GRADIENT OF GENETIC DIVERSITY OF *ERIGERON ANNUUS* IN THE PART OF INVASIVE EUROPEAN RANGE

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**Abstract**

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*Erigeron annuus* (L.) Pers. (Asteraceae) is native species to eastern North America, but has been introduced to Europe and many other temperate regions of the world. In order to assess the impact of spreading history and settlement time on the genetic diversity of invasive populations of *E. annuus*, individual plants were sampled from 16 populations located in Switzerland, Poland, Lithuania and Latvia along the south-northern expansion direction of this species in Europe. One population was collected in native range (New Brunswick, Canada). The analysis of ISSR polymorphism in 253 plants revealed 161 polymorphic bands. The highest genotype variation and genetic diversity parameters were revealed in the populations from Switzerland and Poland, the least – in Latvian populations. All 37 plants from Latvia were clones of the same genotype. The comparison of genetic diversity parameters of populations from different countries showed the decrease of genetic diversity on the south-north direction.

**Keywords**: *Erigeron annuus*, genetic diversity, invasive species, population genetic structure.

**INTRODUCTION**

Invasive plant species cause serious environmental and economic problems nowadays in the entire world, contributing to decline of native biodiversity, ecosystem degradation and economic loss. The negative effects of invasions are now widely noticed, and many studies have been carried out by invasion biologists in many parts of the world to reduce current and future impacts (Pyšek & Richardson, 2010). Ecological research have prevailed in this field for a long time, however, were insufficient to reveal all causes and consequences of biological invasions (Boßdorf et al., 2005). The investigation of neutral genetic variation is also recognized as important instrument to study plant invasions. Molecular markers provide information about invasion pathways, the amount of genetic variation introduced and rapid evolutionary changes in exotic species (Boßdorf et al., 2005; Kupciński et al., 2015; Vy niauskienė et al., 2015). Intraspecific genetic variation may contribute significantly to invasiveness (Ward et al., 2008). Also the process of invasion may significantly affect the patterns of genetic diversity of exotic species (Boßdorf et al., 2005; Parisod et al., 2009). It is known that expansion of some invasive species create a gradient of genetic diversity decreasing along the spatial axis of spreading (Austerlitz et al., 1997). The variation among plants in populations also may be caused by multiple colonizations of the site by propagules from other populations with or without subsequent hybridization (Röelofs & Bachmann, 1995).

The object of our study was an invasive plant species *Erigeron annuus* (L.) Pers. (Asteraceae), also known as daisy fleabane. Plants of *E. annuus* display extensive phenotypic plasticity (Stratton, 1992;
Apomixis is the dominant reproductive mode in this species. It is considered a winter annual, but some plants delay reproduction until the second or third summer. Mother plant produces large numbers of genetically identical minute seeds. Populations of daisy fleabane also frequently contain genotypes that can reproduce sexually (Edwards et al., 2006). *E. annuus* is native in the eastern parts of the United States, and in the 17th century this species was introduced to Europe. The spreading of *E. annuus* in Switzerland has started since 1770 (Frey et al., 2003). About the middle of the 19th century, the species reached Poland (NOBANIS). In Lithuania, *E. annuus* has been known since the end of the 19th century (Gudžinskas, 1997) and now it is in the phase of rapid spreading. The reasons of this process are largely unknown, but climate changes, decreased agricultural activity, appearance of new well-adapted and more invasive genotypes may be implied (Patamsytė et al., 2013). Genetic diversity is considered to be very important for the adaptation of species in the new range and it plays very important role for plants that are introduced into new areas located thousand kilometres from the native range of the species (Dlugosch & Parker, 2008).

Earlier studies on genetic diversity of *E. annuus* showed rather high genetic variability. Edwards et al. (2006) used RAPD to identify genetic variation between and among populations of this species in its native range (North America) and in Europe, where it has been introduced. In this study, high variability of RAPD phenotypes was found. Using AFLP, Trtikova et al. (2011) studied genetic diversity of *E. annuus* populations at different altitudes along several major roads crossing the Alps. This study revealed high genetic differentiation among populations. Authors of this investigation found no evidence for local adaptation to altitude and proposed phenotypic plasticity as important factor in occupying new habitats by this species. Our previous studies on clonal structure of *E. annuus* using DNA markers revealed reduced genetic variation in considerable number of studied populations and wide spread of one clone in populations of Lithuania (Patamsytė et al., 2013). Our aim in this research was 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 settlement of this exotic species in the certain territory had impact on genetic variability of local populations.

**MATERIALS AND METHODS**

**Plant material and DNA extraction**

Sampling included 17 populations of *E. annuus* from Switzerland (4), Poland (6), Lithuania (4), Latvia (2) and Canada (1). The number of plants per population ranged from 12 to 25 (Table 1). A total of 253 plants were analysed in this study. Genomic DNA was extracted from fresh or dried plant leaves using the modified cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990).

**Molecular fingerprinting**

ISSR analyses were performed as described by Patamsytė et al. (2011). Each 10 µL ISSR-PCR contained 1 µL 10 × PCR buffer (Thermo Fisher Scientific Baltics), 100 µM dNTPs, 1 unit *Taq* polymerase (Thermo Fisher Scientific Baltics), 300 µM MgCl₂, 0.4 µM of the primer and approximately 10 ng of DNA. The ISSR-PCR conditions were: 1 cycle of 7 min at 94°C, 32 cycles of 30 s at 94°C, 45 s at 55°C, and 2 min at 72°C, 1 cycle of 7 min at 72°C. The analysis was carried out with five ISSR primers (ISSR-O, ISSR-B, ISSR-C, ISSR-D, ISSR-G), which produced clear and reproducible profiles in agarose gels. All ISSR-PCR reactions were replicated for each primer. In each amplification, a negative control PCR without DNA template was carried out.

For ISSR fingerprinting, PCR products were resolved on 1.2% agarose gels (4 h, 4 V/cm) stained with ethidium bromide. The electrophoretic patterns of the amplification products were recorded using UV transilluminator and BioDocAnalyse software (Biometra, Germany). The size of scored DNA fragments was estimated with DNA size standard (GeneRuler DNA Ladder Mix, Thermo Fisher Scientific Baltics).

**Data analysis**

Amplified bands were scored in a size range from 300 to 1500 bp. The presence of the DNA fragment (band) was represented with “1” and the absence was represented with “0”. Based on this, the ma-
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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 was different for studied countries. The highest average number of ISSR bands (116 ± 1.3) was revealed in Switzerland populations. It ranged from 62 to 116 bands per population. The number of ISSR bands per population in Poland and Latvia was lower (ranging from 44 to 92 bands) than in Switzerland and Canada. The number of ISSR bands in Lithuania was intermediate (ranging from 71 to 90 bands).

The genetic structure of 17 populations of *Erigeron annuus* was revealed by means of *STRUCTURE*, version 2.3.4 (Pritchard et al., 2000) using a Bayesian model-based clustering algorithm adapted to dominant markers (Falush et al., 2007). The procedure was run using the admixture model, with 10 independent replicate runs per K value (number of clusters) ranging from 1 to 20. A burn-in period 50000 was followed by 50000 MCMC iterations.

### Table 1. *Erigeron annuus* populations examined in this study with description of collecting coordinates and habitats

| No. | Population | Code | Sample size | Collecting 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   | Mragowo    | MRA  | 12          | 53°51’55”, 21°17’26”                  | Mowed meadow by road |
| 8   | Suwałki    | 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  | Święta 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°04’15”                  | Unmowed meadow |
| 17  | Kernavė    | KER  | 12          | 54°53’03”, 24°52’29”                  | Meadow on the slope |

RESULTS AND DISCUSSION

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 was different for studied countries. The highest average number of ISSR bands (116 ± 1.3) was revealed in Switzerland populations. It ranged from 62 to 116 bands per population. The number of ISSR bands in Poland and Latvia was lower (ranging from 44 to 92 bands) than in Switzerland and Canada. The number of ISSR bands in Lithuania was intermediate (ranging from 71 to 90 bands).
from 113 (LOC) to 119 (SIG). Very high differences in the number of detected ISSR bands were revealed among six Polish populations. It ranged from 79 (SZC) to 134 (LUA). The average band number detected in Polish populations was 104.8 ± 8.1. In each of two monomorphic Latvian populations, 82 bands were revealed. The average number of bands identified in four studied Lithuanian populations was 90 ± 5.1. One population (NEW) from Canada possessed 95 polymorphic bands.

Various numbers of genotypes were identified in the studied populations (Table 2). Four populations (SZC, DAA, DAB and PAT) were monomorphic. The largest number of genotypes were found in Switzerland populations. It varied between five (ZÜR) and 11 (LOC) per population. Genotypes consisted of different number of clones. The largest genotype was found in Daugavpils populations, where all 37 plants were clones of the same genotype. Twenty two plants of daisy fleabane showed distinctive ISSR banding profiles. The highest proportion of unique genotypes were found in Switzerland 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 2). The mean band richness (Br) was highest in Switzerland populations (1.523 ± 0.024) and the lowest in Lithuanian populations (1.199 ± 0.085). The highest mean Nei’s gene diversity and Shannon’s indices were also in Switzerland 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; I = 0).

Table 2. Genetic characteristics of 17 populations of Erigeron 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 |
| LITHUANIA      |              |                  |                         |         |   |   |
| 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
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The Mantel test of IBD in all studied daisy fleabane populations showed no correlation ($r = 0.134; p = 0.37$). However, when Canadian and Latvian populations were excluded from calculation, there was a significant correlation between the genetic and geographic distances ($r = 0.261; p = 0.03$). These results indicate that populations within regions are not experiencing high rates of gene flow (Baker & 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 Switzerland 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 (Fig. 1), which in general correlates with the time of settlement of this species in 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 create 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 & Dyer, 2011). Baker & 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. 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 (Fig. 2). PCoA analysis showed the grouping of populations from Switzerland. The Polish populations also showed clear grouping tendency. Two Lithuanian populations (PRI and SUD) were closely related to the Polish populations, while 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 $\Delta K$ reached its maximum at $K = 3$ (Fig. 3) suggesting three clusters indicated by white, black and grey colour (Fig. 4). The distribution of populations among clusters did not show clear geographic pattern, however, there

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Fig. 1. Differences in mean band richness (Br) in populations of *Erigeron annuus* from Switzerland, Poland, Lithuania and Latvia

Fig. 2. Principal coordinate analysis of the 17 populations of *Erigeron annuus*. Population names are coded as in Table 1

Fig. 3. Graph from the admixture model of *STRUCTURE* analyses of $\Delta K$ for *Erigeron annuus* populations. $\Delta K$ showed one peak at $K = 3$
are some regional differences. Most individuals (LOC, SIG, SAN, ZÜR, LUA, MRA, LUB, NEW, KER populations) were assigned to grey cluster. All individuals of Latvian populations were allocated to white cluster. Most individuals of PRI and SUD populations were grouped into black cluster. SZC, SUW, SWI and PAT populations showed a high degree of admixture and were not assigned to any of the three clusters. Comparison of genetic structure indicates that some studied populations originated from distinct sources. Single native population (NEW) from Canada was allocated in gray cluster. In spite of the highest genetic variation, the 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). This result correspond 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 & Dyer, 2011).

In general, the STRUCTURE and PCoA results are congruent. Both analyses revealed relatedness 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 with 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 (Peintinger et al., 2012). 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.

CONCLUSIONS

Our results indicate the existence of E. annuus genetic diversity gradient in transect from Switzerland to Latvia. Monoclonal populations from southern Latvia are located at the front of the expansion of the species, while the populations with longer invasion history (Switzerland, partially Poland) exhibited considerably higher genetic diversity. Thus, the settlement time and invasion history of E. annuus in the northern direction had impact on genetic variability of local populations.

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**ERIGERON ANNUUS GENETINĖS ĮVAIROVĖS GRADIENTAS RŪŠIES EUROPOS INVAZINIO AREALO DALYJE**

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Santrauka

Vienmetė šiušelė (*Erigeron annuus* (L.) Pers.) (*Asteraceae*) yra rytinio Šiaurės Amerikos (JAV ir Pietų Kanada) pakraščio vietinė rūšis, kuri buvo introdukuota ir išplito Europoje bei daugelyje kitų pasaulio vietų. Šiame darbe tyrėme, kaip šios rūšies genetinę įvairovę galėjo paveikti jos įsikūrimo ir gyvavimo naujoje teritorijoje trukmė bei invazijos proceso istorija. Tyrimams augalai buvo surinkti iš 16 invazinių populacijų Šveicarijoje, Lenkijoje, Lietuvoje ir Latvijoje. Mūsų nuomone, ši kryptis atitinka vieną iš galimų rūšies plitimo krypčių Europoje. Taip pat buvo iššūkis ir viena *E. annuus* populiacija iš Kanados, New Brunswick provincijos. DNR polimorfizmas buvo nustatytas paprastųjų pasikartojančiųjų sekų intarpų (*angl. Inter-Simple Sequence Repeats* – ISSR) metodu. Ištyrus 253 augalus, buvo nustatytas 161 DNR žymuo, tinkamas tirtų augalų ir populiacijų genetinei įvairovei įvertinti. Didžiausia genetinė įvairovė nustatyta Šveicarijos ir Lenkijos populiacijose. Mažesnė genetinė įvairovė yra būdinga šiušelės populiacijoms Lietuvoje. Įsitikiname, kad vienmetei šiušelė plintant šiaurės kryptimi, genetinės įvairovės lygis invazinėse populiacijose mažėja.