sites without a humus layer (Motiekaitytė, 2001, 2002). However, the primary natural habitats of these communities are established in river valleys (Motiekaitytė, 2001). *E. annuus* penetrates into stable semi-natural communities, such as dry meadows and pastures. Studies have indicated that this species produces allelochemicals (Park et al., 2011; Scharfy et al., 2011). Therefore, daisy fleabane is an excellent species for studying the impact of habitat on allelopathy and genotypic diversity because it occurs in disturbed and stable habitats occupied during the invasion process. Our aim was to compare the population genetic and genotypic diversity of *E. annuus* collected from disturbed and stable habitats and assess the allelopathic properties of populations from different habitats.

3.3.1. Comparison of genetic and genotypic diversity

Plant specimens were collected from 37 populations. Using ISSR analysis of genomic DNA from 684 plants, we detected 100 polymorphic loci. Based on these loci, we found that 23 of 37 populations had at least two genotypes. Genetic diversity of polymorphic populations (Hj, PLP (5 %) and Br) varied between 0.017 (MIN) and 0.326 (KAR) (mean ± SE: 0.163 ± 0.022), 0.21 (MIN) – 0.80 (KAR) (0.59 ± 0.03) and 1.105 (MIN) – 1.748 (KAR) (1.504 ± 0.040) per population, respectively (Table 8). The highest values of genetic diversity parameters were estimated in KAR population (Hj= 0.326; PLP (5 %) = 0.80; Br = 1.748), the lowest – in MIN population (Hj = 0.017; PLP (5 %) 0.21; Br = 1.105) (Table 9).

Study of genotypic diversity of populations revealed that the number of genotypes per population ranged from 1 to 13 (CEK). 107 genotypes (clones) were identified in total. 53.3 % of these genotypes occurred only once. 47.6% of populations from stable habitats were monomorphic (10 of 21), while only 25 % of populations from disturbed habitats were monomorphic (4 of 16). The number of genotypes per population was higher in stable populations (3.1 ± 0.7) than in disturbed populations (2.6 ± 0.3). However, this difference was not statistically significant (U = 153.5; p = 0.66). One genotype was found

TABLE 9. **Genetic and genotypic diversity parameters at 100 ISSR loci for *E. annuus* populations from stable and disturbed habitats**

Population	Population code	G	i (G/N)	E	Hj	Br [12]	PLP 5 % [12]
Populations from stable habitats							
Naujasis Janavas	NJA	1	0.083	1	0	1	0
Marijampolė	MRA	2	0.167	0.973	0.316	1.600	0.60
Bezdonys	BEZ	2	0.167	0.590	0.078	1.470	0.47
Gelgaudiškis	GEL	3	0.250	0.558	0.183	1.590	0.59
Vilnius A	VLNA	1	0.083	1	0	1	0.00
Kena	KEN	6	0.500	0.750	0.231	1.680	0.68
Tarprubežiai	TAR	1	0.077	1	0	1	0
Senoji Varėna	SVA	1	0.067	1	0	1	0
Druskininkai	DRU	1	0.083	1	0	1	0
Patašinė	PAT	1	0.042	1	0	1	0
Prienai	PRI	5	0.208	0.490	0.046	1.205	0.27
Trakai	TRA	8	0.333	0.244	0.179	1.612	0.69
Čekoniškės	CEK	13	0.542	0.477	0.286	1.700	0.74
Dūkštos	DUK	1	0.042	1	0	1	0
Kernavė A	KERA	2	0.167	0.590	0.091	1.550	0.55
Sudervė	SUD	6	0.250	0.290	0.162	1.544	0.66
Aukštadvaris	AUK	1	0.042	1	0	1	0
Nemėžis	NEM	1	0.083	1	0	1	0
Kalistiškės	KLK	1	0.083	1	0	1	0
Ozo g., Vilnius	VLNF	5	0.357	0.603	0.320	1.721	0.74
Jurbarkas A	JURA	3	0.250	0.774	0.256	1.480	0.48
	Mean ± SE	3.095 ± 0.679	0.185 ± 0.032	0.576 ± 0.063	0.195 ± 0.029	1.559 ± 0.043	0.59 ± 0.04
Populations from disturbed habitats							
Pagiriai A	PGA	1	0.083	1	0	1	0
Savanorių pr., Vilnius	VLNB	5	0.167	0.265	0.124	1.547	0.77
Kariotiškių sąv.	KAR	4	0.167	0.713	0.326	1.748	0.80
Pagirių šiltnamiai	PGS	4	0.167	0.324	0.100	1.473	0.70
Gariūnai	GAR	4	0.333	0.429	0.218	1.740	0.74
Oslo g., Vilnius	VLNC	3	0.125	0.395	0.082	1.436	0.72
Mickūnų sąv.	MIC	2	0.083	0.543	0.034	1.205	0.41
Polocko g., Vilnius	BEL	2	0.083	0.543	0.035	1.210	0.42
Kalvarijų g., Vilnius	VLND	2	0.167	0.590	0.104	1.630	0.63
Grigiškės	GRI	4	0.167	0.522	0.262	1.655	0.76
Užukampis	UZK	2	0.083	0.543	0.037	1.220	0.44
Panerių šiltnamiai	VLNE	1	0.042	1	0	1	0
Minsko plentas	MIN	2	0.083	0.543	0.017	1.105	0.21
Fabijoniškių sąv.	VLNG	1	0.042	1	0	1	0
Jurbarkas B	JURB	4	0.333	0.693	0.251	1.480	0.48
Geležinkelio g., Vilnius	VLNH	1	0.042	1	0	1	0
	Mean ± SE	2.625 ± 0.340	0.135 ± 0.023	0.509 ± 0.039	0.133 ± 0.030	1.454 ± 0.064	0.59 ± 0.05

G – number of genotypes; i (G/N) – genotypic diversity; E - evenness index; Hj - expected heterozygosity; Br [12] - band richness with sample size rarefied to twelve individuals; PLP 5% [12] – proportion of polymorphic bands with sample size rarefied to twelve individuals

in 9 populations, eight of which were located in disturbed habitats. This possibly indicates the impact of selection on the divergence of populations from different habitats. Proportion of distinguishable genotypes (i) in populations varied from 0.042 to 0.542 (mean ± SE: 0.163 ± 0.021) (Table 9). Higher mean genotypic diversity was revealed in populations from semi-natural habitats (0.185 ± 0.032 versus 0.135±0.023). Evenness index ranged from 0.244 to 1 (mean ± SE: 0.541 ± 0.036) and also was higher in populations from stable habitats (0.576 ± 0.063 versus 0.509 ± 0.039). However, both these parameters did not differ significantly between two groups of populations (Genotypic diversity: U = 145, p = 0.48; evenness index: U = 113, p = 0.095).

3.3.2. Population genetic structure

Hierarchical AMOVA of all 37 populations showed that genetic differentiation was high (Phi_{PT} = 0.606), with genetic variation partitioned to 11 % among habitats (Phi_{RT} = 0.108) and 50 % among populations within habitats (Table 10).

TABLE 10. **AMOVA results for 37 populations of *Erigeron annuus* from semi-natural and anthropogenic habitats**

Level of variation	Df	Sum of squares	Variance components	% total of variation	p
Among habitats	1	587.896	1.336	11	≤ 0.001
Among populations within habitats	35	4123.272	6.147	50	≤ 0.001
Within populations	647	3150.661	4.870	39	≤ 0.001
Populations from disturbed habitats					
Among populations	20	3268.720	9.781	66	≤ 0.001
Within populations	321	1624.619	5.061	34	
Populations from stable habitats					
Among populations	15	854.552	2.458	34	≤ 0.001
Within populations	326	1526.042	4.681	66	

39 % of the total genetic variance resided within populations. Genetic variances at each hierarchical level were significant (p < 0.001). The AFLP-SURV v. 1.0 analysis revealed a similar value of impact of habitat (Fst = 0.126) on genetic variance (data not shown). Separate analyses of populations from different habitats revealed that populations growing in stable habitats were more differentiated (Fst = 0.647) than populations located in disrupted habitats (Fst = 0.344). Similar results were obtained using standardized set of populations (Fst = 0.586 and Fst = 0.338, respectively). This difference in Fst values among populations from different habitats was statistically significant (U = 4594, p = 0.0001). It may reflect the impact of selection on early germination and seedling establishment, lower propagule pressure in stable habitats and founder effect (Van der Toorn and Pons,

1988; Trtikova et al., 2011). However, if monomorphic populations were excluded from consideration, genetic diversity parameters (Hj, Br, PLP) would not differ significantly between two groups of populations. So, clonality strongly influenced the differentiation among populations (Ivey and Richards., 2001). Trtikova et al. (2011) studied the impact of altitude on the genetic variation pattern of *E. annuus* in the Alps and revealed significant decrease of genotypic diversity with increasing altitude and higher genetic differentiation among highland populations in comparison with lowland populations. Authors concluded that selection of particular genotypes at the altitudinal limit was weak and differences in the distribution of genetic diversity are influenced by occasional sexual reproduction, dispersal and extinction.

The genetic structure and distribution of genetic diversity among all populations were also analyzed using Bayesian clustering method implemented in Structure Harvester programme. After investigation of K in range from K = 1 to K = 20, ΔK showed a large peak at K = 2 (ΔK = 1005.3). Structure analysis grouped all populations in two uneven clusters: smaller red and larger green (Figure 8).

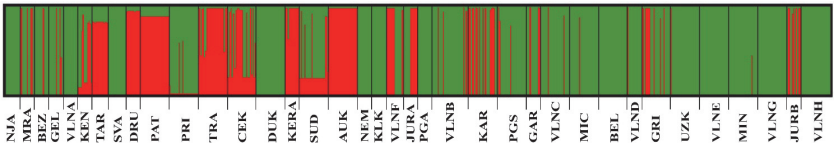


FIGURE 8. **Visual output of the Bayesian analysis of studied populations of *Erigeron annuus* using STRUCTURE software for K = 2.** Populations indicated as in Table 3. Each individual is represented by thin vertical bar. Populations are separated by black lines. NJA-JURA – populations from stable habitats, PGA-VLNH – populations from disturbed habitats

Despite the fact that most populations are fairly admixed, some differences among populations from different habitats are evident. Populations growing in stable habitats showed the tendency to group into the red cluster (KEN, TAR, DRU, PAT, TRA, CEK, KERA, SUD, AUK, VLNF, JURA). Population AUK was genetically homogeneous and was assigned to red cluster. Six of 21 populations (NJA, VLNA, SVA, DUK, NEM, KLK) of stable habitat were also homogeneous but grouped into second (green) cluster. All except three (KAR, JURB and JURB) populations from disturbed habitats were assigned to the green cluster, and five (PGA, BEL, VLNE, VLNG and VLNH) were genetically homogeneous. Eight populations (VLNB, PGS, GAR, VLNC, MIC, VLND, UZK and MIN) showed little admixture. Only three populations (KAR, GRI and JURB) of disturbed habitats were fairly admixed.

Mantel test showed correlation between Nei's genetic distances (Nei 1972) and geographic distances in the group of populations from disturbed habitats (r = 0.532; p = 0.02). However, the IBD pattern was not observed in the populations from stable habitats

(r = - 0.207; p > 0.45), although the IBD was observed for the total number of populations (r = 0.228, p = 0.027). Such pattern of genetic diversity independent of spatial distance in stable habitat populations can result from clonality, strong selection, low gene flow among populations and founder effect (Solé et al., 2004). This situation for stable habitat populations is more reliable, because the spreading into semi-natural habitats is associated with increased competition from native flora and lower available resources (Stratton, 1992; Dietz and Edwards, 2006) and possibly local adaptation (Erfmeier et al., 2011). For example, field experiments on establishment of two *Plantago* species among grasses showed increased selection for early germination (Van der Toorn and Pons, 1988). Statistically significant higher genetic differentiation among populations in stable habitats also supports the impact of founder effect and selection.

3.3.3. Comparison of allelopathic potential

When evaluating allelopathic potential of separate species or populations, it is important to assess the impact of chemical compounds synthesized by invasive species on the growth, development and distribution of other plants. One of the possible approaches is the calculation of juglone index, which is used as an indicator of allelopathic potential (Csiszár, 2009). The allelopathic potential of extracts prepared from plants collected in 10 populations located in stable habitats and 9 populations from disturbed habitats was assessed. The impact of *E. annuus* extracts of different concentrations (1 g/100 ml and 5 g/100 ml) on the *S. alba* shoot and root length is shown in Figure 9. The allelopatic potential of *E. annuus* extracts was assessed in comparison with allelopatic potential of juglone and H_2O (control). The effect of inhibition of disturbed habitats' plant extracts is more expressed on the shoot growth and in the case of 5 g concentration exceeded the effect of 100 mM juglone. The extracts (1 g and 5 g) from plants of disturbed habitat populations had stronger inhibitory effect than extracts from plants of populations and this difference was statistically significant (U = 16, p = 0. 017; U = 0; p = 0.002). After treatment *S. alba* seeds with 5 g extracts from plants of KAR, BEL, VLNB, UZK, MIC, GRI populations, shoot growth was inhibited stronger in comparison with inhibition of 1 mM juglone. Inhibition on the *S. alba* root length of high concentrations extracts of KAR and UZK populations was similar in comparison with 1 mM juglone. Considering that allelopathy is one of invasion mechanisms, it is interesting to notice that inhibition effect was stronger in the populations from disturbed habitats than from stable ones.

The same tendency is seen when juglone indexes of populations originated from semi-natural and anthropogenic habitats are compared (Figure 10). These differences in allelopathic potential of these two group of populations are significant (1 g extracts: U = 88, p < 0.05; 5 g extracts U = 90, p < 0.05).

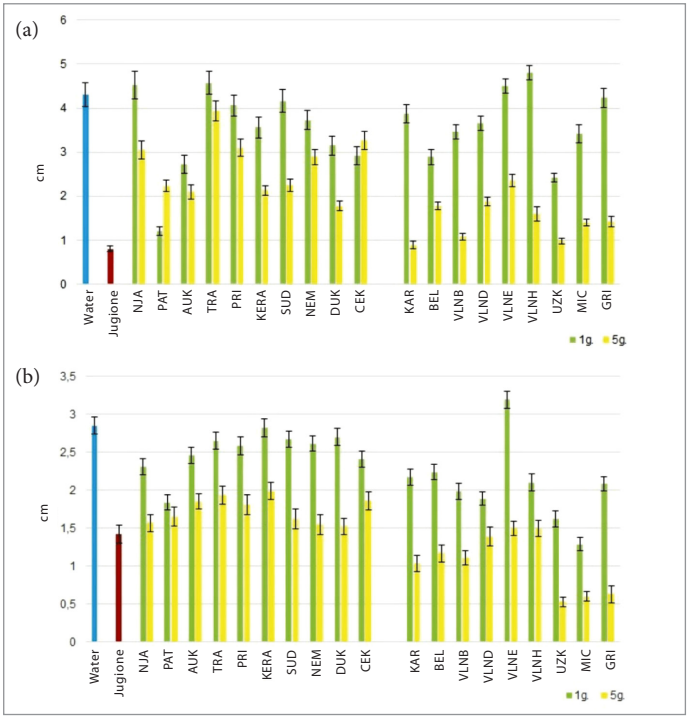


FIGURE 9. **Mean length of *S. alba* roots and shoots after treatment with different concentration extracts of *E. annuus* from different habitats (semi-natural and anthropogenic)** a) mean length of *S. alba* roots (cm); b) mean length of *S. alba* shoots (cm). NJA-CEK – populations from semi-natural habitats; KAR-GRI – populations from anthropogenic habitats

FIGURE 10. **Comparison of juglone index of *E. annuus* populations from different habitats.** NJA-CEK – populations from semi-natural habitats; KAR-GRI – populations from anthropogenic habitats

3.4. Geochemical characterization of habitats

Samples of top soil were collected in the population growing sites. Concentrations of 13 elements were established (data not shown). A dendrogram representing the 19 site groups according to the geochemical composition of the 13 elements showed a pattern in which all of the stable habitat sites except Prienai (PRI) and Aukštadvaris (AUK) were grouped separately from the disturbed habitat sites and occurred on the same branch of the dendrogram (Figure 11). All sites of anthropogenic habitats clustered on the lower part of the dendrogram. Significant differences between habitats according Mann-Whitney test were found in concentrations of the elements Zn, Pb, Cu, Co and Sn.

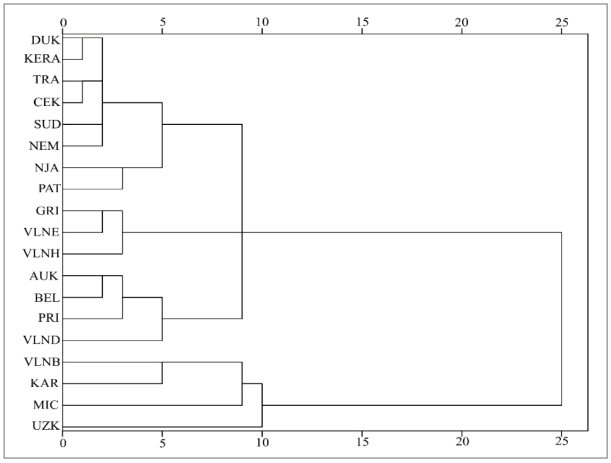


FIGURE 11.
Dendrogram of semi-natural and anthropogenic *E. annuus* populations based on geochemical analysis

In conclusion, we revealed divergence in the genetic diversity and allelopathic properties of populations of *E. annuus* from disturbed and stable habitats, and these differences could be associated with different invasion phases of this species or caused by habitat characteristics. The genotypic diversity analysis of this apomictic species showed higher fraction of monoclonal populations in stable habitats. Our results suggest that selection may have an impact on the distribution of genetic diversity among populations from different habitats.

3.5. Analysis of morphological and phenological variation of *E. annuus* in the common garden experiment

Analysis of morphological characters. To reveal an impact of phenotypic plasticity and local adaptation on the phenotype of *E. annuus*, a common garden experiment was carried out. Eleven populations from different regions of invasive range (Switzerland,

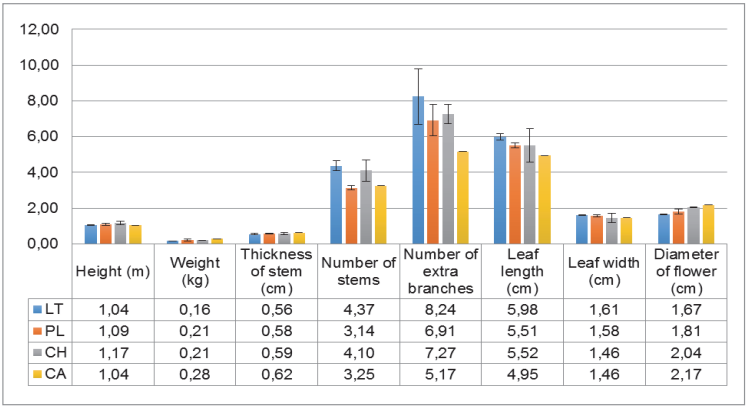
Poland, Lithuania) and one native population were grown together for two years in the experimental plot of Vilnius university Botanical garden. Eight morphological characters were measured. The histograms show (Figure 12) that the mean height (m) of *E. annuus* plants from the studied populations varied from 0.98 ± 0.30 (mean ± SE) to 1.33 ± 0.31. The tallest *E. annuus* plants were in Sigirino (CH1) population, the smallest – in Grigiškės (LT5) population.

The highest mean weight of *E. annuus* plants was in Lublin population (PL3), the lowest – in Szczuczyn (PL1) population. The mean values of this parameter (kg) in these populations were 0.34 ± 0.33 and 0.13 ± 0.05, respectively. The thickness (cm) of the stem at 40 cm height was the largest in Sigirino (CH1) population (0.69±0.10), the lowest (0.49±0.00) – in Zürich (CH3) population. The largest number of stems was detected in Narbutas (LT2) population (5.08 ± 0.31), the smallest – in Sigirino (CH1) population (2.97 ± 1.15). Also the number of extra branches was estimated. Plants from Grigiškės (LT5) population had the highest number of extra branches. It exceeded the mean value for all populations (5.17 ± 1.41) almost three times. The longest and the widest leaves were detected for the plants of Sigirino (CH1) population, the shortest and narrowest – in Zürich (CH3) population. The largest flowers (2.17 ± 0.05) were in New Brunswick (CA) population, the smallest – in Mrągowo (PL2) population.

Analysis of results indicates high phenotypic plasticity of *E. annuus*, which perhaps can play a very important role during the establishment of species in new habitats (Trtikova et al., 2009; Trtikova et al., 2011). However, several populations were found to statistically significantly differ in green weight (LT5 ir PL1), diameter of flower (LT5 and PL1, LT5 and CH1, LT5 and CH2, CH2 and PL1, CA and LT5, CA and PL1, CA and PL2, CA and CH1, CA and CH2), number of stems (LT2 and LT1, LT2 and LT5, LT2 and PL1, LT2 and PL2, LT2 and PL3, LT2 and CH1, LT2 and CA), number of extra branches (LT1 and LT5, LT2 and LT5, LT4 and LT5, LT5 and PL1, LT5 and PL3, LT5 and CH2, LT5 and CA), length of leaves (CH1 and CH3) and width of leaves (LT1 ir CH3). We suppose that this divergence can be the result of the local adaptation. Very interesting interpopulation differences in flower diameter, number of stems and extra branches were revealed. CA population from native region differs from many populations of Lithuania, Poland, Switzerland that represent invasive region (Figure 12). Two Lithuanian populations also showed some peculiarities. A few statistically significant differences in flower diameter and number of extra branches were found between Grigiškės (LT5) population and some other populations. Also statistically significant differences in the number of extra branches were estimated between this population and some populations of Poland, Switzerland, Canada and Lithuania. Narbuto str. population (LT2) had statistically significant higher number of stems in comparison with some populations from Lithuania, Poland, Switzerland and Canada.

Comparison of populations according to the countries is shown in Figure 13.

FIGURE 12. **Variation of morphological traits in the populations of *E. annuus*.** LT1 – Pagiriai, LT2 – Narbuto street, Vilnius, LT3 – Marijampolė, LT4 – Ozo street, Vilnius, LT5 – Grigiškės, PL1 – Szczuczyn, PL2 – Mrągowo, PL3 – Lublin, CH1 – Sigirino, CH2 ± Locarno, CH3 – Zürich, CA – New Brunswick



	Height (m)	Weight (kg)	Thickness of stem (cm)	Number of stems	Number of extra branches	Leaf length (cm)	Leaf width (cm)	Diameter of flower (cm)
LT	1,04	0,16	0,56	4,37	8,24	5,98	1,61	1,67
PL	1,09	0,21	0,58	3,14	6,91	5,51	1,58	1,81
CH	1,17	0,21	0,59	4,10	7,27	5,52	1,46	2,04
CA	1,04	0,28	0,62	3,25	5,17	4,95	1,46	2,17

FIGURE 13. **The comparison of variation in morphological characters of *E. annuus* in the populations from different countries**

Histogram shows different variation of morphological characters in populations from different countries. A few statistically significant differences between countries were detected in the number of stems (LT and PL; LT and CA), number of branches (PL and CA; CH and CA), length and width of leaves (LT and CA; PL and CA) and diameter of flowers (LT and CH; LT and CA; PL and CA).

In conclusion, our analysis of morphological characters indicates the peculiarities of CA (New Brunswick) population from native region. However, this result should be interpreted with caution because only one population from this region was studied.

Analysis of phenological traits. Histogram shows mean number of days till the beginning of phenological stages of *E. annuus* plants from Lithuanian, Polish, Swiss and Canadian populations, which were grown in the common garden (Figure 14). Three phenological phases were assessed: I – stem height ≥ 3 cm, II - buds formation, III – flowering. The beginning of a phenological phase was considered when half of plants have reached it. The largest number of days till the beginning of all phenological phases was fixed in Canadian population (native region). For this population the number of days to achieve I, II, III phases was 55, 70, and 80 respectively. It took fewer days for invasive range populations to reach these phases. The longest time (51 days) till phase I was detected for Lithuanian populations. The biggest mean number of days (62) till phase II was revealed for populations from Poland. The biggest mean number of days (72) till phase III was detected also in Poland populations. However, the differences between the populations are not statistically significant.

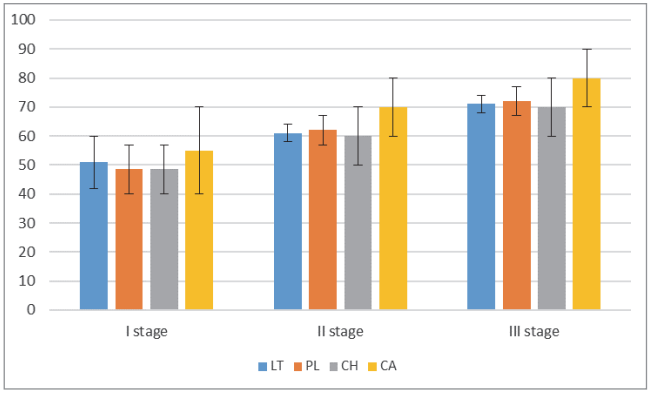


FIGURE 14. **Mean number of days till the beginning of phenological stages of *E. annuus*.** LT – populations from Lithuania. PL – populations from Poland. CH – populations from Switzerland. CA – populations from Canada.

4. CONCLUSIONS

1. Lithuanian populations of *E. annuus* are characterized by a low genetic diversity of RAPD and ISSR loci and a high genetic differentiation possibly caused by the founder effect.
2. There are two genetically different types of *E. annuus* populations in Lithuania. The first population type is represented by a widely spread main clone and some related populations. The second population type is represented by polymorphic populations. The absence of genetic polymorphism in half of populations and the small number of genotypes in the polymorphic populations indicates predominating asexual reproduction of *E. annuus* in Lithuania.
3. Our results indicate the existence of *E. annuus* genetic diversity gradient in the part of invasive European range. There is a tendency of decreasing of genetic diversity in *E. annuus* populations in transect from Switzerland to Latvia.
4. There is statistically significant genetic differentiation ($Phi_{RT} = 0.108$, $p < 0.001$; $F_{ST} = 0.126$) between *E. annuus* populations from stable and disturbed habitats. Populations located in disturbed habitats are less differentiated ($F_{ST} = 0.338$) than those from stable habitats ($F_{ST} = 0.586$) and these differences are statistically significant ($U = 4594$, $p = 0.0001$). The plants from disturbed habitat populations show higher allelopathic potential than those from stable habitats.
5. Although *E. annuus* shows high phenotypic plasticity in its morphology and phenology, which helps to survive and spread in new habitats, genetically determined local adaptation occurs in some populations.

5. PUBLICATIONS AND PRESENTATIONS

Publications in journal with a citation index of the Istitute for Scientific Information database "ISI Web of Science":

1. Patamsytė J, Rančelis V, Čėsnienė T, Kleizaitė V, **Tunaitienė V**, Naugžemys D, Vaitkūnienė V, Žvingila D. Clonal structure and reduced diversity of the invasive alien plant *Erigeron annuus* in Lithuania. Central European Journal of Biology. 2013;8(9):898–911.
2. Patamsytė J, Čėsnienė T, Naugžemys D, Kleizaitė V, **Tunaitienė V**, Vaitkūnienė V, Rančelis V, Mikaliūnaitė R, Žvingila D. Different habitats show similar genetic structure of *Bunias orientalis* L. (*Brassicaceae*) in Lithuania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2013;41(2):396–403.

Publications in other peer-reviewed journals:

1. **Tunaitienė V**, Naugžemys D, Patamsytė J, Žvingila D. Gradient of genetic diversity of *Erigeron annuus* in the part of invasive European range. Botanica Lithuanica. 2015;21(2):81–88.
2. **Tunaitienė V**, Patamsytė J, Čėsnienė T, Kleizaitė V, Naugžemys D, Rančelis V, Žvingila D. Genotypic diversity and clonal stucture of *Erigeron annuus* (*Asteraceae*) in Lithuania. Julius Kuhn Archiv. 2014;443: 200–207.
3. **Tunaitienė V**, Patamsytė J, Čėsnienė T, Kleizaitė V, Naugžemys D, Rančelis V, Žvingila D. Comparison of genetic diversity in two alien plant spacies. Julius Kuhn Archiv. 2012;434 (2):679–686.

Oral presentation

Comparison of genetic diversity in two alien plant species (*Erigeron annuus* and *Bunias orientalis*). Biofuture. Perspectives of nature and life sciences 2012. 2012 12 05. Vilnius. Lithuania.

Poster presentations

1. Genetic diversity of some Polish *Erigeron annuus* populations. **Tunaitienė V**, Patamsytė J, Žvingila D. Science at Faculty of Natural Sciences. the 8th scientific conference. Vilnius. Lithuania. 2014 10 03.
2. Assessment of the allelopathic potential of invasive species daisy fleabane. **Tunaitienė V**, Krivičiūtė K, Patamsytė J, Čėsnienė T, Kleizaitė V, Žvingila D. 8th International Scientific Conference the Vital Nature Sign. Kaunas. Lithuania. 2014 10 24-26.
3. Molecular approach to study clones of invasive apomictic plant species *Erigeron annuus* in Lithuania. **Tunaitienė V**, Patamsytė J, Čėsnienė T, Kleizaitė V, Naugžemys D, Vaitkūnienė V, Rančelis V, Žvingila D. Современное состояние, тенденции развития. рациональное использование и сохранение биологического разнообразия растительного мира. Minsk. Belarus. 2014 09 23-26.
4. Genotypic diversity and clonal structure of *Erigeron annuus* (*Asteraceae*) in Lithuania. **Tunaitienė V**, Patamsytė J, Čėsnienė T, Kleizaitė V, Naugžemys D, Vaitkūnienė V, Rančelis V, Žvingila D. 26th German Conference on Weed Biology and Weed Control. Braunschweig. Germany. 2014 03 11-13.

5. Comparison of geographically remote populations of invasive plant species *Erigeron annuus*. **Tunaitienė V**, Patamsytė J, Česnienė T, Kleizaitė V, Naugžemys D, Vaitkūnienė V, Rančelis V, Žvingila D. 5th Baltic Genetics Congress. Kaunas. Lithuania. 2012 10 19-22.
6. The impact of reproduction strategy of DNA polymorphism in invasive species populations. Tunaitienė V. Science at Faculty of Natural Sciences. the 7th scientific conference. Vilnius. Lithuania. 2012 10 05.
7. Comparison of genetic diversity in two alien plant species. **Tunaitienė V**, Patamsytė J, Česnienė T, Kleizaitė V, Naugžemys D, Vaitkūnienė V, Rančelis V, Žvingila D. 25th German Conference on Weed Biology and Weed Control. Braunschweig. Germany. 2012 03 13-15.

6. SUMMARY IN LITHUANIAN

Įvadas

Invazinės rūšys vertinamos kaip svarbi globalių pokyčių, vykstančių mūsų planetoje. dalis (Bleeker ir kt., 2007). Europos Parlamento ir Tarybos reglamente (ES) Nr. 1143/2014 nurodoma, jog invazinės rūšys yra svetimos rūšys, kurių introdukcija arba plitimas, kaip nustatyta, kelia grėsmę arba daro neigiamą poveikį biologinei įvairovei ir atitinkamoms ekosistemų funkcijoms. Dėl išaugusios migracijos ir besiplečiančios tarptautinės prekybos biologinės invazijos spartėja ir gausėja visame pasaulyje (Mack, 2000; Ward ir kt., 2008). Pavyzdžiui, Europoje užregistruota daugiau nei 12 tūkst. svetimkraščių augalų rūšių (Hulme ir kt., 2010). Invazijų gausėjimas Europoje vyksta beveik eksponentiškai. Per paskutiniuosius 40 metų jų skaičius padidėjo 76 % (Butchart ir kt., 2010). Invazinių augalų tyrimas aktualus ne tik dėl jų sukeliamų didelių ekologinių, bet ir dėl ekonominių bei sveikatos problemų. Tokių rūšių gausėjimas ir jų nulemiami ekonominiai nuostoliai kelia susirūpinimą visame pasaulyje. Pasaulio mastu biologinių invazijų daroma žala vidutiniškai sudaro apie 300 mlrd. JAV dolerių per metus (Luque ir kt., 2014). Vienas aktualiausių biologinių invazijų tyrimų klausimų, kodėl vienos rūšys tampa invazinėmis, o kitos blogai prisitaiko naujoje aplinkoje ir išnyksta? Kadangi rūšies virtimas invazine yra ilgas procesas, tai mūsų dienomis vykstančių procesų tikrosios pasekmės ir mąstai išryškės tik po keleto dešimtmečių. Catford ir kt. (2009) nurodė, kad iš 29 invazijas aiškinančių hipotezių. 26 yra ekologinės. Vis dėlto aiškėja, kad vien ekologiniai požymiai nepaaiškina, kodėl vienos rūšys tampa invazinėmis, o kitos – ne. Atsižvelgiant į invazijos proceso daugiastadijiškumą, manoma. kad svetimkraštės rūšies virtimas invazine neretai priklauso ir nuo evoliucinių genomo pokyčių, kuriuos ji patiria delsimo fazėje (Clements ir Ditommaso, 2010). Įsikuriant rūšiai naujoje vietoje įvyksta ir genetiniai pokyčiai, kuriuos lemia genų dreifas, mutacijos, hibridizacija su vietinėmis rūšimis ir kt. priežastys (Bossdorf ir kt., 2005; Ainouche ir kt., 2009; Nagy ir Korpelainen, 2015). Šie genetiniai pokyčiai gali nulemti įvairius rūšies biologijos pokyčius, tarp jų – invazyvumo padidėjimą (Ellstrand ir Schierenbeck, 2000; Ellstrand,

2009). Svetimkraščių augalų rūšių inventorizacija rodo, kad Lietuvoje jų yra daugiau nei 500, dažniausiai tai – žoliniai augalai (Gudžinskas, 1994, 1995, 1997, 1999). Viena iš šiuo metu sparčiausiai Lietuvoje plintančių svetimkraščių rūšių – vienmetė šiušelė (*Erigeron annuus*) (L.) Pers yra šio disertacinio darbo tyrimo objektas. Tai didelio invazyvumo rūšis, kilusi iš Šiaurės Amerikos rytinės pakrantės (Stratton, 1992). XVII amžiuje kaip dekoratyvinis augalas pateko į Europos botanikos sodus (Rothmaler, 1995). Nors vienmetė šiušelė paplito ir kituose žemynuose, tačiau Europoje ji ypač gausi ir per pastaruosius 30 metų jos plitimas labai spartėja (Frey, 2003). Rūšis triploidinė (3n=27) apomiktinė, retkarčiais dauginasi lytiškai (Edwards ir kt., 2006).

Manoma, kad į Lietuvą vienmetė šiušelė (dar vadinama šiauriniu šemeniu) pateko iš Vakarų Europos XIX amžiaus pabaigoje kaip dekoratyvus augalas. Jis buvo pradėtas sodinti kapinėse, prie sodybų, vėliau ėmė plisti ir į natūralias ekosistemas. Dažniausiai šios rūšies augalai įsikuria pakelėse, apleistuose laukuose, prie upių, kaip piktžolės plinta miestuose. Nors tyrimų, iliustruojančių *E. annuus* poveikį aplinkai, beveik nėra. nekyla abejonių, jog gebėjimas konkuruoti su vietinėmis rūšimis, toleruoti šienavimą, subrandinti didžiulius kiekius sėklų bei sintetinti alelopatinius junginius gali lemti šios rūšies augalų plitimą (Stratton, 1992; Trtikova, 2009; Nazaruk, 2010).

Jau 1951 metais paskelbtame M. Natkevičaitės straipsnyje "Lietuvos TSR adventyvinė flora" pabrėžiama, koks svarbus yra svetimkraščių ("užneštinių") augalų pažinimas. Ir nors invazijų problema Lietuvoje darosi vis aktualesnė ir yra paskelbtas Lietuvos Respublikos Aplinkos ministerijos įsakymas "Dėl introdukcijos, reintrodukcijos ir perkėlimo tvarkos, invazinių rūšių organizmų kontrolės ir naikinimo tvarkos, invazinių rūšių kontrolės tarybos sudėties ir nuostatų, introdukcijos, reintrodukcijos perkėlimo programos patvirtinimo" (Valstybės žinios. 2002-08-20. Nr. 81-3505), tačiau iki šiol invazinių augalų rūšių populiacijų molekulinių genetinių tyrimų palyginti atlikta nedaug (Patamsytė ir kt., 2011. Vyšniauskienė ir kt., 2011; Zybartaite ir kt., 2011; Danusevičius ir kt., 2013; Kupcinskiene ir kt., 2013; Vyšniauskienė ir kt., 2013; Vyšniauskienė ir kt., 2015). Invazinių rūšių populiacijų dinamikos ir vystymosi tendencijų žinojimas yra svarbus norint prognozuoti tokių rūšių poveikį vietinėms ekosistemoms, o taip pat ieškant tinkamiausių jų plitimą kontroliuojančių būdų. Tyrimams *E. annuus* buvo pasirinkta kaip šiuo metu sparčiai plintanti rūšis, kuri atstovauja invazijomis gausiai *Asteraceae* šeimai ir kuri pasižymi savitomis biologinėmis savybėmis (dideliu fenotipiniu plastiškumu, dominuojančia agamospermija bei retkarčiais vykstančiu lytiniu procesu).

Tikslas buvo įvertinti *E. annuus* populiacijų genetinę struktūrą, invazyvumą ir adaptaciją prie naujų augaviečių.

Pagrindiniai tyrimų uždaviniai:

1. Ištirti *E. annuus* Lietuvos populiacijų genetinę struktūrą bei genotipinę įvairovę RAPD ir ISSR metodais.
2. Naudojant ISSR metodą nustatyti, ar skiriasi kai kuriose Europos šalyse skirtingu laiku įsikūrusių *E. annuus* populiacijų genetinė įvairovė.

3. Palyginti *E. annuus* populiacijų. įsikūrusių skirtingose augavietėse (antropogenizuotose ir pusiau natūraliose), alelopatinį potencialą bei genetinę diferenciaciją.
4. Naudojant bendro sklypo metodą. Nustatyti, ar lokali adaptacija. ar fenotipinis plastiškumas lemia tam tikrus morfologinius ir fenologinius *E. annuus* populiacijų skirtumus.

Ginamieji teiginiai

1. *Erigeron annuus* populiacijoms Lietuvoje būdinga didelė genetinė diferenciacija, kurią galėjo nulemti pradininko efektas.
2. *E. annuus* Lietuvos populiacijose vyrauja genotipai, kurie dauginasi nelytiniu būdu.
3. *E. annuus* populiacijoms plintant šiaurės kryptimi mažėja molekulinė genetinė įvairovė.
4. Antropogenizuotų ir pusiau natūralių augaviečių *E. annuus* populiacijoms būdinga genetinė diferenciacija ir alelopatinių savybių skirtumai.
5. Nors *E. annuus* pasižymi dideliu fenotipiniu plastiškumu, leidžiančiu rūšiai prisitaikyti prie naujų sąlygų, tačiau kai kurioms populiacijoms būdinga ir galimai genetiškai sąlygojama lokali adaptacija.

Rezultatų apibendrinimas

Iš 328-ių vienmetės šiušelės augalų tik 18 turėjo unikalius molekulinius fenotipus. Labiausiai paplitusį kloną sudarė 88 genetiškai vienodi individai. Genetinių atstumų tarp populiacijų rezultatai, gauti RAPD metodu. reikšmingai koreliavo su genetiniais atstumais. gautais naudojant ISSR analizės duomenis ($r = 0,91$. $p < 0,05$). Remiantis gautais DNR polimorfizmo tyrimo rezultatais. visas ištirtas Lietuvos *E. annuus* populiacijas galima padalinti į dvi grupes. Beveik pusė jų (remiantis RAPD duomenimis – 48,3 %; ISSR – 51,7 %) buvo polimorfinės. Genetinės diferenciacijos koeficientas (G_{ST}). RAPD analizės duomenimis yra 0,58, o ISSR – 0,64. Atlikti *E. annuus* Lietuvos populiacijų tyrimai atskleidė mažą genetinę įvairovę populiacijų viduje ir didelę populiacijų diferenciaciją. Genetinio polimorfizmo nebuvimas pusėje tirtų populiacijų ir nedidelis genotipų skaičius polimorfinėse populiacijose rodo. kad Lietuvoje šiušelės populiacijose vyrauja apomiktinis dauginimasis.

Atlikus kai kuriose Europos šalyse skirtingu laiku įsikūrusių *E. annuus* populiacijų genetinės įvairovės tyrimą nustatyta, kad didžiausias vidutinis lokusų skaičius ($116 \pm 1,3$) buvo Šveicarijos populiacijose. Abi tirtos Latvijos populiacijos buvo monomorfinės. Šveicarijos populiacijose buvo nustatytas ir didžiausias genotipų skaičius. Tuo tarpu visi Latvijos populiacijų individai buvo vieno genotipo. Ta pati tendencija išryškėjo nustačius vidutinį lokusų gausumą. Didžiausias vidutinis lokusų gausumas (Br) buvo Šveicarijos populiacijose, mažiausias – Lietuvos ir Latvijos (atitinkamai $1,523 \pm 0,024$, $1,199 \pm 0,085$ ir 1,0) Taigi apibendrinant matyti, jog egzistuoja *E. annuus* genetinės įvairovės gradientas Šveicarijos – Latvijos kryptimi, ir kad *E. annuus* plintant šiaurės kryptimi, genetinė įvairovė invazinėse populiacijose mažėja. Toks genetinės įvairovės mažėjimas, kaip anksčiau minėta, gali būti paaiškinamas įsikūrimo vienoje ar kitoje šalyje trukme.

Palyginus *E. annuus* populiacijų, įsikūrusių skirtingose augavietėse (antropogenizuotose ir pusiau natūraliose) genetinę diferenciaciją nustatyta, kad populiacijos. įsikūrusios pusiau natūraliose augavietėse, buvo labiau diferencijuotos (F_{st} = 0,647) palyginti su antropogenizuotų augaviečių populiacijomis (F_{st} = 0,344). Tai gali būti susiję su atrankos poveikiu, nes vietinių augalų užimtose pusiau natūraliose augavietėse įsitvirtinti daug sunkiau ir tai galbūt pavyksta tik tam tikrų genotipų individams. Didesnę populiacijų diferenciaciją taip pat tai gali nulemti mažesnis *E. annuus* reprodukcinės medžiagos kiekis, patenkantis į pusiau natūralias augavietes bei pradininko efektas. Be genetinės diferenciacijos buvo palygintas ir skirtingose augavietėse (antropogenizuotose ir pusiau natūraliose) įsikūrusių populiacijų alelopatinis potencialas. Antropogenizuotų augaviečių populiacijų augalų juglono indeksas buvo didesnis palyginti su pusiau natūralių populiacijų augalų.

Dviejų metų rezultatų analizė rodo, kad *E. annuus* būdingas didelis fenotipinis plastiškumas, kuris, matyt, ir vaidina svarbiausią vaidmenį rūšies įsikūrimo naujame areale pradžioje. Pavyzdžiui, buvo stebimas didelis kai kurių populiacijų augalų aukščio, svorio, stiebų skaičiaus, lapų pločio varijavimas. Vis dėlto buvo nustatyti ir statistiškai reikšmingi kai kurių populiacijų augalų morfometrinių rodiklių skirtumai, pavyzdžiui, pagal augalų svorį, graižo skersmenį, stiebų skaičių, pagrindinio stiebo papildomų šakų skaičių, lapo ilgį ir plotį. Tai rodo, kad kai kuriose populiacijose įvyko gamtinės atrankos palaikomi genetiniai pokyčiai, kurie nulėmė adaptaciją prie vietos sąlygų.

Išvados

1. *Erigeron annuus* populiacijoms Lietuvoje būdinga palyginti nedidelė RAPD ir ISSR lokusų genetinė įvairovė ir didelė genetinė diferenciacija, kurią galėjo nulemti pradininko efektas.
2. Nustatyti du *E. annuus* Lietuvos populiacijų tipai. Pirmajam tipui priklauso plačiai paplitusio pagrindinio klono ir jam giminingos populiacijos, antrajam – polimorfinės. Tai, kad pusė tirtų populiacijų yra monomorfinės, o polimorfines sudaro nedidelis genotipų skaičius, rodo, kad *E. annuus* populiacijose vyrauja nelytinis dauginimasis.
3. *E. annuus* plintant iš Vakarų Europos rytiniu Baltijos pakraščiu šiaurės kryptimi jos populiacijų molekulinė genetinė įvairovė mažėja.
4. Nustatyta statistiškai reikšminga genetinė diferenciacija (Phi_{RT} = 0,108, p < 0,001; F_{ST} = 0,126) tarp *E. annuus* populiacijų iš pusiau naturalių ir antropogenizuotų augaviečių. *E. annuus* antropogenizuotų augaviečių populiacijos mažiau genetiškai diferencijuotos (F_{ST} = 0,338) palyginti su pusiau natūralių augaviečių populiacijų genetine diferenciacija (F_{ST} = 0,586), (U = 4594, p = 0,0001). Antropogenizuotų populiacijų augalai taip pat pasižymėjo didesniu alelopatiniu potencialu. Tai rodo, kad yra genetiniai ir fiziologiniai skirtumai tarp skirtingose invazijos fazėse esančių populiacijų augalų.
5. Nors *E. annuus* būdingas didelis fenotipinis plastiškumas, leidžiantis rūšiai prisitaikyti prie naujų sąlygų, tačiau kai kurioms populiacijoms būdinga ir galimai genetiškai sąlygota lokali adaptacija.

Darbo mokslinis naujumas ir reikšmė

Pirmą kartą atlikti vienmetės šiušelės Lietuvos populiacijų genetinės struktūros tyrimai, nustatyta genetinė ir genotipinė įvairovė. Šie tyrimai atlikti naudojant dviejų tipų DNR žymenis. Atlikus kai kurių Europos šalių *E. annuus* populiacijų molekulinius genetinius tyrimus, nustatytas genetinės įvairovės gradientas invazinio arealo dalyje, į kurią įeina ir Lietuva. *E. annuus* populiacijų iš antropogenizuotų ir pusiau natūralių augaviečių genetinės įvairovės palyginimas parodė šių dviejų populiacijų grupių genetinę diferenciaciją, kuri gali būti susijusi su ekotipų formavimusi skirtingose invazijos proceso stadijose. Pirmąkart įvertintas *E. annuus* invazinių populiacijų iš skirtingų augaviečių alelopatinis potencialas ir nustatyta, kad jis skiriasi priklausomai nuo augavietės. Naudojant bendro sklypo metodą geografiniu požiūriu skirtingos kilmės populiacijų morfometriniams ir fenologiniams tyrimams atlikti parodyta, kad nepaisant rūšiai būdingo didelio fenotipinio plastiškumo, kai kurioms populiacijoms būdingi ir lokalios adaptacijos požymiai. Šie požymiai gali būti svarbūs rūšies invazyvumui.

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