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RAPD analysis of genetic diversity among Lithuanian populations of *Impatiens glandulifera*

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Abstract

In some Asian countries, the seeds of Himalayan balsam or policeman's helmet, *Impatiens glandulifera* Royle are harvested as a food source. First introduced to Europe in 1838–1839, *I. glandulifera* quickly became a garden favourite and later on a prodigious weed. Nowadays *I. glandulifera* is highly invasive in almost whole Europe and occurs in various habitats. So far, little has been known about the genetic diversity of *I. glandulifera* in the Baltic region. The objective of this study was to evaluate the genetic variability of Lithuanian populations of *I. glandulifera* differing in geography or habitats by the randomly amplified polymorphic DNA (RAPD), using 8 primers. At the species level, all DNA bands (188) were polymorphic. Among populations of *I. glandulifera* genetic parameters ranged in the following intervals: 40–56% of polymorphic DNA bands, 0.115–0.165 for Nei's gene diversity, 0.179–0.255 for Shannon's information index. Pairwise genetic distances between populations ranged in the interval 0.088–0.259. AMOVA showed significant genetic differentiation of *I. glandulifera* populations in Lithuania ($\Phi_{PT} = 0.511$, $p \leq 0.01$). Percentage of polymorphic DNA bands (generated by RAPD primers) of the populations correlated negatively with the site's mean temperature for vegetation season ($r = -0.535$, $p < 0.015$). Only for one population (Palanga population located near the Baltic sea) out of 20, significant relations were found between this population genetic and geographic distances to the other populations ($r = -0.755$, $p < 0.001$). Genetic distance-based cluster analyses for 400 individuals indicated 4 major groups of populations, among which there was no clear geographical pattern. Our RAPD analyses indicate multiple introduction of this species in Lithuania. Presumably several different ways of invasion of *I. glandulifera* took place: natural run and predisposing it intentional and unintentional dispersal by human.

Key words: Himalayan balsam, *Balsaminaceae*, polymorphic DNA, molecular markers, invasion, alien species, neophytes, habitats.

Introduction

Over 900 *Impatiens* species are known worldwide (Chen, 2001; Janssens et al., 2007) and some of them (*Impatiens walleriana*, *I. hawkeri*, *I. balsamina*, and *I. glandulifera*) are common in cultivation. In Nepal, the seeds of Himalayan balsam or policeman's helmet, *I. glandulifera* are harvested as a food source, their oil is used for cooking (Morgan, 2007). *Impatiens* spp. are the first choice for summer ornamental planting. First introduced to Europe (England) from Nepal in 1838–1839, *I. glandulifera* quickly became a garden favorite (Morgan, 2007; Helmisaari, 2010). Despite being very attractive, *I. glandulifera*, has become a noxious weed (Chittka, Schürkens, 2001). The species' prolific self-seeding soon led it to escape from the gardens into surrounding land. It has gradually colonized many parts of Europe (Helmisaari, 2010). The same occurred in North America. Several other *Impatiens* genus species have also become naturalized over parts of Europe and the United States, including *I. parviflora* and *I. balfourii* (Schmitz, Dericks, 2010).

In the 40's of the last century, *I. glandulifera* was described as an ornamental plant cultivated in the gardens of Lithuania. Two decades later, this species was characterised by the ability to naturalize and escape from private sites (Gudžinskas, Sinkevičienė, 1995). Nowadays, *I. glandulifera* together with *I. parviflora* are among the most aggressive alien plant species in the Baltic States (Priede, 2009).

In Central and West Europe, many studies have been devoted to evaluate *I. glandulifera* geographical distribution, habitat description (Helmisaari, 2011), pathways of introduction, alien status (Tokarska-Guzik, 2003), reproduction and life cycle (Beerling, Perrins, 1993), physiology and biochemistry (Ugoletti et al., 2009), resistance to frosts (Skalova et al., 2011), dispersal (Galil et al., 2006), pressure on indigenous organisms

(Chittka, Schürkens, 2001), economic effects (Hulme et al., 2009). In the Baltic States, *I. glandulifera* research is mainly concentrated on morphology, geography and phytocenology of this species (Gudžinskas, Sinkevičienė, 1995; Priede, 2009).

It has gradually been recognized that ecological attributes alone are insufficient to explain why some plant species become invasive. This has led to more comprehensive genetic studies of introduced species. Different mechanisms might be implicated in generating the genetic variation underlying rapid adaptive evolution and the colonization of new habitats. Till now, the exact source of genetic variation causing traits important to successful invasions remains uncharacterized (Dortmontt et al., 2011). One of the possible solutions is to use the cheap and beneficial technique in order to get the information of genetic variations present in populations. The numerous studies indicate the existence of a vast range of knowledge obtained by RAPD (randomly amplified polymorphic DNA) markers (Williams et al., 1990). The RAPD technique, a simple, inexpensive, and rapid method, requiring a small amount of plant material, has been widely used in plants for genotype identification and population biology (Barcaccia et al., 2006; Jonavičienė et al., 2009; Jones et al., 2009). These molecular techniques help distinguish populations from each other, measure the genetic variability, determine the genetic relationship (Edwards et al., 2006).

The present study was aimed to evaluate the genetic variation of *Impatiens glandulifera* in Lithuania and determine the possible link between population genetic diversity and geographical location as well as habitat features.

Materials and methods

Plant material. Selection of *I. glandulifera* populations for our study was based on earlier studies on the distribution of this species in Lithuania indicating the highest density of sites in southern and eastern parts of the country (Gudžinskas, Sinkevičienė, 1995). This was the reason to include more populations from the mentioned regions in genetic analyses. Thus, three populations were taken from Vilnius and seven were sampled around Kaunas city. To reflect geographical ranges of all Lithuania, the other populations were selected through remainder territory of the country and if possible close to the borders (geographical distances between populations are provided in result chapter). The populations were named after the sampling sites. A total of 20 populations (20 individuals in each) of *Impatiens glandulifera* were selected (Table 1). Latitudinal (North) gradient covered range of examined plants from North (56°20′) to South (54°41′), longitudinal (East) gradient extended from West (21°07′) to East (25°30′), altitude ranged between 40 and 100 m. Climatic parameters of the population sampling sites were as follows: 5.4–6.8°C for annual mean temperature, 11.1–12.5°C for vegetation period mean temperature, 821–963 mm for annual rainfall and 631–793 mm for rainfall during vegetation period (climate data were obtained from the meteorological stations closest to the sampling site). Habitats were characterized according to the population size, water source presence and distance, proximity to path/road and intensity of traffic, presence of the residence/building. Light conditions were evaluated according to the prevailing life forms of neighbouring species.

Table 1. Geographical location and climate characteristics of Lithuanian populations of *Impatiens glandulifera*

Population	Latitude N	Longitude E	Altitude m	Annual mean temperature °C	Vegetation period mean temperature °C	Annual rainfall mm	Vegetation period rainfall mm
Rokiskis	55°58′	23°52′	85	6.1	12.3	821	631
Juodkrante	55°33′	21°07′	40	6.8	12.5	928	685
Palanga	55°55′	21°30′	56	6.7	11.9	962	721
Belvederis	55°40′	23°23′	55	5.4	11.1	899	706
Jurbarkas	55°40′	22°46′	55	5.4	11.1	899	706
Raudondvaris	54°56′	23°46′	63	6.5	12.5	847	696
Kaunas-Marvele	54°53′	23°49′	55	6.5	12.5	847	696
Kaunas-Lampedziai	54°54′	23°49′	54	6.5	12.5	847	696
Kaunas-A.Sanciai	54°52′	23°56′	54	6.5	12.5	847	696
Kaunas-Zaliakalnis	54°54′	23°55′	57	6.5	12.5	847	696
Vaisvydava	54°50′	24°01′	55	6.5	12.5	847	696
Girininkai	55°40′	21°30′	57	6.5	12.5	847	696
Jieznas	54°35′	24°10′	80	6.5	12.5	847	696
Kruonis	54°45′	24°14′	64	6.5	12.5	847	696
Varena-Ziurai	56°20′	23°13′	64	6.5	12.4	833	657
Jonava	55°40′	24°16′	55	6.5	12.5	847	696
Anyksciai-Malgazatavas	55°29′	25°30′	100	6.3	12.3	847	657
Vilnius-Fabijoniskes	54°44′	25°14′	73	6.5	12.4	963	793
Vilnius-Visoriai	54°45′	25°15′	74	6.5	12.4	963	793
Vilnius-Snipiskes	54°41′	25°17′	70	6.5	12.4	963	793

Sampling was carried out during the period July 21–August 1, 2010. Aboveground parts of the plants were cut, sealed in separate bags, cooled and on the same day transferred to the laboratory. Healthy, undamaged leaves from the top of the shoots were cut and frozen under −20°C. Molecular analyses were carried out at the Department of Biology, Vytautas Magnus University.

DNA extraction and randomly amplified polymorphic DNA (RAPD) – polymerase chain reaction (PCR). Total DNA was extracted from frozen leaves. Approximately 100–150 mg of plant material was ground in liquid nitrogen and transferred to 200 µl Tris-EDTA buffer with 400 µl lysis solution (Genomic DNA Purification Kit, KO512, "Fermentas", Lithuania). The concentration and purity of DNA samples were determined spectrophotometrically ("Eppendorf BioPhotometer", Germany), other details of DNA extraction are described earlier (Areškevičiūtė et al., 2006). RNase A/T1 Mix (0.5 µl; ENO551, "Fermen-

tas", Lithuania) was applied. For RAPD analysis 8 oligonucleotide primers (Barcaccia et al., 2006) of 10 bp length were used ("Biomers.net GmbH", Germany). The mixture of the PCR reaction had a final volume of 25 µl and contained 100 ng of genomic DNA, 1 × *Taq* buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 20 pmol of each primer, 1.25 U *Taq* DNA polymerase ("Fermentas", Lithuania).

RAPD analyses. DNA amplification was performed in Eppendorf Mastercycler® ("Eppendorf", Germany) according to the following program: first denaturation for 2 min at 94ºC; 35, 40 or 45 cycles of denaturation for 30 s at 94ºC, primers annealing for 35 s at 32ºC or 34ºC (depending on primer: the same as melting temperature or lower), extension for 1 min at 72ºC and final extension for 2 min at 72ºC. The reaction products were fractionated by electrophoresis in 1.5% agarose gel with ethidium bromide and UV light photographs of the gels with DNA bands were taken using "Herolab" transluminator (Germany). The length of DNA bands was estimated according to the gene ruler Gene Ruler™ 100 bp DNA Ladder ("Fermentas", Lithuania).

Statistical analyses. A binary matrix reflecting the presence (1) or absence (0) of the DNA bands (generated by RAPD primers) was analyzed. Because RAPD products are mainly dominant markers, the method yields information at the phenotype rather than the genotype level. Each band was considered to be a locus with the dominant allele present (allele-based approach), reviewed in more detail by Nybom and Bartish (2000). The band was assumed to be monomorphic if it was detected in all the samples investigated. The percentage of polymorphic bands (% P) was calculated by dividing the number of polymorphic bands at the population or species level by the total number of bands surveyed. Nei's gene diversity (h) and Shannon's index for genetic variation (I) were calculated for each population using analysis of molecular variation (AMOVA) in *GenAlEx* (Genetic Analysis in *Excel*) v. 6.1 package (Peakall, Smouse, 2006). Estimates of genetic differentiation were based on allelic frequency data and calculated as G_{ST} according to Nei (1973). The variance components and their significance levels for variation among populations and within population were obtained using data of 400 individuals. Pairwise genetic distances between populations were calculated according to the percentage disagreement method. Based on it, unweighted pair group method with arithmetic mean (UPGMA) was applied. Genetic distances among populations (Nei, 1973; 1978) based on RAPD data were estimated using *PopGene* program. According to these data dendrograms were prepared. Cluster analysis is more sensitive to related individuals. To complement it multivariate approach was chosen, because principal component analysis (PCA) is more informative regarding distances among major groups. PCA was performed using *GenAlEx v 6.41* program (Peakall, Smouse, 2006). Spearman's correlations were calculated to relate populations' genetic distance to geographical distance also to relate genetic variability to climatic features. For correlation analyses *Statistica v. 7.0* program (StatSoft Inc., USA, 2004) was used.

Results

Habitat features of populations selected for RAPD analyses. Population size (estimated according to the length of transect through the population) was as follows: smaller (< 100 m) and intermediate sized (100–500 m) ones were prevailing, comprising 40% each, while 500–1000 m long populations were not frequent (20%). According to water source and its proximity, the populations were subdivided into following groups: for 45% of the populations there was no water basin in the vicinity, 40% of populations grew parallel to the dike/stream and 5% (1 population) grew in overmoistured site. According to light intensity estimated by the life form of neighbouring plants, 65% of the populations grew in an open place with shrubs/separate trees, 25% of the populations were found in open places with herbaceous plants only and 10% were located near the park/forest edges. The populations examined differed in traffic intensity/road vicinity: 50% of the populations grew along blacktop road of the city/town/village with low intensity traffic, 15% of the populations were located along blacktop road with intensive traffic and for remainder of the populations (35%) parallel road/path was absent. Estimating human impact classification of habitats according to their proximity to any type of shelter showed that 65% of the populations grew in the backyards of the estates/houses/farms with escapes from managed plot, 10% (2 populations) were located in landfills and for 25% absence of any type of shelter was characteristic.

Characteristics of bands generated by RAPD. In the first stage of investigation, thirty 10-mer RAPD primers were tested. RAPD revealed a multi-locus banding pattern. For the second stage, 8 most informative primers which amplified scorable and reproducible DNA bands (with annealing temperature ranging from 32°C to 36°C) were applied for the analysis of 20 populations (Table 2). The selected primers generated the DNA bands whose size was 0.13–3.10 kb. The shortest bands were amplified by 269 primer, while the longest by OPA-20 (Table 2). The number of the bands generated by individual primers was 18–30, being the lowest for 340 primer and the highest for 516 primer, the total number of DNA bands per species was 188.

The selected RAPD primers differed in various characteristics of obtained DNA bands including size and total number of DNA bands for individual populations. Relations among the populations defined by PCA analyses or genetic distance and UPGMA-based dendrograms depended on the primer used.

Population-specific bands were documented for the following 5 populations: Palanga (1 band), Raudondvaris (1 band), Vaisvydava (1 band), Vilnius-Visoriai (1 band) and Belvederis (2 bands). Percentage of polymorphic bands, amplified by individual primers for population ranged from 0 to 100. According to individual primers, the highest level of polymorphic loci (100%) was obtained with 340 primer for Jieznas population, but with some primers (with OP-A20 in Vilnius-Fabijoniskes and Kruonis populations and with 250 in Kruonis population) polymorphic loci were not obtained.

Molecular variance among populations and within populations. For individual populations the number of polymorphic bands generated by distinct primers ranged between 0 and 20, forming a total of 76–106 polymorphic bands (Table 3). At the species level, all bands (188) were polymorphic. According to the DNA bands generated by all of the 8 primers, the least polymorphic (40.4%) was Kaunas-A. Sanciai population and the most polymorphic (56.4%) was Vilnius-Snipiskes population, for all populations the mean value of polymorphic DNA percentage was 46.12. The highest Nei's

gene diversity (0.165) was characteristic of Vilnius-Snipiskes and the lowest of Vilnius-Visoriai (0.115), as a mean for all populations it was 0.268. Extremes of Shannon's index (0.255 and 0.179) were characteristic of the same populations as in the Nei's gene diversity case.

Table 2. Size and number of DNA bands generated by individual RAPD primers per population and characteristics of RAPD primers used for *Impatiens glandulifera*

Name of the primer	Sequence of the primer 5'→3'	Annealing T °C	Size of DNA bands bp	Total number of DNA bands	Ranges of the band numbers per population	Mean of band numbers per population (Mean ± CI*)
OPA-20	GTT GCG ATC C	32	350–3100	21	0–17	7.2 ± 2.2
OPD-20	ACC CGG TCA C	34	200–1700	19	8–18	14.6 ± 1.3
222	AAG CCT CCC C	34	250–2500	21	4–12	8.0 ± 1.1
250	CGA CAG TCC C	34	150–3000	25	0–15	7.5 ± 1.8
269	CCA GTT CGC C	34	130–2500	25	5–18	13.0 ± 1.4
340	GAG AGG CAC C	34	210–1900	18	8–18	13.5 ± 1.0
474	AGG CGG GAA C	34	250–2000	29	9–16	9.1 ± 1.4
516	AGC GCC GAC G	36	200–2000	30	6–20	13.9 ± 1.7
All 8 primers				188		

*CI – confidence interval; n = 20, $p \leq 0.05$

Table 3. Genetic parameters of the populations of *Impatiens glandulifera* growing in Lithuania (h – Nei's gene diversity, *I* – Shannon's information index) according to 8 RAPD primers

Population	Number of polymorphic bands	Percentage of polymorphic bands	Nei's gene diversity h	Shannon's index *I*
			Mean ± CI*	Mean ± CI
Rokiskis	95	50.5	0.144 ± 0.080	0.225 ± 0.115
Anyksciai-Malgazatavas	95	50.5	0.148 ± 0.080	0.228 ± 0.115
Jonava	85	45.2	0.127 ± 0.076	0.198 ± 0.111
Vilnius-Visoriai	79	42.0	0.115 ± 0.073	0.179 ± 0.112
Vilnius-Snipiskes	106	56.4	0.165 ± 0.081	0.255 ± 0.116
Vilnius-Fabijoniskes	98	52.1	0.151 ± 0.080	0.234 ± 0.115
Kaunas-A. Sanciai	76	40.4	0.123 ± 0.078	0.188 ± 0.113
Kaunas-Marvele	80	42.6	0.119 ± 0.072	0.187 ± 0.107
Kaunas-Lampedziai	83	44.2	0.129 ± 0.078	0.200 ± 0.113
Kaunas-Zaliakalnis	84	44.7	0.133 ± 0.081	0.203 ± 0.116
Girininkai	77	40.9	0.122 ± 0.078	0.186 ± 0.114
Vaisvydava	87	46.3	0.149 ± 0.083	0.226 ± 0.120
Jieznas	85	45.2	0.142 ± 0.082	0.216 ± 0.118
Kruonis	78	41.5	0.117 ± 0.074	0.183 ± 0.109
Varena-Ziurai	81	43.1	0.123 ± 0.076	0.190 ± 0.111
Raudondvaris	84	44.7	0.121 ± 0.075	0.189 ± 0.109
Belvederis	94	50.0	0.131 ± 0.075	0.207 ± 0.109
Jurbarkas	85	45.2	0.134 ± 0.079	0.206 ± 0.114
Palanga	90	47.9	0.136 ± 0.079	0.210 ± 0.114
Juodkrante	92	48.9	0.140 ± 0.077	0.218 ± 0.113
All populations	76–106	40–56	0.268 ± 0.068	0.419 ± 0.088
Mean for 20 populations		46.1 ± 0.9		

*CI – confidence interval; n = 20, $p \leq 0.05$

Genetic differentiation (G_{ST}) by individual primers between populations was found to be in the range 0.337 (primer 516) and 0.663 (primer 250). Eight RAPD primers-based analyses of molecular variance among individuals within populations and among populations revealed that all 400 individuals examined were of different RAPD phenotype. The variation among the populations was negligibly higher than within populations (respectively, 51% and 49%; Table 4).

Pairwise genetic distances between populations ranged in the interval 0.088–0.259 (Table 5). The most distinct were Kaunas-Zaliakalnis and Jonava populations (0.259), Jurbarkas and Palanga populations (0.247), Jonava and Belvederis (0.247) populations. The highest genetic similarity was identified between the following pairs of populations: Kruonis and Vilnius-Fabijoniskes (0.088), Belvederis and Vilnius-Fabijoniskes (0.113), also Kaunas-Marvele and Vilnius-Fabijoniskes (0.118).

UPGMA dendrograms prepared according to individual primers (data are not shown in figures) revealed different, most distinct populations: Jonava and Kaunas-Lampedziai (by 222 primer), Jieznas and Rokiskis (by 250 primer), Vilnius-Visoriai and Anyksciai-Malgazatavas (by 269 primer), Vaisvydava and Kaunas-Zaliakalnis (by 340 primer), Vaisvydava (by 474 primer), Girininkai (by 516 primer), Jurbarkas (by OPA-20 primer), Jonava (by OPD-20 primer).

Table 4. Statistical analyses of molecular variance among populations and within populations of *Impatiens glandulifera* in Lithuania

Source	df	SS	MS	Est. var.	%
Among populations	19	6150.663	323.719	15.446	51
Within populations	380	5622.850	14.797	14.797	49
Total	399	11773.513		30.243	100

Note. Significant levels are based on 1000 permutations; df – degree of freedom, $p \leq 0.01$.

UPGMA dendrograms based on all 8 primers (Fig. 1) indicated Jonava and Vaisvydava as the most distinct populations. In this dendrogram, the rest of the populations might be split into 3 branches: the 1st cluster comprised Varena-Ziurai, Palanga, Vilnius-Visoriai, Vilnius-Snipiskes, the 2nd cluster consisted of Girininkai, Kaunas-Zaliakalnis, Raudondvaris, Belvederis, Kaunas-Lampedziai, Kaunas-A. Sanciai, and the 3rd group included Jurbarkas, Jieznas, Juodkrante Kaunas-Marvele, Kruonis, Vilnius-Fabijoniskes, Anyksciai-Malgazatavas, Rokiskis populations.

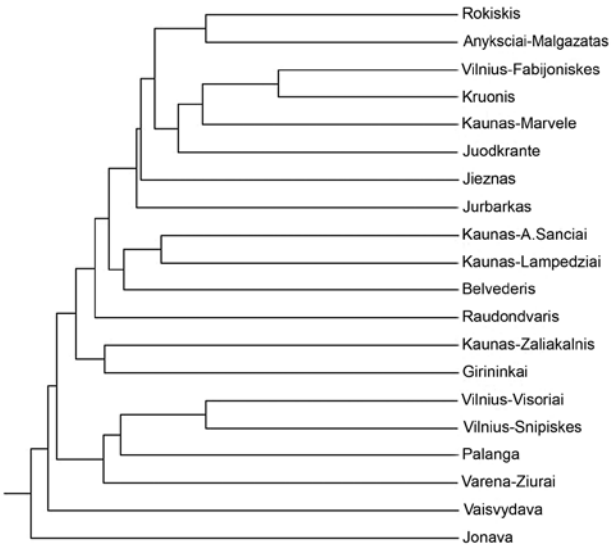

Figure 1. Dendrogram of genetic relationships among 20 populations of *Impatiens glandulifera* based on 8 RAPD primers using the UPGMA algorithm and the genetic distances

Table 5. Based on 8 RAPD primers genetic distances (Nei, 1978) and geographical distances (km) among Lithuanian populations of *Impatiens glandulifera*

		Geographical distance, km																			
	Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Genetical distance	1	x	128.0	130.0	142.8	144.4	144.0	159.3	160.8	160.7	158.9	159.2	155.7	172.1	154.0	206.2	163.1	173.7	203.3	282.6	284.6
	2	0.122	x	73.0	92.2	95.4	93.1	105.6	105.2	105.7	104.1	107.8	101.5	119.3	95.2	150.4	106.5	129.0	156.0	257.2	251.5
	3	0.176	0.186	x	77.3	84.8	74.7	31.0	31.8	33.2	27.8	37.7	29.4	53.1	35.4	97.3	31.9	95.3	95.3	223.4	207.2
	4	0.159	0.136	0.208	x	6.7	3.6	91.1	92.4	92.6	89.2	82.6	76.6	65.3	57.3	72.3	96.9	135.9	166.1	298.2	281.9
	5	0.151	0.170	0.214	0.122	x	10.5	93.2	93.2	94.1	90.9	88.0	80.2	71.8	63.6	68.6	100.8	129.6	169.1	301.6	284.4
	6	0.13	0.146	0.188	0.163	0.155	x	89.5	89.8	91.0	88.7	85.3	77.9	68.1	59.7	69.2	96.9	126.5	165.5	297.6	281.1
	7	0.171	0.142	0.189	0.168	0.178	0.162	x	7.1	9.4	4.0	12.6	10.6	38.1	28.4	89.5	8.7	36.2	75.0	212.1	192.7
	8	0.153	0.124	0.177	0.164	0.183	0.118	0.165	x	2.2	3.0	10.8	12.9	36.2	29.5	88.0	9.3	36.1	74.0	212.1	192.0
	9	0.162	0.158	0.157	0.214	0.212	0.153	0.142	0.138	x	5.1	12.1	12.1	38.3	31.1	89.5	7.9	35.1	72.8	211.3	192.0
	10	0.180	0.174	0.259	0.243	0.207	0.191	0.198	0.144	0.221	x	12.3	10.5	37.0	28.9	87.4	9.4	38.9	75.2	212.5	194.6
	11	0.175	0.165	0.226	0.207	0.218	0.172	0.162	0.170	0.169	0.169	x	10.7	29.1	21.7	77.5	19.4	59.5	80.8	219.6	199.8
	12	0.185	0.182	0.229	0.222	0.192	0.177	0.189	0.177	0.162	0.218	0.222	x	31.1	19.2	79.4	20.4	61.8	85.9	223.6	203.9
	13	0.151	0.146	0.217	0.182	0.180	0.154	0.200	0.139	0.173	0.176	0.181	0.194	x	24.3	50.5	46.4	85.8	106.8	247.0	224.8
	14	0.156	0.152	0.178	0.198	0.179	0.088	0.161	0.129	0.148	0.160	0.196	0.188	0.153	x	70.5	35.1	75.3	100.7	239.0	217.2
	15	0.176	0.168	0.214	0.130	0.195	0.184	0.163	0.156	0.211	0.202	0.202	0.206	0.164	0.182	x	91.9	123.4	150.9	293.6	270.4
	16	0.175	0.157	0.184	0.158	0.174	0.156	0.152	0.152	0.186	0.217	0.184	0.231	0.177	0.175	0.178	x	43.9	64.2	198.2	179.9
	17	0.165	0.191	0.247	0.201	0.204	0.113	0.165	0.150	0.155	0.237	0.176	0.191	0.210	0.174	0.218	0.197	x	37.1	173.1	154.5
	18	0.161	0.146	0.204	0.208	0.196	0.166	0.193	0.128	0.145	0.193	0.205	0.209	0.155	0.168	0.210	0.218	0.187	x	142.5	118.5
	19	0.186	0.187	0.248	0.169	0.154	0.186	0.195	0.207	0.242	0.242	0.237	0.162	0.196	0.206	0.183	0.225	0.240	0.247	x	37.1
	20	0.158	0.146	0.165	0.157	0.151	0.142	0.144	0.130	0.176	0.159	0.170	0.221	0.172	0.132	0.197	0.165	0.225	0.153	0.207	x

Note. Population name: 1 – Rokiskis, 2 – Anyksciai-Malgazatavas, 3 – Jonava, 4 – Vilnius-Visoriai, 5 – Vilnius-Snipiskes, 6 – Vilnius-Fabijoniskes, 7 – Kaunas-A. Sanciai, 8 – Kaunas-Marvele, 9 – Kaunas-Lampedziai, 10 – Kaunas-Zaliakalnis, 11 – Girininkai, 12 – Vaisvydava, 13 – Jieznas, 14 – Kruonis, 15 – Varena-Ziurai, 16 – Raudondvaris, 17 – Belvederis, 18 – Jurbarkas 19 – Palanga, 20 – Juodkrante.

UPGMA method using genetic distance matrix (bootstrap values obtained after 1000 iterations) revealed all different RAPD phenotypes in the case of each population individuals (i.e. 400 RAPD phenotypes; data are not provided in figure). In this dendrogram individuals split into 4 major clusters: 1st cluster is represented by individuals from Kaunas-A. Sanciai, Raudondvaris; 2nd – from Juodkrante, Girionys, Kaunas-Lampedziai, Rokiskis; 3rd – from Jonava, Kaunas-Zaliakalnis, Kaunas-Marvele, Jurbarkas, Varena-Ziurai, Vaisvydava, Vilnius-Visoriai, Anyksciai-Malgazatavas and 4th – from Kruonis, Jieznas, Palanga, Vilnius-Snipiskes, Vilnius-Fabijoniskes, Belvederis. The main groups in the dendrogram based on genetic relationships among the populations did not coincide with the clusters in the dendrogram prepared on the basis of individual genetic relations.

According to the individual primers, RAPD data percentage of variation of populations explained by the first 3 principal components was high enough and ranged 68.23–80.52% (respectively, 29.04–36.79% by the 1st prin-

cipal component, 17.40–27.45% by the 2nd, 12.79–19.19% by the 3rd). PCA parameters of all examined individuals (400 individuals) were lower and ranged 58.93–67.52% (respectively, 23.96–30.34% by the 1st, 18.32–22.66% by the 2nd, 15.39–18.03% by the 3rd principal component).

According to the generalised data of 8 primers (Fig. 2), percentage of variation of populations explained by the first 3 principal components was 58.17% (22.78% by the 1st principal component, 18.02% by the 2nd and 17.37% by the 3rd) and the most distinct populations were Juodkrante, Palanga, Vaisvydava.

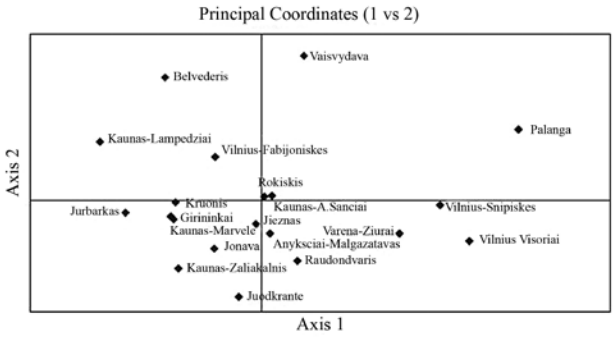

Figure 2. Principal coordinate analyses of RAPD (8 primers) data for 20 Lithuanian populations of *Impatiens glandulifera*

PCA (2nd and 3rd variable axe) performed by RAPD primer 474 allowed us to separate Palanga from three main clusters of populations, 1st being three populations from Vilnius, 2nd being Kaunas-related and Varena-Ziurai populations and the 3rd comprising the rest of the studied populations with an intermediate geographical position between Palanga and Kaunas, Vilnius cities (Fig. 3).

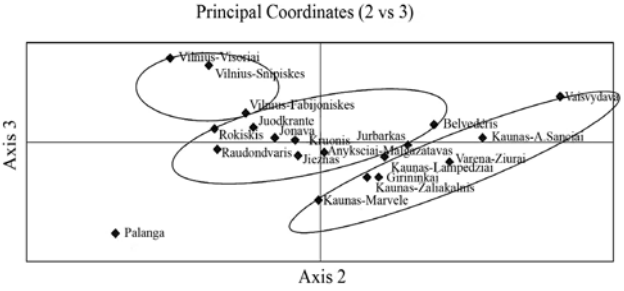

Figure 3. Principal coordinate analyses of RAPD (474 primer) data for 20 Lithuanian populations of *Impatiens glandulifera*

Only few significant correlations were obtained between genetic and geographic distances of *I. glandulifera* populations. The percentage of polymorphic DNA bands (generated by RAPD primers) of the populations correlated negatively with the site's mean temperature for vegetation season ($r = -0.535$, $p < 0.015$). Only for one (Palanga population located near the Baltic sea) out of 20 populations significant relations were found between this population genetic and geographic distances to the other populations ($r = -0.755$, $p < 0.001$).

Subdivision of the sampling sites according to various habitat features (population size, water source presence and distance, proximity to path/road and intensity of traffic, presence of the residence/building also light conditions) did not reveal obvious relations to the genetic parameters of the populations.

Discussion

Plant sampling revealed that there is still a strong interest in *Impatiens glandulifera* cultivation in small villages and big cities of Lithuania. It is in support for documented in Poland 100 plots of active cultivation of *I. glandulifera* (Tokarska-Guzik, 2003). According to our study, intervention of Himalayan balsam occurs into various types of habitats, very often invaded areas are backyards or gardens and in some cases Himalayan balsam might be observed even in the fields of wheat or oats. Many new localities of *I. glandulifera* in Lithuania have been found compared to previous data (Gudžinskas, Sinkevičienė, 1995). It shows that all sites of Himalayan balsam taken together might approach one hundred. Such calculations are in agreement with 134 plots of *I. glandulifera* documented for the neighbouring country Latvia (Priede, 2009). Ongoing studies in other parts of Europe are permanently alerting about increasing number of alien plant species (Hulme et al., 2009; Helmisaari, 2010; Dortmontt et al., 2011). The current invasion of several neophytes including *I. glandulifera* might be promoted by prolonged vegetation season and rise of minimal temperatures (Priede, 2009; Schmitz et al., 2010). Similarly to the Latvian study, our sites of Himalayan balsam were not very big in size.

In Central Europe, *I. glandulifera* is attributed to riparian alien species (Chittka, Schürkens, 2001; Galil et al., 2006; Hejda, Pyšek, 2006). The distribution patterns of invasive neophytes coincide with the river valleys as a migration corridors and spatial distribution of human settlements as well as road networks. Over the last two decades, ongoing socio-economic changes like increasing intensity of international and national transport, urban area extensions due to private sector growth and soil erosion accompanying it, also higher attention paid to ornamental horticulture, extended possibilities for plant exchanges, enlarged number of tourists visiting natural sites are factors facilitating invasion of alien species in the Baltic States. Our study together with the data of Latvia shows that in the Baltic countries the distribution pattern and spread of *I. glandulifera* are predominantly determined by anthropogenic rather than riparian factors (Priede, 2009). Lithuanian data support the hypothesis that transport is one of the most important routes for migration of this species. According to our habitats used for genetic studies, a major *I. glandulifera* invasion occurs from the yards along dikes excavated in parallel with roads.

The different molecular techniques that have been used for alien species analysis worldwide include: amplified fragment length polymorphism (AFLP; Hatcher et al., 2004; Jahodova et al., 2007), microsatellites (Durka et al., 2004; Provan et al., 2007), inter simple sequence repeat (ISSR; Hatcher et al., 2004; Patamsytė et al., 2011), RAPD (Edwards et al., 2006; Patamsytė et al., 2011; Vyšniauskienė et al., 2011) and many other DNA analysis methods recently reviewed by Dortmontt et al. (2011). For *Impatiens* species, sequencing of chloroplastic and nuclear DNA also microsatellites (Li et al., 2008) were used for analyzing their phylogenetic and evolution processes (Janssens et al., 2007). Invasion-related features of this species were analyzed exploring mainly AFLP and microsatellite techniques (Hatcher et al., 2004; Jahodova et al., 2007; Provan et al., 2007). Genetic diversity of *I. glandulifera* has never been estimated by RAPD primers.

A compilation of numerous plant studies using RAPD markers for evaluating population differentiation shows that in outcrossing taxa estimates of inter-population diversity were closely correlated with maximum

geographic distance between the sampled populations (Nybom, Bartish, 2000). Our data revealed that the populations most distinct according to genetic distance were not the most contrasting ones according to their geography and vice versa. It might be due to the relatively small size of the country (the biggest distance between the selected Lithuanian populations was 300 km in East–West direction, and the most contrasting North–South populations were only at 200 km distance). The lack of correlation between genetic and geographical distance indicates predominantly human-mediated dispersion. It is in agreement with the data obtained for *Heracleum* taxa in Europe (Jahodova et al., 2007). Application of RAPD primer 474 allowed us to separate Palanga from three distinct geographical regions of Kaunas, Vilnius and the rest of the sites with different historical development patterns. This indicates that in some cases individual primer analysis might be more informative than combined analysis. Significant relations between Palanga population genetic and geographic distances to the other populations show that possible route of *I. glandulifera* invasion in Lithuania might occur along East–West (Palanga–Vilnius) transect. Both vicinity to the river Nemunas and one of the major motorways of Lithuania might be the factors which contributed to the spread of *I. glandulifera*. Presumably several different ways of invasion of *I. glandulifera* took place in Lithuania: natural run and predisposing it intentional and unintentional dispersal by human. Seeds might be obtained from various countries. Moreover, exchanges between gardeners inside the country complicate explanation of invasion routes of *I. glandulifera*. Our assumptions about multiple introduction are in agreement with the conclusions of genetic studies carried out on the other invasive species (Durka et al., 2004; Patamsytė et al., 2011). Summarized population studies by RAPD show that annual taxa allocate most of the genetic variability among populations, contrary to long-lived taxa which retain most of their genetic variability within populations (Nybom, Bartish, 2000). Our study evidenced that annual *I. glandulifera* intra- and interpopulation variabilities were very similar. In parallel to our Himalayan balsam, some other invasive species of Lithuania were assayed by RAPD. When compared to Lithuanian *Lupinus polyphyllus* (Vyšniauskienė et al., 2011) and *Bunias orientalis* (Patamsytė et al., 2011), percentage of polymorphic loci, Nei's gene diversity, pairwise genetic distances between populations were of an intermediate size and interpopulation variability was higher for *Impatiens glandulifera*.

Temperature might be an important factor determining the spread of the alien species (Beerling, Perrins, 1993). In Czech Republic, seedling differences in frost sensitivity depending on temperature at the seed source were observed among the populations of *I. glandulifera* (Skalova et al., 2011). Himalayan balsam data obtained by us might be in support to these findings: percentage of polymorphic DNA bands (obtained by RAPD primers) of populations correlated negatively with the mean site temperature for vegetation season. Our results show that adaption of populations to more northern conditions, might be related to higher genetic variability.

Conclusions

1. Analyses of *Impatiens glandulifera* populations using 8 RAPD primers show that the data obtained by individual primers in some cases might be more valuable in providing information about geographic location of populations.

2. The populations most distinct according to genetic distance were not the most contrasting ones according to their geography and vice versa.

3. RAPD analyses indicate multiple ways of introduction of this species in Lithuania.

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Bitinės sprigės (*Impatiens glandulifera* Royle) Lietuvos populiacijų genetinė įvairovė pagal atsitiktinai pagausintą polimorfinę DNR

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Santrauka

Kai kuriose Azijos šalyse natūraliai augančios bitinės sprigės (*Impatiens glandulifera* Royle), anglų vadinamos „Himalajų sprige“ arba „policininko šalmu“, sėklų aliejus naudojams maistui. 1838–1839 m. įvežta į Europą (Angliją), bitinė sprigė greitai tapo populiariu darželių augalu, o netrukus virto piktžole. Pastaruoju metu bitinė sprigė tapo invaziniu augalu beveik visoje Europoje ir toliau skverbiasi į naujas augavietes. Iki šiol Pabaltijo šalių bitinės sprigės genetinė įvairovė nebuvo tyrinėta. Darbo tikslas – įvertinti bitinės sprigės Lietuvos populiacijų genetinį kintamumą atsitiktinai pagausintos polimorfinės DNR (APPD) metodu. Panaudojus 8 APPD pradmenis išanalizuota 20 bitinės sprigės populiacijų (kiekvienoje po 20 individų), besiskiriančių geografine padėtimi arba augavietės ypatumais. Nustatyta, kad polimorfinių DNR atkarpų skaičius svyravo 40–56 %, Nei genetinės įvairovės rodiklis prilygo 0,115–0,165, Šanono informacijos indeksas – 0,183–0,255. Tarppopuliacinė įvairovė buvo didesnė (51 %) už vidupopuliacinę. Populiacijų polimorfinių atkarpų procentas didėjo ($r = -0{,}535$, $p < 0{,}015$), mažėjant vidutinei vietovės vegetacijos sezono temperatūrai. Iš visų tirtų 20 populiacijų tik vienos Palangos populiacijos genetinis atstumas su kitomis populiacijomis didėjo, mažėjant šios populiacijos geografiniam atstumui su kitomis populiacijomis ($r = -0{,}755$, $p < 0{,}001$). APPD genetinius atstumus vaizduojančioje dendrogramoje išsiskiria 4 pagrindinės grupės, tarp kurių nėra aiškaus geografinio pasiskirstymo. Molekuline genetika (APPD metodu) paremti Lietuvos bitinės sprigės tyrimų duomenys rodo, kad į mūsų kraštą ji buvo įvežta ne vieną kartą. Šios rūšies plitimas vyksta keliais būdais: gamtiniu, kurį gali įvairiai keisti tikslingai ir netikslingai žmonių platinamos arba pernešamos sėklos.

Reikšminiai žodžiai: *Balsaminaceae*, polimorfinė DNR, APPD, invazija, svetimkraščiai augalai, DNR žymenys, molekuliniai pradmenys.