

Comparison of genetic diversity in two alien plant species

Vergleich der genetischen Vielfalt von zwei nicht-einheimischen Pflanzenarten

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Summary

Bunias orientalis and *Erigeron annuus* are two alien herbal plant species invading native ecosystems in Lithuania. Both species have similar introduction and invasion history but differ in their life history traits and strategies of reproduction. We used random amplified polymorphic DNA (RAPD) data to compare the genetic diversity in invasive populations of these species. 42 RAPD bands were identified in *B. orientalis* and 70 RAPD bands in *E. annuus*. All six populations of *B. orientalis* were polymorphic, while only four of eight study populations of *E. annuus* were polymorphic. The highest differences between species were established at the level of RAPD phenotypes. 67 RAPD phenotypes were found among 90 plants of *B. orientalis* but only 10 RAPD phenotypes were identified among 89 plants of *E. annuus*. This study shows that the level of genetic diversity in populations of different invasive species at the same geographic area may vary considerably.

Keywords: *Bunias orientalis*, *Erigeron annuus*, genetic diversity, RAPD phenotype

Zusammenfassung

Bunias orientalis und *Erigeron annuus* sind zwei nicht-einheimische Pflanzenarten, die in litauische natürliche Ökosysteme eindringen. Eindringungs- und Invasionswege beider Arten sind ähnlich. Unterschiede liegen in den Entwicklungs- und Reproduktionsbesonderheiten. In dieser Arbeit haben wir anhand der Ergebnisse von random amplified polymorphic DNA (RAPD) die genetische Vielfalt von verschiedenen Populationen dieser nicht-einheimischen Arten verglichen. 42 RAPD Banden wurden bei *B. orientalis* und 70 bei *E. annuus* festgestellt. Alle sechs *B. orientalis*-Populationen waren polymorph; bei *E. annuus* waren es nur vier von acht Populationen. Die größte Differenz zwischen den Arten zeigten die RAPD-Phänotypen. Bei 90 *B. orientalis*-Pflanzen wurden 67 RAPD-Phänotypen identifiziert aber nur 10 RAPD-Phänotypen bei 89 *E. annuus*-Pflanzen. Die Ergebnisse zeigen, dass die genetische Vielfalt der nicht-einheimischen Pflanzenarten auf demselben geographischen Territorium wesentliche Unterschiede aufweisen kann.

Stichwörter: *Bunias orientalis*, *Erigeron annuus*, genetische Vielfalt, RAPD-Phänotypen

1. Introduction

Genetic diversity is important for the adaptation of plant species to changing environments. The role of this genetic factor may be critical for plants that are introduced into new areas located thousand kilometers from the native range of the species. Though the impact of genetic diversity on the invasion success is the topic of many studies, it still remains relevant (LAMBRINOS, 2001; BARRETT et al., 2008). Many invasive species have a reduced genetic variation in their introduced ranges, while many others do not (DLUGOSCH and PARKER, 2008). According to theoretical predictions, alien plant species suffer from reduction in genetic diversity provoked by founder effects. In an analytic review of numerous studies of invasive organisms, DLUGOSCH and PARKER (2008) assert that there are significant losses of both allelic richness and heterozygosity in introduced populations. On the other hand, there is now growing recognition that in the time of invasion lag phase, genetic changes take place and restore the certain level of genetic diversity which is necessary for alien species to invade native ecosystems at a new range (ELLSTRAND and SCHIERENBECK, 2000; SCHIERENBECK and ELLSTRAND, 2009; WARD et al., 2009). The level of genetic diversity of populations is also shaped by plant life history traits, especially the breeding system (HAMRICK and GODT, 1996). Biological peculiarities of species may influence the threshold of genetic diversity necessary for expansion in a new area. In this work, we studied the level of genetic diversity in two alien herbal plant species invading native ecosystems in Lithuania. *Bunias orientalis* L. (Brassicaceae) is a perennial grass currently invasive in Central Europe

and common in Lithuania (GUDŽINSKAS, 1997; BIRNBAUM, 2006). It is native to Caucasus and southern Russia (BIRNBAUM, 2006). This outcrossing species is pollinated by different insects. *B. orientalis* can reproduce sexually by seeds and vegetatively via fragments of roots (STEINLEIN and DIETZ, 2002). In contrast, *Erigeron annuus* (Asteraceae) is an apomictic winter annual, producing large numbers of minute seeds that are genetically identical to the mother plant. This species is originated from North America. In the 17th century, it was introduced to Europe (EDWARDS et al., 2006). Most populations of *E. annuus*, however, contain several genotypes that can reproduce sexually (EDWARDS et al., 2006). Both species were introduced in Lithuania at the end of 19th century (GUDŽINSKAS, 1997) and now are in the phase of rapid spreading. To compare the genetic diversity in sexual (*B. orientalis*) and agamosperous (*E. annuus*) species, we used the RAPD technique which shows sufficiently high resolution in discriminating closely related genotypes of both species (DIETZ et al., 1999; EDWARDS et al., 2006).

2. Materials and methods

2.1 Population sampling

Plants were collected from six populations of *Bunias orientalis* and eight populations of *Erigeron annuus*. The total number of studied plants for each species was similar: 90 plants of *B. orientalis* and 89 plants of *E. annuus*.

2.2 DNA extraction, RAPD-PCR and electrophoresis

DNA was extracted from young leaves using cethyl-trimethyl-amonium bromide (CTAB; DOYLE and DOYLE, 1990). DNA samples were quantified with „BioPhotometer“ and diluted with TE buffer to concentration of 10 ng/μL. Each 20 μL RAPD-PCR reaction volume contained 2 μL 10xPCR buffer, 200 μM dNTPs, 1 unit Taq polymerase, 300 μM MgCl₂, 0.4 μM of the primer and 20 ng of template DNA. The RAPD-PCR were carried out for 4 min at 94 °C, followed by 45 cycles of 1 min at 94 °C, 1 min at 35 °C, and 1 min at 72 °C, followed by a final extension step of 5 min at 72 °C. RAPD-PCR products were resolved on a 1.5 % agarose gel (4 V/cm for 4 h) and stained with ethidium bromide. All reactions were repeated at least twice. A negative control sample containing all components of PCR mixture except the genomic DNA was included to ensure that no contamination occurred. Only clear and reproducible DNA bands were scored for analysis. To increase the accuracy of scoring, RAPD profiles of five individuals of different populations were analysed side-by-side in the same agarose gel.

2.3 Data analysis

Amplified bands were scored in a size range from 0.46 to 2.1 kb. A binary data matrix of RAPD phenotypes was constructed. The presence of the DNA fragment (allele) was represented with “1” and the absence was represented with “0”. Differences in RAPD phenotypes of study individuals were used to calculate genetic distance. The POPGENE v.1.32 software (YEH et al., 1999) was used to calculate parameters of molecular diversity within populations: Nei’s gene diversity, the percentage of polymorphic loci (P) and Shannon’s diversity index (I). An unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on pairwise genetic distances (NEI and LI, 1979) was used to assess genetic relationships among accessions and to present the results as dendrograms. Bootstrap analysis was performed using TRECON for Windows (VAN DE PEER and DE WACHTER, 1994) with 1000 replicates to evaluate the reliability of the dendrograms topology.

3. Results

3.1 Genetic diversity estimates of *B. orientalis*

Using RAPD analysis with three primers, we identified 42 reliable DNA bands in *B. orientalis* (Tab. 1). The number of RAPD bands resolved by one primer ranged from 13 to 15. 66 % of loci polymorphic was at species level. The size of DNA bands varied from 520 to 1500 bp. The polymorphism of plants from individual populations was lower. 60.7 % of polymorphic bands were detected in the Belmontas population, 39.3 % in the Seredžius population, 42.7 % in the Darsūniškis population, 46.4 % in the Viduklė population, 42.9 % in the Vilnius population and 39.3 % in the Vilkpėdė population (Tab. 2).

Tab. 1 Sequences of RAPD primers used in the study of *B. orientalis* and polymorphism of scored DNA bands.

Tab. 1 Für die Untersuchungen von *B. orientalis* verwendete RAPD-Primer mit jeweiligen Primersequenzen und Polymorphismus von ausgewählten DNA-Fragmenten.

Primer	Sequence 5'→3'	Size of DNA bands (bp)	Number of DNA bands		Polymorphism, %
			polymorphic	monomorphic	
Roth A03	AGTCAGCCAC	520-1150	12	3	80
Roth A04	AATCGGCTG	580-1100	10	4	71
Roth A05	AGGGGTCTG	800-1500	6	7	46
		Sum	28	14	
		Average	9.33±3.06	4.67±2.08	66±17.62

The mean value of polymorphism within populations was $45.24 \pm 8.0\%$. The summary value of genetic variation of Shannon index for all loci determined on the basis of RAPD analysis was 0.227 ± 0.036 . The highest level of DNA polymorphism was exhibited by the Belmontas population, the lowest variability was detected in the Vilkpėdė and Seredžius populations. The extreme values of Nei's genetic diversity were established also in the Belmontas (0.185) and Vilkpėdė population (0.135). The UPGMA cluster analysis was carried out on the basis of estimated genetic distances and dendrograms were drawn which show the genetic relationships among individual plants studied (Fig.1). The RAPD-based dendrograms show that clusters are heterogeneous; they are composed of small groups of genetically related individuals. Some plants from different populations showed identical RAPD phenotypes, which means that the level of DNA polymorphism in populations was not high. On the other hand, the use of three primers could be insufficient to discriminate all individuals. The clustering of genotypes in the dendrograms was independent of geographical distance among populations.

Tab. 2 Estimates of genetic diversity at RAPD loci of *B. orientalis* populations.

Tab. 2 Genetische Variabilität der RAPD-Loci von *B. orientalis*-Populationen.

Population	Plants per population	Number of RAPD phenotypes	P ¹ %	h ²	I ³
Belmontas	20	18	60.7	0.185	0.280
Seredžius	20	14	39.3	0.116	0.178
Darsūniškis	14	14	42.7	0.145	0.217
Viduklė	14	13	46.4	0.167	0.248
Vilnius	8	7	42.9	0.165	0.240
Vilkpėdė	14	9	39.3	0.135	0.201
Average			45.24±8.0		
Summary of genetic variation statistics for all loci				0.152±0.025	0.227±0.036

1 – percentage of polymorphic loci, 2 – Nei's (1973) gene diversity, 3 – Shannon's information index

3.2 RAPD variation in *E. annuus*

70 RAPD bands were detected by the three primers in 89 plants of *E. annuus*. Approximately 77 % of these bands were polymorphic at species level (Tab. 3).

Tab. 3 Sequences of the RAPD primers used in the study of *E. annuus* and the polymorphism of scored DNA bands.

Tab. 3 Für die Untersuchungen von *E. annuus* verwendete RAPD-Primer mit den jeweiligen Sequenzen und Polymorphismus von gewählten DNA-Fragmenten.

Primer	Sequence 5'→3'	Size of DNA bands (bp)	Number of DNA bands		Polymorphism, %
			polymorphic	monomorphic	
Roth A 03	AGTCAGCCAC	460-1700	20	3	87
Roth A 04	AATCGGGCTG	480-1750	19	7	73
Roth A 05	AGGGGTCTTG	460-2100	15	6	71
		Sum	54	16	
		Average	18±2.65	5.33±2.08	77±8.72

The size of scored bands was in the range of 460-2100 bp. The comparison of the study populations showed that only half of them were polymorphic (Užutrakis, Vilnius B, Jurbarkas, Lielius). The other four populations (Vilnius A, Vilnius C, Kulautuva, Betygala) were monomorphic (Tab. 4).

Tab. 4 Estimates of genetic diversity at RAPD loci of *E. annuus* populations.

Tab. 4 Genetische Variabilität der RAPD-Loci von *E. annuus*-Populationen.

Population	Plants per population	Number of RAPD phenotypes	P ¹ %	h ²	I ³
Užutrakis	20	2	46.3	0.183	0.270
Vilnius A	20	1	0	0	0
Vilnius B	14	2	61.1	0.253	0.370
Jurbarkas	14	2	44.4	0.122	0.188
Lielius	8	3	27.8	0.115	0.165
Vilnius C	14	1	0	0	0
Kulautuva	12	1	0	0	0
Betygala	12	1	0	0	0
Average			22.5±25.6		
Summary of genetic variation statistics for all loci				0.0841±0.2	0.1242±0.146

1 – the percentage of polymorphic loci, 2 – Nei's (1973) gene diversity, 3 – Shannon's information index

The UPGMA dendrogram constructed using the Nei and Li (1979) genetic distance coefficient is shown in Figure 1. Among 89 plants included in this study, only 10 RAPD phenotypes (clones) were identified. One RAPD phenotype was identified in four populations. Vilnius A, Vilnius C and Kulautuva populations were composed of the same clone. This RAPD phenotype was detected also in Lielius population together with two more RAPD phenotypes. Other populations consisted of one to three clones. For example, the Betygala population was made of one clone whereas the Vilnius B population was made of two clones.

4. Discussion

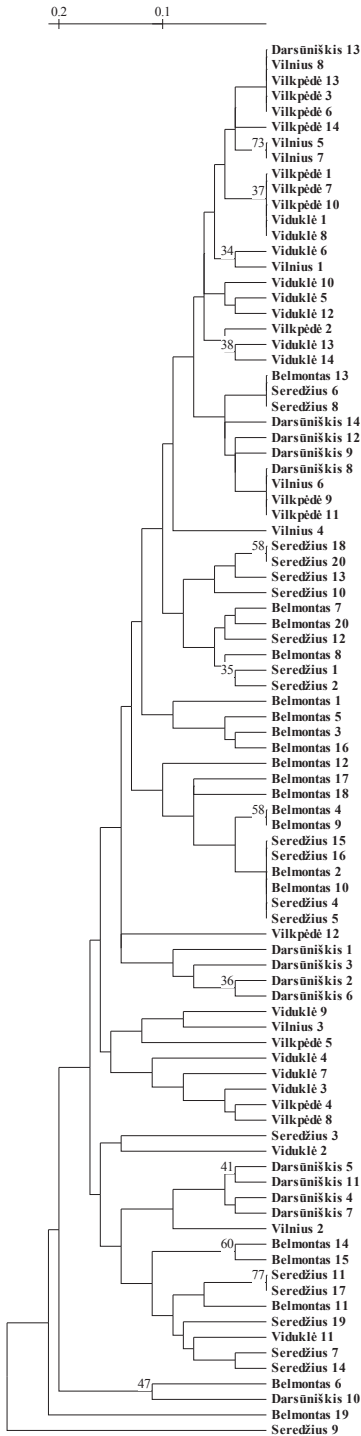
Study of genetic variation in two alien species with different mode of reproduction and similar history of spreading in Lithuania showed rather high level of RAPD loci polymorphism at species level in both species: *B. orientalis*: 66 % and *E. annuus*: 77 %. In the present study, we used a rather small number of primers for the analysis. Nevertheless, these three primers were preselected from 26 primers by the

number and reproducibility of DNA bands. Using these primers, 42 RAPD bands were identified in *B. orientalis* and 70 RAPD bands in *E. annuus*. Similar number of primers was used by EDWARDS et al. (2006) in the study of North American and European populations of *E. annuus*. These authors analyzed 39 stable polymorphic RAPD bands produced using two primers. The number of identified RAPD phenotypes in populations of sexually reproducing species *B. orientalis* and apomixtic species *E. annuus* was very different. 67 RAPD phenotypes were identified among 90 plants of *B. orientalis*, while in *E. annuus*, only 10 RAPD phenotypes were identified among 89 accessions. Average polymorphism of RAPD loci in populations of *B. orientalis* was also two times higher (45.2 %) than in populations of *E. annuus* (22.5 %). Half of the populations of *E. annuus* analyzed in this study were monomorphic. Other four populations consisted of 2-3 clones (Fig. 2). It is worth noting that genetic distances (GD_{xy}) among these clones were high. For this reason, DNA polymorphism of RAPD loci in such polymorphic populations was also rather high, e. g. 61.1 % in Vilnius B population which consisted of two clones (GD_{xy} = 0.72). However, in spite of lack of genetic diversity in most populations, the contemporary situation of this species in Lithuania shows very good performance in local conditions. *E. annuus* is in a phase of intensive spreading which started in Lithuania about three decades ago. Apomixis in this species may maintain so called 'general purpose' genotypes with broad environmental tolerance which enables *E. annuus* to colonize a wide range of habitats (BAKER, 1965; TRTIKOVA et al., 2011). It is well known that, despite the reduction of genetic variation, some invasive populations show increased growth rate and intensive expanding (LI et al., 2006; DLUGOSCH and PARKER, 2008). The spreading of *B. orientalis* also takes place in Lithuania. In the third volume of the Flora of the Lithuanian SSR (1961), this species is considered as rather rare. Now it is common in some habitats such as flooded meadows and railways surroundings (GUDŽINSKAS, 1997; BIRNBAUM, 2006). Obviously, the success or failure of establishment of a species in a given environment depends on the interaction of the genotype and the environment (ELLSTRAND, 2009). There are many factors influencing invading process and they may differ among species. However genetic diversity as a parameter of species genetic pool in given territory is important for the invasion. Our pilot study shows that the level of genetic diversity at which the species can start invasive spreading in to native ecosystems may be species specific.

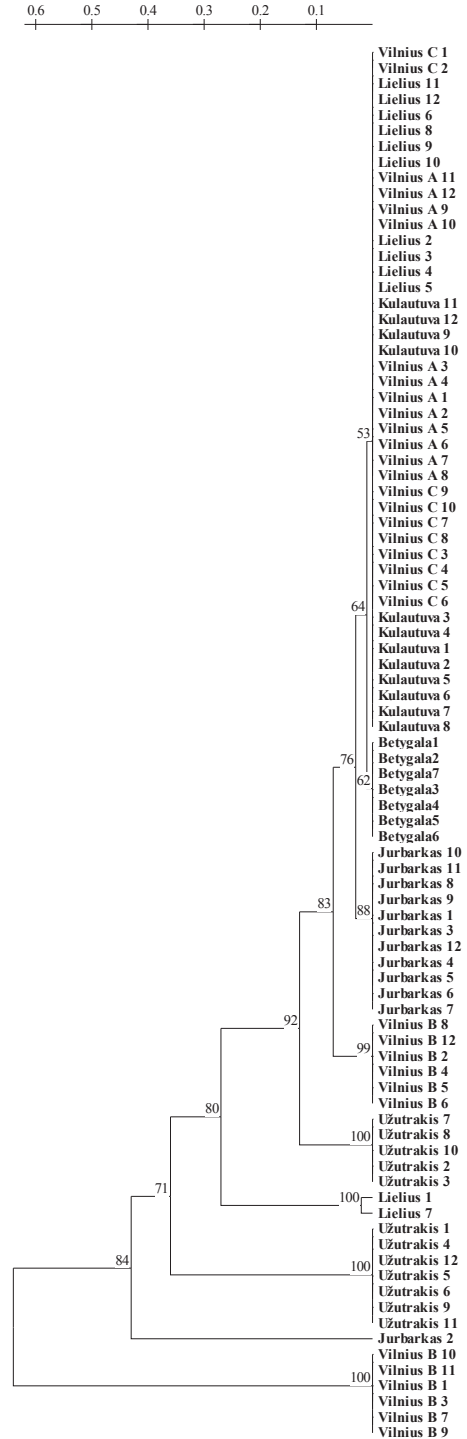
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Fig. 1 UPGMA dendrograms of *B. orientalis* (A) and *E. annuus* (B) accessions, constructed using RAPD based genetic distance matrixes, estimated according to NEI and LI (1979). The top x-axis indicates values of Nei and Li's genetic distance coefficient. The numbers on the branches indicate bootstrap values above 30 % (1000 replications).

Abb. 1 UPGMA-Dendrogramme für *B. orientalis* (A) und *E. annuus* (B) zusammengestellt anhand der durch RAPD erhaltenen genetischen Distanzmatrixen und berechnet nach NEI und LI (1979). Die x-Achse zeigt die Werte der Koeffizienten der genetischen Distanz von Nei und Li. Zahlen über den Dendrogrammzweigen bezeichnen bootstrap-Werte über 30 % (1000 Wiederholungen).



A



B

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