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Different Habitats Show Similar Genetic Structure of *Bunias orientalis* L. (Brassicaceae) in Lithuania

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Abstract

We studied genetic diversity within and among populations of warty cabbage (*Bunias orientalis* L.), which is an alien species in Lithuania and other Baltic countries. In Lithuania, this weed colonises two main types of habitats: railway/roadsides and meadows on riversides. The aim of this study was to assess the genetic structure of invasive populations of *B. orientalis* in Lithuania and consider the impact of diverse habitats on the partitioning of genetic diversity using inter-simple sequence repeat (ISSR) markers. An analysis of molecular variance (AMOVA) carried out on the basis of ISSR showed that there is high genetic differentiation (46%) among populations of *B. orientalis*, which is probably caused by the founder effect and limited gene flow. However, we observed no impact of habitat on the genetic difference among populations. Similar levels of ISSR polymorphic loci were observed in riverside (P = 31.67%) and railway/roadsides (P = 30.51%) populations. UPGMA cluster analysis and principal coordinate analysis (PCoA) also did not show grouping of studied populations according to habitat type. High genetic differentiation among populations, as indicated by ISSR markers, confirm multiple independent introductions of this species in Lithuania.

Keywords: alien species, biological invasions, invasion history, molecular markers

Introduction

Biological invasion is a multistage process influenced by many factors (Sakai *et al*., 2001). Intra-specific genetic variation may contribute significantly to the invasiveness of an alien species (Wang *et al*., 2012; Ward *et al*., 2008). The founding events usually establish only a fraction of the genetic variance that occurs in the source population(s) (Dlugosch and Parker, 2008; Nei *et al*., 1975). In spite of frequent loss of genetic diversity, populations of some invaders may evolve rapidly (Andrew *et al*., 2012; Dlugosch and Parker, 2008; Ellstrand and Schirenbeck 2000) and adapt to local conditions (Riis *et al*., 2010).

Our study focuses on *B. orientalis*, which is commonly referred to as warty cabbage, hill mustard or Turkish rocket. It is native to the south-west part of Russia, the Caucasus, and western Siberia, where it grows in grasslands, at the sunny edges of forests, in dry meadows and near rivers (Birnbaum, 2006; Laiviņš *et al*., 2006). *B. orientalis* is invasive agricultural weed in Central Europe and expands to other parts of the continent (Dietz *et al*., 1999; Mirek and Piękoš-Mirkowa, 1996). The species is also spreading in North America and Asia. In Baltic countries, especially Latvia and Estonia, *B. orientalis* is common (Birnbaum, 2006; Laiviņš *et al*., 2006). The first record of this species in Lithuania is from 1898 (Gudžinskas, 1997). At present, *B. orientalis* is not evenly spread through the whole country. It usually colonises two types of habitats: railways/roadsides and meadows on riversides. These two *B. orientalis* locations could be influential in determining the means by which the species spreads, which may result in local adaptation of genotypes from populations localised in these habitats. The first type of habitat, railways/roadsides, is strongly influenced by human activity and is more or less artificial, while a fraction of the populations in meadows on riversides in Lithuania are localised in more natural or semi-natural habitats. Ecological differences between habitats may result in genetic structuring of populations through the action of selection or through limited gene flow (Ward, 2006). On the other hand, roads and river valleys are well known as corridors for the spreading of invasive plant species (Christen and Matlack, 2006; Galil *et al*., 2007; Hejda and Pyšek, 2006). To assess the level of genetic differentiation among populations and the possible impact of habitat on genetic differentiation of *B. orientalis* populations, we used DNA markers as valuable

tools in invasive plant population studies (Jasieniuk and Maxwell, 2001). These techniques have also been applied in studies of genetic differentiation among populations existing in different ecological conditions (Owuor *et al*., 1997; Patamsytė *et al*.; 2010; Reisch *et al*., 2003, 2005; Trtikova *et al*., 2011). In our previous study, we identified molecular markers suitable for genomic DNA analysis of *B. orientalis* using the RAPD and ISSR techniques (Patamsytė *et al*., 2011). RAPD and ISSR assays do not require sequence information for primer synthesis; these techniques are quick and usually identify many polymorphic loci. Moreover, ISSRs exhibit the specificity of microsatellite markers and amplification at higher-stringency conditions, which enables high reproducibility of this assay (Ge *et al*., 2003; Słomka *et al*., 2011). Microsatellite markers usually considered to be selectively neutral, are known to be linked to coding regions and possibly mark gene rich regions (Kojima *et al*., 1998; Reddy *et al*., 2002). In this study, we used ISSR to answer the following questions: Is there genetic differentiation among populations of *B. orientalis*? How does the genetic diversity correlate with habitat type of study populations? Is there differentiation among populations from different habitats that differ in ecological conditions? Can we consider the spreading of *B. orientalis* in Lithuania to be the result of multiple introductions?

Material and methods

Plant material

Bunias orientalis (L.) is a diploid (2n=14), mainly outcrossing perennial grass (Dietz *et al*., 1999). To study the genetic diversity within and among populations of *B. orientalis*, nineteen populations from the central and eastern regions of Lithuania (where this species is mainly spread) were chosen (Tab. 1). Plants from populations that were predominantly localised in two different habitats – riverside meadows and railway/roadsides – were collected. These two types of habitat vary according temperature, water fluctuations and environmental pollution. Characteristic properties of studied riverside populations are high water and low temperature fluctuations during growing season and low environmental pollution. In contrast high fluctuations of temperature, environmental pollution, and low fluctuations of water are typical for railway/roadside habitats. Young, disease-free, undamaged leaves were collected from at least 8 different plants per population. A total 309 of plants were sampled and analysed in this study.

DNA isolation and ISSR-PCR analysis

The samples (approximately 100 mg fresh leaves) were processed on-site using Bashing Bead™ and Xpedition™ Sample Processor Technologies (Zymo Research). The samples were homogenised in Xpedition™Lysis/Stabilization solution, and genomic DNA was isolated in a laboratory using a Xpedition™ Plant/Seed DNA Mini prep kit (Zymo Research) following the manufacturer instruc-

Tab. 1. Populations of *Bunias orientalis* analysed in this study and they habitats

Population			Site coordinates	Sample size	Habitat
No.	Name	Code			
Riverside					
1	Vilnius A	VILA	54°40'51", 25°20'45"	20	Meadow near river
2	Seredžius A	SERA	55°04'47", 23°25'31"	20	Meadow near river
3	Darsūniškis	DAR	54°44'14", 24°07'06"	14	Meadow near river
4	Seredžius B	SERB	55°04'19", 23°21'21"	15	Meadow near river
5	Pamerkiai	PAM	54°24'59", 24°54'45"	20	Riverside
Railway/roadside					
6	Viduklė	VID	55°24'56", 22°50'52"	14	Meadow near railway line
7	Vilnius B	VILB	54°38'09", 25°12'40"	14	Railway bed
8	Vokė	VOK	54°38'01", 25°07'45"	12	Meadow near railway line
9	Kaišiadorys	KAI	54°51'46", 24°28'01"	11	Railway bed
10	Lazdėnai	LAZ	54°44'45", 24°56'45"	18	Meadow near railway line
11	Kariotiškės	KAR	54°40'35", 25°00'22"	9	Railway bed
12	Marijampolė	MAR	54°33'31", 23°21'59"	8	Railway bed
13	Obeliai	OBE	55°57'11", 25°49'15"	20	Meadow near road
14	Molėtai	MOL	55°13'17", 25°26'10"	20	Meadow near highway
15	N. Vilnia	NVIL	54°41'39", 25°26'53"	20	Railway bed and meadow near railway
16	Žąsliai	ZAS	54°50'16", 24°34'59"	22	Railway bed and meadow near railway
17	Lentvaris	LEN	54°38'40", 25°03'19"	20	Bush and meadow near railway
18	Kaunas	KAU	54°52'51", 23°56'04"	20	Railway bed
19	Kurniškės	KUR	54°51'45", 24°30'47"	12	Railway bed

tions. Five ISSR primers, selected in previous study and generating clear and reproducible ISSR banding patterns, were chosen for the analysis of *B. orientalis* (Patamsytė *et al*., 2011). ISSR-PCR was performed in a 10 µl reaction volume containing approximately 10 ng of genomic DNA, 1 unit recombinant Taq DNA polymerase (Thermo Fisher Scientific Baltics), 1µl 10·PCR buffer with KCl (100 mM Tris-HCl, 500 mM KCl, 0.8% Nonidet P40), 300 µM $MgCl_2$, 200 µM dNTPs and 0.4 µM of the primer. The PCR program consisted of an initial 7 min denaturation at 94 °C, followed by 32 cycles of 30 s at 94 °C, 45 s at a primer-specific temperature, and 120 s at 72 °C. The last reaction extension step was performed at 72 °C for 7 min. ISSR-PCR products were separated on 1.5% agarose gels stained with ethidium bromide. The GeneRuler™ DNA Ladder Mix, 100-10000 bp (Thermo Fisher Scientific Baltics), was used for DNA band sizing. All of the samples were analysed at least twice in different ISSR-PCR experiments. Samples from a few different populations were usually analysed within the same gel, and the monomorphic bands, though not included in population variability data analysis, were used as size and reproducibility criteria to ensure correct loci scoring among populations. All of the amplifications also contained negative control (without DNA) samples. We also applied the data evaluation recommendations of Bonin *et al*. (2004) to calculate the error rate in the genotyping analysis and followed the suggestions of these authors to minimise this error. The error rate was 6.4% (169 differences in 2640 comparisons).

Statistical analysis

ISSR profiles were documented using the BioDocAnalyse gel documentation system (Biometra, Germany). An example of typical ISSR profiles detected with different primers is shown in Fig. 1. Each clear and reproducible DNA fragment was scored across all samples as either present (1) or absent (0). Based on these data, a binary matrix was generated. The parameters of molecular diversity within populations (the percentage of polymorphic loci (P), Nei's gene diversity ($h=1-\Sigma p^2_i$), Shannon's information index ($I=-\Sigma p_i \log_2 p_i$), coefficient ofgenetic differentiation ($G_{ST} = (H_T-H_S)/H_T$) and gene flow ($Nm=0.5(1-G_{ST})/G_{ST}$) were estimated using the computer program POPGENE version 1.32 (Yeh and Boyle, 1999). This program was also used for cluster analysis, which was carried out using Nei's genetic distance with an unweighted pair group method and arithmetic mean (UPGMA).

To estimate pairwise genetic distances (Φ_{ST}) between populations and their levels of significance, analysis of molecular variance (AMOVA) was carried out. The binary matrix based on ISSR profiles was used in AMOVA with the computer program GenAIEx version 6.5 (Peakall and Smouse, 2012). For each analysis, 999 permutations were carried out to obtain significance levels. To assess possible habitat influence on genetic differentiation among populations, an additional AMOVA was performed, in which the grouping of populations was based on their reliance on particular habitats. For correlation analyses, the STATIS-

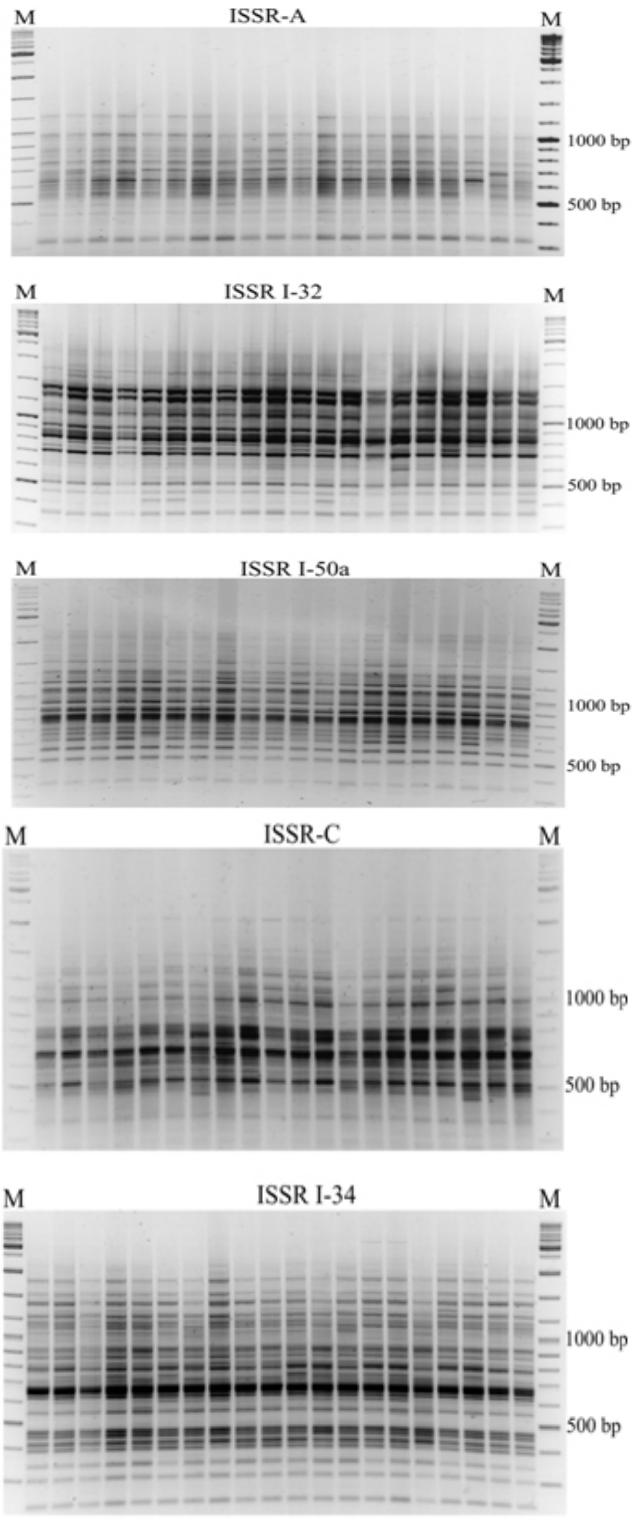

Fig. 1. ISSR profiles obtained from individual plants of *B. orientalis* using five oligonucleotide primers (ISSR-A, ISSR-C, ISSR I-32, ISSR I-34, ISSR I-50a). M-molecular size marker (The GeneRuler™ DNA Ladder Mix, 100-10000 bp)

TICA version 8.0 program (StatSoft. Inc., 2008; www.statsoft.com) was used.

Results

ISSR polymorphism at species level

The genetic variability study of *B. orientalis* was based on an analysis of 309 individuals from 19 populations (approx. 16 individuals/population) located in two main habitat types. Eighty-eight reliable ISSR loci were identified in this study using five preselected primers (Tab. 2). The average percentage of polymorphic loci at species level was 55%. In spite of moderate genetic diversity, 299 genotypes were identified among all studied plants. Ten genotypes that had two identical clones were identified in six populations (MOL, ZAS, PAM, VID, LEN and KUR). No clones were shared between populations. The size of scored DNA fragments ranged from 390 to 1800 bp. The used primers generated between 40% and 64.71% polymorphic loci. Primers based on dinucleotide repeats (ISSR-A and ISSR-C) revealed the highest polymorphism. This parameter was highest (64.71%) with the ISSR-C primer based on (AG) repeat.

Molecular diversity within populations

The number of polymorphic loci displayed per population ranged from 5 (KUR) to 21 (LAZ), and the percentage of polymorphic bands per population ranged from 10.42% to 43.75% respectively (Tab. 3). The mean level of polymorphic loci in analysed populations of *B. orientalis* was 30.81%. The Nei's gene diversity values and Shannon's information index values were also lowest in the KUR population (h = 0.039; I = 0.058) and highest in the LAZ population (h = 0.164; I = 0.243). In the small KUR population, only nine different genotypes were identified among twelve studied individuals. In this population, three distinct ISSR patterns were each shared by a different pair of plants sampled near each other. In the LAZ population, all 18 individuals were genetically different. The MOL population also showed low genetic diversity (P = 20.83%; h = 0.081; I = 0.119). The ZAS population contained more genotypes than any other population examined in this study: 21 genotypes were identified among 22 studied individuals. Some of the 48 polymorphic loci were fixed in certain populations. For example, the ISSR-A_{970} locus was fixed in 15 populations, and the ISSR-C_{850} locus was fixed in all except one MAR population (data not shown).

Genetic differentiation of populations

The mean G_{ST} for all loci was 0.507, which indicates that 50.7% of the genetic diversity occurs among populations and 49.3% occurs within populations (data not shown). The gene flow estimated from G_{ST} was 0.487. Similar results were obtained with AMOVA. AMOVA revealed that 46% of the total genetic variance occurred among populations and that 54% occurred among individuals within populations (Tab. 4). The variation in ISSR profiles among and within populations was highly significant (P< 0.001). The pairwise genetic differentiation of populations did not reveal reliance on geographic distance (r = −0.08; P = 0.05). The lowest Φ_{ST} = 0.10 was established between the SERA and ZAS populations (Tab. 5). The largest Φ_{ST} value was 0.72, between VOK and KUR populations. Upon arranging the *B. orientalis* populations in two groups according to their origin (meadows on riversides and railway/roadsides) (Tab. 1), no molecular differentiation among populations from different habitats was observed. This same result was obtained when these two groups were different in size (5 riverside populations versus 12 railway/roadsides populations) and when they were equal in size (5 riverside and 5 arbitrarily chosen railway/roadsides populations). Similar levels of ISSR polymorphism were observed in riverside (P = 31.67%) and railway/roadsides populations (P = 30.51%) (Tab. 3).

The relationships among individuals and populations were also analysed using UPGMA cluster analysis and principal coordinate analysis (PCoA). Neither analysis revealed any grouping of populations according to geographic location or habitat type. The UPGMA dendrogram produced using Nei's genetic distance is shown in Fig. 2. The dendrogram consists of three clusters and a group of three populations (KUR, SERB and MAR) located separately from these clusters. All clusters are heterogeneous and

Tab. 2. Oligonucleotide primers used and ISSR loci revealed in *Bunias orientalis*

Primer	Primer sequence (5'-3')	Annealing temperature (°C)	Approx. size of DNA fragments (bp)	No. of loci	No. of polymorphic loci	P (%)
ISSR-A	$CTC(GT)_8$	51	570-1200	11	7	63.63
ISSR-C	$(AG)_8TG$	51	490-1250	17	11	64.71
ISSR I-50a	$CCA(GCT)_4$	46	480-1500	20	8	40.00
ISSR I-34	$(AGC)_4GG$	46	390-1800	25	15	60.00
ISSR I-32	$(AGC)_4C$	39	470-1450	15	7	46.67
			Total	88	48	
			Mean ± SE	17.60 ± 2.36	9.60 ± 1.54	55.00 ± 4.94

P (%): percentage of polymorphic loci; SE: standard error

consist of populations from different habitat types. For example, the first cluster includes two riverside populations (VILA and DAR) and four railway/roadside populations (VID, KAI, NVIL and VOK). The second cluster includes two riverside populations (SERA and PAM), three railway populations (ZAS, VILB and LAZ) and two populations sampled in meadows near highways (MOL and OBE). The third small cluster includes exclusively railway/roadsides populations (KAR, LEN and KAU). The remaining unclustered populations also originate from different habitat types. Populations sampled in KUR and MAR are located near the railway, while the SERB population is located near a river. The PCoA also shows a similar heterogeneous pattern of population grouping (Fig. 3). The population grouping in the PCoA plot was similar to that in the UPGMA dendrogram. PCoA revealed no grouping by habitat type. For example, two geographically related riverside populations, SERA and SERB, are located separately in both the UPGMA dendrogram and PCoA plot. Likewise, the MAR, SERB and KUR populations are most differentiated from the main group of populations according to both analyses. In the PCoA of individual genotypes, approximately 44.81% of the total variation is described by the first two axes. This assay revealed that genotypes of the same population are grouped in rather compact groups; however, there is considerable population overlap (data not shown).

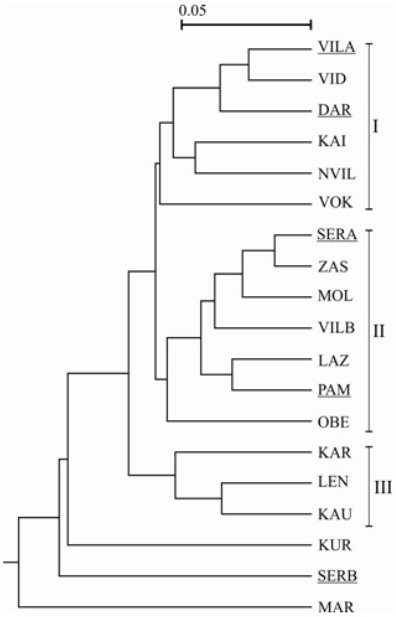

Fig. 2. UPGMA dendrogram constructed using genetic distance matrix based on data from 48 polymorphic ISSR markers identified on 309 *B. orientalis* individuals. Riverside populations codes are underlined. Population codes are given as in Tab. 1

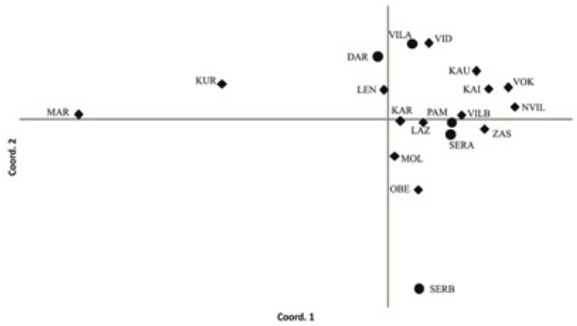

Fig. 3. Principal coordinate analysis (PCoA) plot based on ISSR markers showing similarity and genetic variation among nineteen populations of *B. orientalis*. Diamonds indicate railway/roadside populations, circles – riverside populations. Population codes are given as in Tab. 1

Tab. 3. Estimates of genetic diversity parameters of nineteen populations of *B. orientalis*. Population codes are given as in Tab. 1

Populations	P (%)	Number of polymorphic loci	h	I
Riverside				
VILA	31.25	15	0.124	0.182
SERA	41.67	20	0.167	0.245
DAR	29.17	14	0.104	0.155
SERB	33.33	16	0.109	0.165
PAM	22.92	11	0.096	0.139
Mean ± SE	31.67 ± 3.05	15.20 ± 1.46	0.120 ±0.013	0.177 ± 0.018
Railway/roadside				
VID	31.25	15	0.121	0.178
VILB	27.08	13	0.110	0.161
VOK	27.08	13	0.109	0.159
KAI	29.17	14	0.111	0.163
LAZ	43.75	21	0.164	0.243
KAR	25.00	12	0.105	0.151
MAR	27.08	13	0.115	0.165
OBE	33.33	16	0.146	0.209
MOL	20.83	10	0.081	0.119
NVIL	39.58	19	0.141	0.211

Populations	P (%)	Number of polymorphic loci	h	I
ZAS	29.17	14	0.112	0.163
LEN	41.67	20	0.137	0.206
KAU	41.67	20	0.162	0.239
KUR	10.42	5	0.039	0.058
Mean ± SE	30.51 ± 2.44	14.64 ± 1.17	0.118 ± 0.009	0.173 ± 0.013
Mean of all populations	30.81 ± 1.93	14,79 ± 0.93	0.119 ± 0.007	0.174 ± 0.011

P (%): percentage of polymorphic loci; h: Nei's gene diversity; I: Shannon's information index; SE: standard error

Tab. 4. Analysis of molecular variance (AMOVA) for 309 individuals of *B. orientalis* sampled from nineteen populations using 48 markers

Source of variation	df	SS	MS	Est. Var.	P-value	%
Among regions	1	44.934	44.934	0.000		0
Among populations	17	692.073	40.710	2.357	<0.001	46
Within populations	290	798.016	2.752	2.752	<0.001	54
Total	308	1535.023		5.109		100

df: degrees of freedom; SS: sum of squares; MS: mean squares; Est. Var.: variance component estimates; P-value: significance (P < 0,001) based on permutation (999 replicates); %: percentage of total variance

Tab. 5. Pairwise genetic distance (Φ_{ST}) and geographic distance among populations of *B. orientalis* included in this study (Φ_{ST} values are shown below the diagonal and geographic distance in kilometres are shown above diagonal)

	VILA	SERA	DAR	SERB	PAM	VID	VILB	VOK	KAI	LAZ	KAR	MAR	OBE	MOL	NVIL	ZAS	LEN	KAU	KUR	
VILA		131.6	79.5	135.0	40.8	179.4	10.2	15.0	60.3	26.8	22.6	128.7	144.1	59.6	6.7	52.0	19.7	93.6	57.3	VILA
SERA	0.39		59.4	4.6	121.6	51.7	125.7	120.9	71.3	104.9	111.5	59.5	179.4	129.1	137.0	79.6	115.2	39.8	74.3	SERA
DAR	0.24	0.35		61.5	62.5	110.9	71.7	66.4	26.9	53.6	57.3	52.5	173.0	99.9	85.8	32.0	60.8	20.4	29.7	DAR
SERB	0.61	0.37	0.59		124.0	50.3	129.0	124.1	74.8	108.3	114.7	57.5	184.0	133.6	140.5	83.0	118.4	42.5	77.8	SERB
PAM	0.41	0.35	0.49	0.55		172.8	30.9	27.9	57.9	37.1	28.3	101.3	180.0	95.0	46.7	51.1	27.4	81.9	56.3	PAM
VID	0.20	0.42	0.39	0.60	0.436		174.4	169.8	119.7	153.0	160.5	102.0	195.4	165.1	184.0	128.0	164.3	91.0	122.4	VID
VILB	0.41	0.26	0.44	0.53	0.47	0.42		5.4	54.7	21.5	14.4	119.7	151.4	66.4	16.8	46.4	10.9	87.0	51.9	VILB
VOK	0.41	0.34	0.40	0.57	0.58	0.42	0.40		50.1	17.5	9.4	114.3	152.8	67.7	21.7	41.8	5.7	82.0	47.4	VOK
KAI	0.38	0.35	0.35	0.54	0.51	0.41	0.32	0.36		33.6	40.9	79.0	147.7	73.0	65.8	8.4	44.6	34.2	3.2	KAI
LAZ	0.40	0.26	0.33	0.47	0.31	0.42	0.35	0.37	0.40		9.8	104.4	144.4	60.4	32.6	25.3	12.9	66.9	30.7	LAZ
KAR	0.48	0.28	0.46	0.55	0.54	0.51	0.34	0.46	0.44	0.37		106.1	152.0	67.2	29.2	32.6	3.7	72.6	38.2	KAR
MAR	0.56	0.56	0.54	0.65	0.66	0.55	0.58	0.64	0.61	0.52	0.57		220.1	151.7	135.3	84.4	109.1	52.0	81.9	MAR
OBE	0.44	0.31	0.45	0.44	0.50	0.44	0.29	0.48	0.38	0.37	0.41	0.44		85.1	141.1	146.7	152.6	168.1	145.9	OBE
MOL	0.52	0.21	0.46	0.40	0.50	0.55	0.42	0.51	0.50	0.39	0.40	0.59	0.37		57.5	69.0	67.7	102.6	70.3	MOL
NVIL	0.31	0.37	0.40	0.50	0.42	0.27	0.41	0.41	0.27	0.44	0.49	0.57	0.34	0.48		57.6	26.3	99.5	62.7	NVIL
ZAS	0.47	0.10	0.41	0.43	0.35	0.47	0.29	0.39	0.36	0.32	0.40	0.61	0.39	0.29	0.37		36.3	42.0	5.7	ZAS
LEN	0.59	0.44	0.51	0.61	0.58	0.59	0.53	0.53	0.49	0.47	0.41	0.61	0.55	0.49	0.53	0.43		76.3	41.9	LEN
KAU	0.44	0.38	0.37	0.57	0.56	0.45	0.43	0.37	0.36	0.42	0.31	0.58	0.47	0.46	0.43	0.42	0.31		37.4	KAU
KUR	0.67	0.57	0.60	0.70	0.69	0.69	0.71	0.72	0.71	0.57	0.67	0.70	0.66	0.65	0.63	0.58	0.60	0.60		KUR

Discussion

In the study of *B. orientalis*, we observed rather different levels of genetic variation in the populations ranging from 10.42% (KUR population) to 43.75% (LAZ population). The mean intra-populational ISSR polymorphism was rather low (30.81%), which implies a possible reduction of genetic diversity during the process of invasion (Dlugosch and Parker, 2008). It is worthwhile to mention that there is no published analysis of the genetic structure of *B. orientalis* in its native range. However, previous plant population studies indicate that genetic variability strongly depends on the species life history traits (Nybom and Bartish, 2000). *B. orientalis*, as an allogamous and perennial plant species, should maintain a high level of genetic variability in its native range. Additionally, high polymorphism of RAPD loci in a single invasive population of *B. orientalis* was reported by Dietz *et al*. (1999). In our study, we determined that genetic variation was almost equally partitioned between and within populations using ISSR markers. The average G_{ST} estimate was 0.507, ranging from 0.172 to 0.992 for different loci (data not shown).

AMOVA also detected significant population differentiation. The obtained distribution of genetic variation shows deviation from the variance pattern established previously for outcrossing plant species. The estimate of population differentiation obtained in our study (46%) is higher than the average Φ_{ST} values reported by Nybom (2004) for outcrossers (0.27) and short-lived perennials (0.41). Increased genetic differentiation in *B. orientalis* populations can be explained by the impact of random genetic processes during invasion. Though *B. orientalis* is an outbreeding species, the plants are self-fertile (Birnbaum, 2006). The founder effect and lack of gene flow in the new range may increase the probability of inbreeding because a low density of plants at a given site restricts mating opportunities. In this situation, some alleles associated with incompatibility may also be lost (Byers, 1995). In our study, the mean gene flow (N_m) estimated from G_{ST} was 0.487. This N_m estimate indicates limited gene flow among populations (Li and Jin, 2006). The absence of correlation between geographic distance and pairwise Φ_{ST} of populations suggests multiple introductions of the species (Ge *et al*., 2003).

B. orientalis occupies two main types of habitats in Lithuania: riversides and railways/roadsides. The colonisation of these habitats may be associated with different ways of spreading of this alien species and/or the selection mechanism. The spreading of *B. orientalis* on riversides in Lithuania may be associated with the rather wide network of flow mills that were exploited until the middle of the 20th century and that employed water engines. The transportation and processing of grain and forage contaminated with warty cabbage seeds also may have promoted the establishment of founding populations of this species near railways and roadsides. The role of the transport network as one of the most important factors in spreading has been emphasised for *Impatiens glandulifera*, another alien species in Lithuania (Zybartaite *et al*., 2011). The two types of *B. orientalis* habitat are rather different regarding water availability, light and the impact of environmental pollution. In some previous studies, it was found that the ecological properties of habitats influenced population genetic structure (Owuor *et al*., 1997; Patamsytė *et al*., 2010; Reisch *et al*., 2003). On the other hand, other publications do not show evidence of adaptive genetic differentiation by means of molecular markers (Holderegger *et al*., 2006;Volis *et al*., 2003). The finding that there was no evidence of differentiation (AMOVA, UPGMA and PCoA results) in populations of *B. orientalis* colonising different habitats could also mean that both habitats are equally prone to plant invasions, and show the same "corridor" structure.

The specific vegetation conditions of railway/roadside populations, which experience increased environmental pollution, may result in the appearance of new DNA banding patterns due to a genotoxic effect of environmental pollutants (Čėsnienė *et al*., 2010; Fedorova *et al*., 2007; Słomka *et al*., 2011). However, we did not find higher ISSR variation in plants growing near railways or roads in comparison with plants growing on riversides.

Conclusions

Our study confirms multiple introductions of *B. orientalis* in Lithuania. An analysis carried out using ISSR markers revealed that there is high genetic differentiation among *B. orientalis* populations, which is probably caused by the founder effect and limited gene flow. However,we observed no impact of different ecological conditions on the distribution of ISSR markers and genetic differentiation among populations in two habitat types. Additional studies of genetic diversity in native populations of *B. orientalis* are necessary to elucidate the mechanisms of invasion for this species.

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