Genotypic diversity and clonal structure of *Erigeron annuus* (Asteraceae) in Lithuania

Genetische Vielfalt und Klonstruktur von Erigeron annuus (Asteraceae) in Litauen

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DOI 10.5073/jka.2014.443.023

Abstract

This study was conducted to assess the clonal structure and genetic diversity of alien herbaceous plant species *Erigeron annuus*. The global warming and changes in agriculture practice in the past few decades were favourable for the expansion of this species in Lithuania. We used RAPD and ISSR assays to assess genetic variation within and among 29 populations of *E. annuus*. A total of 278 molecular markers were revealed. Our study detected reduced level of genetic diversity of invasive populations of *E. annuus*. Significant differences in DNA polymorphism among populations of *E. annuus* were also found. Some populations of this species are composed of genetically identical plants, while others were polymorphic. Clonal diversity of study populations ranged from 0.083 to 0.4 for both DNA marker systems. The Simpsons diversity index values ranged from 0.0 to 0.636. The average number of genotypes per population established using both assays was about 1.7. Out of 328 *E. annuus* individuals only 16 showed unique RAPD and 14 unique ISSR banding patterns. The remaining plants were clones of different size. The most common genotype of *E. annuus* identified in our study was represented by predominate in nine populations.

Keywords: Clonal structure, DNA markers, *Erigeron annuus*, invasive plants, ISSR, RAPD

Zusammenfassung

Die Untersuchungen verfolgen das Ziel, die genetische Struktur der nicht-einheimischen Pflanzenart *Erigeron annuus* auszuwerten. Globale Erwärmung sowie Veränderungen in der landwirtschaftlichen Praxis der letzten Jahrzehnte waren bedeutsam für die Verbreitung dieser Art in Litauen. Für die Feststellung der genetischen Verteilungsvielfalt in den 29 *E. annuus* Populationen haben wir uns der RAPD und ISSR -Methoden bedient. Insgesamt wurden 117 RAPD - und 161 ISSR - Loci festgestellt. Die Untersuchungen haben die verringerte genetische Vielfalt der *E. annuus* Populationen aufgezeigt. Außerdem wurden bedeutende DNR polymorphe Unterschiede zwischen *E. annuus* Populationen angetroffen. Einige Populationen dieser Art bestanden aus genetisch identischen Pflanzen, während die anderen polymorphem waren. Die Klonvielfalt der untersuchten Populationen schwankte zwischen 0.083 und 0.4 bei der Verwendung von beiden DNR-Signifikanten. Die durchschnittliche Genotypen Zahl in der Population betrug etwa 1.7 bei der Verwendung von beiden Signifikanten. Nach der Untersuchung der 328 *E. annuus* Individuen wurden 16 unikale RAPD - und 14 unikale ISSR - Phänotypen festgestellt. Die übriggebliebenen Pflanzen waren Klonen von unterschiedlicher Größe. Der am meisten verbreitete *E. annuus* Genotyp wurde in neun Populationen ermittelt.

Stichwörter: DNS- Marker, *Erigeron annuus,* ISSR, Klonstruktur, invasive Pflanzen, RAPD

Introduction

Daisy fleabane (*Erigeron annuus* (L.) Pers.) is a winter annual invasive plant species which was introduced from North America into Europe in the 17th century (EDWARDS *et al.*, 2006). Now it is one of the 150 most widespread alien plant species in Europe (LAMBDON *et al.*, 2008). In the native range this species is an inhabitant of tall grass prairies, while in the new area it is abundant on roadsides and ruderal places (TRTIKOVA *et al.*, 2011). *E. annuus* is triploid apomictic plant, which forms embryos through meiotic diplospory (MCDONALD, 1927; STRATTON, 1991). Production of apomictic seeds is one of mechanisms of clonal reproduction (CHUNG *et al.*, 2006). Some populations, however, show rather high polymorphism and contain local genotypes, suggesting that sexual reproduction does occur occasionally (EDWARDS *et al.*, 2006; TRTIKOVA *et al.*, 2011). Asexual reproduction may be favorable for spread of *E. annuus*, because it allows establishing populations from a single

propagule and may also maintain genotypes with broad environmental tolerance, called according BAKER (1965) 'general purpose' genotypes. *E. annuus* achieved Lithuania probably from Western Europe at the end of the 19th century, i. e. more than two hundred years later than some regions of west Europe. Here it was introduced as ornamental plant. A few decades later according botanical literature it invaded natural ecosystems (MOWSZOWICZ, 1939). Now this species is in a phase of intensive spread, which started in Lithuania few decades ago. Expansion of *E. annuus* as other neophytes might be promoted by global warming (prolonged vegetation season, rise of minimal temperatures) and anthropogenic factors, such as changes in agriculture practice in the past few decades, increasing international trade, extension of urban area (SCHMITZ *et al.*, 2010; ZYBARTAITE *et al.*, 2011). *E. annuus* in Lithuania is generally found in roadsides, abandoned fields, ruderal places, and lawns, near lakes, rivers and as weed in towns of the southern and southern-eastern regions. Asexual mode of reproduction implies the existence of clonal structure in populations of apomictic plants and low genetic diversity (LOVELESS and HAMRICK, 1984). However numerous studies have confirmed the existence of many multilocus genotypes in populations of apomictic plants (VAN DER HULST *et al.*, 2003). *E. annuus* is an example of species possessing clonality and sexual reproduction. Investigations using RAPD markers revealed a high diversity at the level of the RAPD phenotypes in North American and West European populations (EDWARDS *et al.*, 2006). RAPD and AFLP markers have been used to study genetic diversity, population structure and local adaptation of *E. annuus* in the Swiss Alps (TRTIKOVA *et al.*, 2011). Edwards *et al.* (2006) supposed that most of the genotypes now present in Europe have been produced through sexual reproduction that has occurred since its arrival. Despite of this, several common RAPD phenotypes were also found on both continents. EDWARDS *et al.* (2006) also suggested that certain genotypes of *E. annuus* have a much stricter agamospermy than others. The possibility to reproduce sexually and asexually and invade new area makes this species very interesting model plant for adaptation studies. Because this species achieved Lithuania later, than west and central Europe countries, it is interesting to know if this had some influence on the clonal diversity and species genetic structure. Furthermore, the study of molecular variation of *E. annuus* is important for understanding the invasion process of apomictic plants and for management of invasive populations in new area.

So, the present study was carried out to study clonal structure and to determine the extent of molecular variation and distribution of molecular phenotypes in invasive populations of *E. annuus* in Lithuania.

Material and Methods

Plants of *E. annuus* were collected from 29 *E. annuus* sites and were considered as representatives of 29 populations (Užutrakis, Giedraičiai, Vilnius A, Naujasis Janavas, Vilnius B, Pagiriai A, Roduka, Daniliškės, Marijampolė, Pagiriai B, Naujoji Vilnia, Bezdonys, Gelgaudiškis, Kėdainiai, Jurbarkas, Vilnius C, Lielius, Babtai, Vilnius D, Kulautuva, Kalvarija, Mikytai, Betygala, Kavarskas, Svėdasai, Kamajai, Kena, Mažeikiai, Pervalka). A total of 328 plants were sampled. From 5 to 12 plants were collected from each population. The highest density of this invasive plant is concentrated in southern and south-eastern part of country. For this reason the considerable part of study populations are from this region.

DNA was extracted from fresh leaves using cethyl-trimethyl-amonium bromide (CTAB) (DOYLE and DOYLE, 1990). RAPD and ISSR analyzes were performed as described in PATAMSYTĖ *et al.* (2011). All reactions were repeated at least twice. A negative control PCR without DNA template was carried out in each amplification. The selection of primers for reproducibility and polymorphism of DNA profiles was performed before analysis of all material on the 10 samples from different populations. Primers generating complex or weak profiles were discarded. Amplifications were resolved on a 1.5% agarose gel (4 h, 4 V/cm), stained with ethidium bromide and photographed using BioDocAnalyse system.

The presence of the DNA fragment (allele) was represented with ''1'' and the absence was represented with ''0''. Clonal diversity of populations was assessed according frequently used two parameters: G/N – clonal diversity, where G – the number of genets and N is the total number of individuals sampled and D – Simson's diversity index (ELLSTRAND and ROOSE, 1987; 2003; DEV *et al.*, 2010). Genetic diversity was analyzed at population and species level. The percentage of polymorphic loci (P), population genetic differentiation coefficient (G_{ST}) (NEI, 1973) were calculated using POPGENE v. 1.31 software (YEH *et al.*, 1999). Analysis of molecular variance (AMOVA), significance of Φ_{PT} values using permutation test with 999 permutations, was carried out using GenAlEx v. 6.4 (PEAKALL and SMOUSE, 2006).

Results

In the present study we used RAPD and ISSR markers to study genetic diversity and clonal structure of *E. annuus*. Amplified bands were scored in a size range from 440 to 2200 bp. A total of 117 reproducible RAPD and 161 ISSR bands were detected in the 328 individuals at the species level using preselected 4 RAPD and 5 ISSR primers. The polymorphism of RAPD markers at the species level was 60.5 ± 8.2% and ISSR markers – 71.7 ± 9.2% (Tab. 1).

Tab. 1 Primers used in the RAPD and ISSR analyses of *E. annuus,* size, number and polymorphism of DNA markers.

Tab. 1 *Die in der E. annuus RAPD- und ISSR-Analyse verwendeten Primer, Größe, Zahl und Polymorphismus der identifizierten Loci.*

Primer	Sequence 5'→3'	Size of DNA fragments (bp)	Number of DNA bands		P, %
			Total	Polymorphic	
RAPD					
Roth-A 03	AGTCAGCCAC	460-1700	29	15	51.7
Roth-A 04	AATCGGGCTG	440-1900	30	19	63.3
Roth-A 05	AGGGGTCTTG	440-2100	29	16	55.2
Roth-A 07	GAAACGGGTG	560-2100	29	20	70.0
		Sum	117	70	
		Average	29.3±0.5	17.5±2.4	60.5±8.2
ISSR					
ISSR_O	$GAG(CAA)_5$	620-2200	36	29	80.6
ISSR_B	$(AG)_8CG$	600-1900	30	24	80
ISSR_C	$(AG)_8TG$	500-1600	21	15	71.4
ISSR_D	$(AG)_8$	480-1700	38	26	68.4
ISSR_G	$(GCC)_5$	500-1650	36	21	58.3
		Sum	161	115	
		Average	32.2±6.9	23±5.3	71.7±9.2

DNA polymorphism at population level was considerably lower than at species level. Similar results were obtained for both RAPD and ISSR analyses. RAPD assay revealed 15 monomorphic populations of 29 studied while ISSR analysis showed no polymorphism in 14 populations of 29 studied. The average polymorphism of RAPD markers at population level including monomorphic populations was 26.01% and ISSR polymorphism was 23.63%. The percentage of polymorphic RAPD bands within polymorphic populations varied between 25.71% (Lielius) and 78.57% (Kena) (Fig. 1). The extreme values of ISSR polymorphism within the same group of populations were 10.43% (Vilnius C) and 69.57% (Daniliškės). The second largest ISSR polymorphism showed Kena population (62.61%) (Fig. 2).

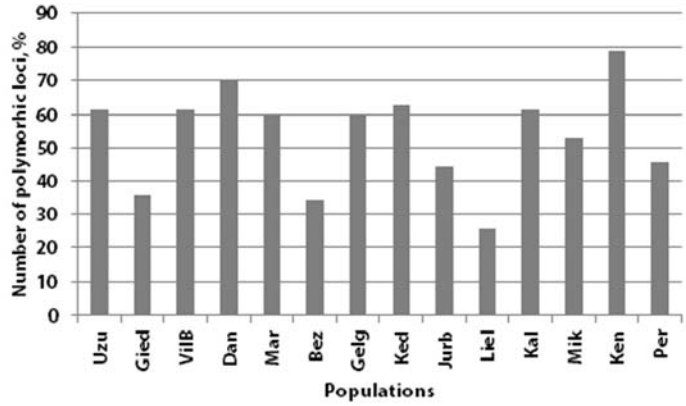

Fig. 1 Number of polymorphic loci (%) revealed by RAPD. Population codes: Uzu-Užutrakis, Gied-Giedraičiai, VilB-Vilnius B, Dan-Daniliškės, Mar-Marijampolė, Bez-Bezdonys, Gelg-Gelgaudiškis, Ked-Kėdainiai, Jurb-Jurbarkas, Liel-Lielius, Kal-Kalvarija, Mik-Mikytai, Ken-Kena, Per-Pervalka.

Abb. 1 *Polymorphismus der RAPD- Loci in den* E. annuus *polymorphen Populationen.*

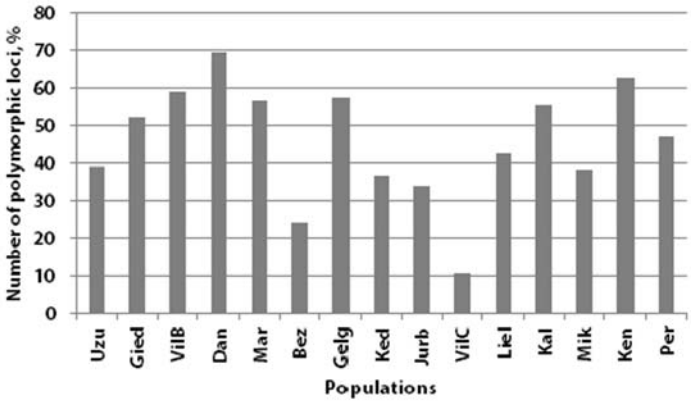

Fig. 2 Number of polymorphic loci (%) revealed by ISSR. Population codes: Uzu-Užutrakis, Gied-Giedraičiai, VilB-Vilnius B, Dan-Daniliškės, Mar-Marijampolė, Bez-Bezdonys, Gelg-Gelgaudiškis, Ked-Kėdainiai, Jurb-Jurbarkas, VilC-Vilnius C, Liel-Lielius, Kal-Kalvarija, Mik-Mikytai, Ken-Kena, Per-Pervalka.

Abb. 2 *Polymorphismus der ISSR -Loci in den* E. annuus *polymorphen Populationen.*

Tab. 2 Variation in clone structure among populations of *Erigeron annuus* in Lithuania revealed by RAPD and ISSR assays.

Tab. 2 *Die Vielfalt der Klonstruktur in den untersuchten* E. annuus *Populationen, festgestellt nach den RAPD- und ISSR- Signifikanten.*

	Total samples (N)	**Number of genotypes (G)**		**Clonal diversity (G/N)**		**Simpson's diversity index (D)**	
		RAPD	**ISSR**	**RAPD**	**ISSR**	**RAPD**	**ISSR**
Užutrakis	12	2	3	0.167	0.250	0.530	0.621
Giedraičiai	12	2	2	0.167	0.167	0.167	0.167
Vilnius A	12	1	1	0.083	0.083	0	0
N.Janavas	12	1	1	0.083	0.083	0	0
Vilnius B	12	2	2	0.167	0.167	0.545	0.545
Pagiriai A	12	1	1	0.083	0.083	0	0
Roduka	12	1	1	0.083	0.083	0	0
Daniliškės	12	4	4	0.333	0.333	0.455	0.455
Marijampolė	12	2	2	0.167	0.167	0.530	0.530
Pagiriai B	12	1	1	0.083	0.083	0	0
N.Vilnia	12	1	1	0.083	0.083	0	0
Bezdonys	12	2	2	0.167	0.167	0.167	0.167
Gelgaudiškis	12	4	3	0.333	0.250	0.636	0.439
Kėdainiai	12	2	2	0.167	0.167	0.409	0.409
Jurbarkas	12	2	2	0.167	0.167	0.167	0.167
Vilnius C	12	1	2	0.083	0.167	0	0.167
Lielius	12	2	2	0.167	0.167	0.303	0.303
Babtai	6	1	1	0.167	0.167	0	0
Vilnius D	10	1	1	0.100	0.100	0	0
Kulautuva	12	1	1	0.083	0.083	0	0
Kalvarija	12	2	2	0.167	0.167	0.545	0.545
Mikytai	5	2	2	0.400	0.400	0.400	0.400
Betygala	7	1	1	0.143	0.143	0	0
Kavarskas	12	1	1	0.083	0.083	0	0
Svėdasai	12	1	1	0.083	0.083	0	0
Kamajai	12	1	1	0.083	0.083	0	0
Kena	12	4	4	0.333	0.333	0.636	0.636
Mažeikiai	12	1	1	0.083	0.083	0	0
Pervalka	12	2	2	0.167	0.167	0.530	0.530
Average		1.7	1.7	0.154	0.157	0.208	0.210

The populations consisted of different number of genotypes (genets). The variability of RAPD and ISSR patterns was rather low. Out of 328 fleabane individuals collected in all 29 sites, only 16 showed unique RAPD and 14 unique ISSR patterns. The remaining plants were clones of different size (28 RAPD and 32 ISSR patterns were observed in two or more accessions). The largest clone according RAPD analysis data consisted of 109 accessions. This genotype was shared among 9 populations (data not shown). Clonal diversity ranged from 0.083 to 0.4. The Simpson's diversity index values varied from 0.0 to 0.636 (Tab. 2).

All the parameters of genetic differentiation obtained using RAPD and ISSR markers were rather similar and indicated a high level of genetic differentiation among populations**.** An AMOVA revealed that 51.1% (Φ_{PT} = 0.511) of the total variation established using RAPD analysis occurred among populations and 48.9% occurred within the population. When analysis was carried out on the basis of ISSR data, the 59.5% (Φ_{PT} = 0.595) of the total variation was found among populations and 40.5% occurred within populations. The coefficient of genetic differentiation between populations (G_{ST}) was 0.58 for RAPD markers and 0.64 for ISSR markers. The estimated values of Nm from G_{ST} were 0.358 and 0.278 respectively, which suggested that the gene flow in *E. annuus* was low.

Discussion

To get more precise and reliable data we used two types of DNA markers. An analysis of molecular data indicated a high level of genetic differentiation (G_{ST} = 0.64 for ISSR, G_{ST} = 0.58 for RAPD). Similar values of population differentiation were revealed by AMOVA. The Φ_{PT} value for RAPD markers was 0.511, and for ISSR markers 0.595. TRTIKOVA *et al.* also identified high level of genetic differentiation among populations from Switzerland. Nearly half of the total genetic diversity in this study was established among populations (G_{ST} = 0.46). A higher differentiation of Lithuanian populations in comparison with Swiss lowland populations possibly can be explained by more expressed founder effect and reduced gene flow among them.

Our study of Lithuanian populations of environmental weed *E. annuus* using RAPD and ISSR markers also revealed reduced genetic diversity at population level in comparison with previous results obtained for this species. Previously EDWARDS *et al.* (2006) reported that most of the populations of this species from Northern America and Western Europe were multiclonal and possessed high levels of genotypic diversity within populations (EDWARDS *et al.*, 2006). In the other study, TRTIKOVA *et al.* (2011) found, that 83% of studied populations from Switzerland were polymorphic. A detailed comparison of Lithuanian populations with western and central European populations is complicated because of different assays used and different number of loci studied. Nevertheless at the population level the trend towards reduced genetic variability in Lithuanian populations is evident. Only about 50% of Lithuanian populations were polymorphic. The fact that one RAPD phenotype is common for about 33% of studied individuals also indicates low genetic variation of *E. annuus* populations in Lithuania. The estimate of average clonal diversity (*G/N*) as parameter of sexual recruitment in Lithuanian populations was rather low (G/N = 0.154 for RAPD; G/N = 0.157 for ISSR) in comparison with the mean (0.17) for the clonal species (ELLSTRAND AND ROOSE 1987). Nevertheless RAPD analysis of studied plant material identified 16 unique genotypes and ISSR analysis – 14 unique genotypes. This result one more time supports the view that most of apomictic plants in an introduced range consist of various genotypes (EDWARDS *et al.*, 2006; LOOMIS and FISHMAN, 2009). EDWARDS *et al.* (2006) pointed out that some genotypes of *E. annuus* have a stronger tendency towards agamospermy than others. For clonal invasive plants phenotypic plasticity in the introduced range may be the most suitable strategy of adaptation to local conditions (PARKER *et al.*, 2003; LOOMIS and FISHMAN, 2009). Uniparental reproduction (e.g. selfing or asexual reproduction by clonal propagation and apomixis) allows establishment after dispersal when founder group sizes are very small (BARRETT *et al.*, 2008). EDWARDS *et al.* (2006) also noticed that specific European RAPD phenotypes were not imported from North America populations but most likely were generated by genetic recombination in the process of occasional sexual

reproduction. Only part of diversity of western European populations was introduced into Lithuania and rare sexual reproduction did not compensate the impact of founder effect. It is known that expansion of invasive species create a gradient in genetic diversity decreasing along the spatial axis of spreading (AUSTERLITZ *et al.*, 1997). For this reason it is expected that populations at the front of an expansion should be lower in diversity than those at the core of the expansion (KLOPFSTEIN *et al.*, 2006; BAKER and DYER, 2011). This idea can be used to explain the possible trend for lower genetic diversity in invasive population of *E. annuus* in the northern direction. This species probably start its spreading through Europe from France in the seventeenth century and at the end of 19th century achieved Lithuania. Although precise information concerning date and way of introduction of *E. annuus* into Lithuania does not exist, it seems likely that it has been distributed here as an ornamental plant or as contamination of seeds of other ornamental plants (PATAMSYTĖ *et al.*, 2013). *E. annuus* is still very rare in Northern Lithuania and in Latvia, which suggests that the front of an expansion of this species divides Lithuanian territory in two parts: with and without of daisy fleabane.

Acknowledgement

This research was founded by Research Grant (No. LEK 07/2012) from the Research Council of Lithuania.

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