Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora

Naturalizované rostliny mají menší genom než neinvadující druhy: cytometrická analýza nepůvodních druhů české květeny

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Genome size has been suggested as one of the traits associated with invasiveness of plant species. To provide a quantitative insight into the role of this trait, we estimated nuclear DNA content in 93 alien species naturalized in the Czech Republic, belonging to 32 families, by using flow cytometry, and compared it with the values reported for non-invading congeneric and confamilial species from the Plant DNA C-values database. Species naturalized in the Czech Republic have significantly smaller genomes than their congeners not known to be naturalized or invasive in any part of the world. This trend is supported at the family level: alien species naturalized in the Czech flora have on average a smaller genome than is the mean value for non-invading confamilials. Moreover, naturalized and non-invading species clearly differed in the frequency of five genome size categories; this difference was mainly due to very small genomes prevailing and intermediate to very large genomes under-represented in the former group. Our results provide the first quantitative support for association of genome size with invasiveness, based on a large set of alien species across a number of plant families. However, there was no difference in the genome size of invasive species compared to naturalized but non-invasive. This suggests that small genome size provides alien plants with an advantage already at the stage of naturalization and need not be necessarily associated with the final stage of the process, i.e. invasion.

K e y w o r d s: alien plants, confamilials, congeners, C-value, flow cytometry, genome size, invasive species, large genome constraint hypothesis, nuclear DNA content, plant invasions

Introduction

The numbers of invasive species in various parts of the world continue to increase, representing a serious threat to biodiversity worldwide (e.g. Meyerson & Mooney 2007, Blackburn et al. 2009, Hulme et al. 2009b, McGeoch et al. 2010). As a result, biological invasions have been receiving serious attention from both scientists and practitioners and research in invasive plant and animal species has been increasing exponentially (e.g. Crall et al. 2006, Pyšek et al. 2006, 2008, Lambdon et al. 2008, Ricciardi & MacIsaac 2008, Chytrý et al. 2009, DAISIE 2009, Davis 2009). The knowledge of ecological impacts on

native biodiversity and ecosystem functioning improved dramatically in the last decade
(e.g. Levine et al. 2003, Gaertner et al. 2009, Hejda et al. 2009a, Nentwig et al. 2010, Vilà
et al. 2010), and it is now widely recognized that invasive species incur serious economic
costs (Binimelis et al. 2007, Kettunen et al. 2009, Vilà et al. 2010). The awareness of the
magnitude of problem is stimulating not only management efforts (Keller et al. 2007,
Richardson et al. 2007, Hulme et al. 2008, 2009a, Simberloff 2009, McGeoch et al. 2010),
but also research aimed at deeper understanding of underlying processes and determinants
of naturalization and invasiveness (e.g. Pyšek et al. 2008, 2009a, Blackburn et al. 2009,
Davis 2009, Essl et al. 2009, Štajerová et al. 2009). Within this research realm, an effort to
identify biological and ecological traits conferring invasiveness is as well established as
the field of invasion biology itself (see Pyšek & Richardson 2007 for a review). Recent
developments, including the development of new technologies (Richardson & Pyšek
2008), in particular molecular techniques, now make it possible to include traits that were
until not long ago impossible to consider in multispecies studies focusing on determinants
of invasiveness (Pyšek & Richardson 2007). The amount of nuclear DNA (genome size) is
one of the traits for which knowledge has improved dramatically in the last decade, largely
due to the advent and spread of flow cytometry (Kron et al. 2007, Ekrt et al. 2009).

    Genome size is a fundamental biological parameter involved in the scaling of both
plants and animals (Gregory 2005). DNA is known to play not only a qualitative (i.e.
genic) role but also a quantitative one because of its direct and sequence-independent
influence on cellular (and by extension, organismal) characteristics. Correlations between
genome size and plant traits are many and range from nuclear and cell volumes through the
duration of cell cycle (both meiotic and mitotic) up to seed size and specific leaf area
(reviewed by Leitch & Bennett 2007, see also Loureiro et al. 2010). Through concomitant
changes in cellular parameters, genome size affects several aspects of a plant's develop-
ment. Minimum generation time (i.e. time to flowering) and life history (i.e. whether
ephemeral, annual or perennial) are illustrative examples of developmental traits con-
strained by the amount of nuclear DNA. On average, ephemerals (plants completing their
life cycle in less then seven weeks) have been shown to possess the smallest genomes, fol-
lowed by annuals, whereas obligate herbaceous perennials have the highest DNA amounts
(Bennett 1972). Whereas species with small genomes can display any developmental life
history, their large-genome counterparts are restricted to an obligate perennial life history.
Large genomes also impose constraints on ecological behaviour, influencing where a plant
may grow and its chances of survival in a changing world (Knight et al. 2005, Vidic et al.
2009). In addition, traits associated with genome size (seed size and mass, and the rate of
developmental processes in particular) may co-determine the life strategy adopted by the
plant (i.e. whether competitor, stress tolerator or ruderal). In their study on 156 weedy
angiosperm species, Bennett et al. (1998) showed that the probability of being recognized
as a weed decreases with increasing genome size.

    On the same conceptual basis, small genomes have been suggested as a prerequisite for
plant invasiveness because species with low nuclear DNA content usually produce many
light seeds and their establishment is fast (Rejmánek 1996). In addition, invasions typi-
cally occur in disturbed habitats (Davis et al. 2000, Chytrý et al. 2005, 2008) and small
genomes have been shown to represent an evolutionary advantage in time-limited environ-
ments (Bennett 1987). In his "theory of seed plant invasiveness", Rejmánek listed a low
amount of nuclear DNA among the most important factors contributing to the invasiveness

of seed plants (Rejmánek 1996, 2000, Rejmánek et al. 2005). Experimental support for this conclusion comes mainly from comprehensive studies on genome size variation in the genus *Pinus* (Wakamiya et al. 1993, Grotkopp et al. 2002, 2004). Invasiveness of pines, particularly of wind-dispersed species, was shown to be negatively associated with both genome size and seed mass (Grotkopp et al. 2002). Smaller genomes in invasive species as compared to their non-invasive congeners have also been found in some other genera such as *Senecio* (Lawrence 1985) or *Acacia* (Mukherjee & Sharma 1990), although the number of analyzed invasive species was usually quite low. In addition, a negative relationship was observed between the genome size of three *Briza* species and the invaded area (Rejmánek 1996).

Despite the pieces of evidence mentioned above for the role of genome size in plant invasions, a systematic study aimed at comparing genome sizes in invasive plant species and their non-invasive counterparts across different taxonomic groups is still lacking. To fill this gap, we determined nuclear DNA amounts in a representative set of alien species occurring in the Czech Republic and compared their genome size values with those of non-invasive congeners and confamilials. Specifically, we addressed the following questions: (i) What is the distribution of holoploid genome sizes in alien species and how it differs from the general pattern found in angiosperms? (ii) Which factors affect the genome size of alien species? Is genome size related to the invasion status? (iii) Do alien species differ in genome size from their non-invasive congeners and confamilials?

Material and methods

Analyzed species

The species set included 93 neophytes (alien species introduced after 1500 A.D.; see Pyšek et al. 2002, 2004) occurring in the flora of the Czech Republic. They belonged to 70 genera and 32 families according to the Angiosperm phylogeny group classification (Stevens 2001). Seeds were collected in the field during 2005–2007 (see Electronic Appendix 1). Seedlings were germinated in a growth chamber and cultivated in the experimental garden of the Institute of Botany, Academy of Sciences, Průhonice, Czech Republic (49°59'30''N, 14°34'00''E, ca 320 m a.s.l.). Fresh young leaf tissue was used for genome size estimation. Herbarium vouchers are kept at PRA.

Species' invasion status in the Czech Republic (casual; naturalized; invasive) was taken from Pyšek et al. (2002). The vast majority of species were naturalized, only three (*Ambrosia trifida*, *Bidens connata* and *Panicum miliaceum*) were casual; for the sake of simplicity, all the species analyzed are further referred to as 'naturalized'. Of these naturalized species, 41 were invasive and 49 naturalized but not invasive (sensu Richardson et al. 2000, Pyšek et al. 2004). Each species was further characterized (see Table 1) by its life history (annual; monocarpic perennial; polycarpic perennial) and moisture score. The moisture score was calculated by using data from Hejda et al. (2009b); this paper and associated database give, for species alien to the Czech Republic, information on habitats in which they occur in their native range. These habitats were classified using a 5-degree ordinal scale (1-dry, 3-mesic, 5-humid, with 2 and 4 representing transitions) and average value was used as the moisture score. Of our species set, habitat data for 58 species were available in Hejda et al. (2009b); for remaining species we used the average Ellenberg's indicator

value for moisture (Ellenberg et al. 1992), transformed to a 5-degree scale as follows: 1–3=1, 4=2, 5=3, 6–7=4, 7–9=5.

Genome size estimation

Holoploid genome sizes (C-values sensu Greilhuber et al. 2005) were determined using propidium iodide flow cytometry following the simplified two-step protocol as described by Doležel et al. (2007). Briefly, young intact leaf tissue of the analyzed plant was chopped together with an appropriate internal reference standard in 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20; Otto 1990). The sample was filtered through 42-μm nylon mesh, and incubated 10 min at room temperature. The staining solution consisted of 1 ml of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$) supplemented with propidium iodide and RNase IIA (both at final concentrations of 50 μg/ml) and β-mercaptoethanol (2 μl/ml). The fluorescence intensity of isolated nuclei (5000 particles) was recorded using Partec CyFlow SL cytometer equipped with a diode-pumped solid state laser 532 nm (Cobolt Samba, 100 mW output power). Each sample was analyzed at least three times on different days; only analyses with a between-day fluctuation below 3% were considered.

The following species were used as internal reference standards (Doležel et al. 2007): *Solanum lycopersicum* 'Stupické polní rané' (2C = 1.90 pg), *Glycine max* 'Polanka' (2C = 2.30 pg), *Bellis perennis* (2C = 3.46 pg), *Zea mays* 'CE-777' (2C = 5.47 pg), *Pisum sativum* 'Ctirad' (2C = 8.76 pg) and *Vicia faba* 'Inovec' (2C = 26.92 pg). *Pisum sativum* 'Ctirad' (Doležel et al. 1998) served as a primary reference standard, with 2C-value of 8.76 pg as recommended by Greilhuber et al. (2007). Genome sizes of other reference species were calibrated against *Pisum*, based on three measurements on different days. For each analyzed plant, internal standard was selected so that its genome size was close to but not overlapping with that of the analyzed sample.

Ploidy levels of analyzed naturalized plants were inferred from chromosome numbers taken from various karyological databases and flora handbooks, including Goldblatt & Johnson (1979), Marhold et al. (2007), Flora of the Czech Republic (Hejný & Slavík 1988–1992, Slavík 1995–2000, Slavík & Štěpánková 2004), the database of the flora of the Czech Republic (CzechFlor) and the internal karyological database of plants of the Czech Republic (both held at the Institute of Botany AS CR, Průhonice). Monoploid genome sizes (1Cx-values) were calculated as 2C-values / ploidy level.

Reference genome size data

To compare the genome size of naturalized alien plants in the Czech flora with non-invasive species, 2C-values and ploidy levels for plants from corresponding genera and families that are not reported to be naturalized or invasive were extracted from the Plant DNA C-values database (Bennett & Leitch 2005). Species in this reference data set are referred to as 'non-invading' to reflect not only that they are not invasive (in the sense of Richardson et al. 2000) but neither naturalized, i.e. they do not successfully enter the invasion process. The selection of non-invading congeners and confamilials was made by omitting from the Plant DNA C-values database any species reported as naturalized or invasive in any part of the world, based on the updated database of Weber (2003) and other sources. In some cases, the ploidy level taken from the Plant DNA C-values database was corrected so that the basic chromosome number (x) was the same for both naturalized species and their

non-invading congeners. Reference genome size data were available for 45 congeneric and 31 confamilial non-invading counterparts.

Statistical analysis

Comparison of genome size categories between naturalized plants of the Czech flora and non-invading species taken from the Plant DNA C-values database was done by G-test on a contingency table (e.g. Crawley 2002, p. 548–550).

The effect of invasion status (41 invasive vs 51 non-invasive species, the latter including 48 naturalized and three casual; *Oxybaphus nyctagineus* was excluded because of non-available moisture data), life history and moisture score on 2C-values was analyzed by general linear model. The most parsimonious model was selected by a stepwise procedure, beginning with the maximal model (containing all predictors and all their possible interactions) and proceeding by the elimination of non-significant terms, using deletion tests. This was done by an automatic step-wise process of model simplification of deviance tables, based on Akaike Information Criterion (AIC) (program Spotfire S-Plus v. 8.1, TIBCO Software Inc. 2008; e.g. Crawley 2002). Observed power of the chosen model (e.g. Steidel & Thomas 2001) was computed for $\alpha = 0.05$ in SPSS v. 18 (SPSS Inc. 2010).

Paired t-tests (Sokal & Rohlf 1995) were used for comparisons of 2C- and Cx-values of naturalized aliens with corresponding mean values of their non-invading congeners, and of mean 2C-values of naturalized aliens with corresponding mean values of their non-invading confamilials. All 2C-values were ln-transformed to normalize the data, and then checked for homogeneity of variance. The general linear model was checked by plotting standardized residuals against fitted values, and by normal probability plots (Crawley 1993).

Results

Genome size variation in naturalized alien species

Flow cytometric analyses yielded histograms with mean coefficients of variation (CVs) of 3.18% and 2.50% for the sample and internal reference standard, respectively (Fig. 1).

Genome size values were determined in one APG family (*Phrymaceae*) and 66 species for the first time (Table 1). 1C-values of analyzed plants varied from 0.24 pg in *Sisymbrium loeselii* to 15.27 pg in *Rudbeckia laciniata*, spanning ~64-fold range. The majority of naturalized species possessed low nuclear DNA amounts, with mean 1C-value of 1.93 pg and median of 1.17 pg. The distribution of genome sizes was highly skewed (Fig. 2). The most common were plants with 1C-values between 0.4 and 0.6 pg; other classes were much less frequent, and only four species had 1C-values above 4.4 pg. The same pattern was obtained when the species analyzed were attributed to five genome size categories as defined by Leitch et al. (1998). Very small genomes (1C ≤ 1.4 pg) were represented by 49 species, small genomes (1.4 pg < 1C ≤ 3.5 pg) by 36 species, intermediate genomes (3.5 pg < 1C ≤ 14.0 pg) by seven species, and large genomes (14.0 pg < 1C ≤ 35.0 pg) by one species. Plants with very large genomes (1C > 35 pg) were not present in our species set. Compared to non-invading species (Table 2), naturalized species had more
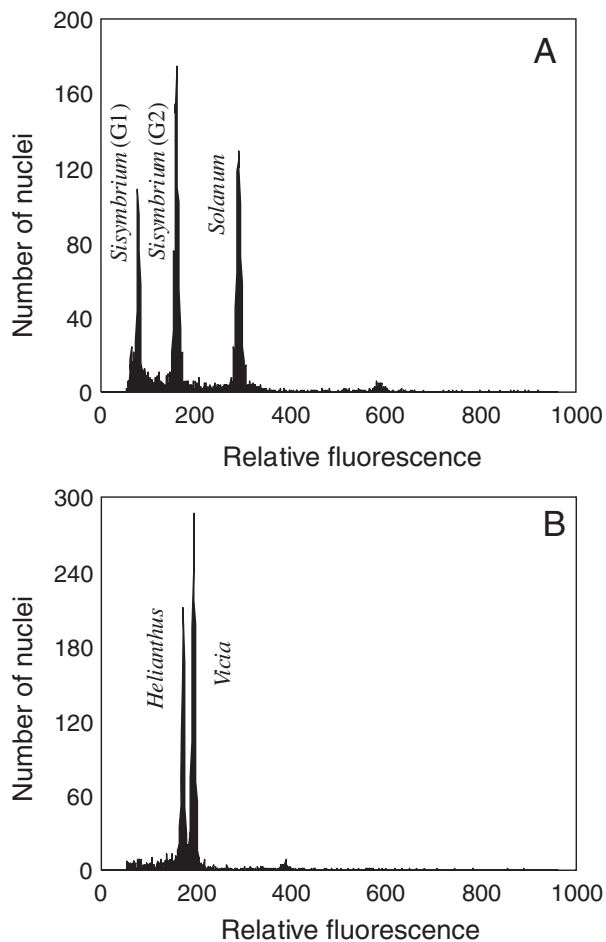
Fig. 1. – Flow cytometric histograms showing genome size determination in species with very small (*Sisymbrium altissimum*, 2C = 0.52 pg; panel A) and large (*Helianthus tuberosus*, 2C = 23.89 pg; panel B) genomes. Nuclei of both the analyzed sample and internal reference standard were isolated, stained with propidium iodide and analyzed simultaneously. *Solanum lycopersicum* and *Vicia faba*, respectively, were used as reference standards.

often very small and small genomes, and less often intermediate to very large genomes than expected by chance (G-test on contingency table: $\chi^2 = 61.15$, df = 2, P < 0.0001).

Ploidy levels in the species set analyzed varied from diploid to dodecaploid (Table 1), and monoploid genome sizes (1Cx-values) ranged from 0.12 pg in 8x *Juncus tenuis* to 4.38 in 2x *Virga strigosa* (= 38-fold range).

Factors affecting genome size of naturalized alien species

None of the examined explanatory variables (invasion status, life history, moisture score) had a significant effect on genome size (full model: F = 1.141; df = 11, 80; NS; observed power = 0.486).
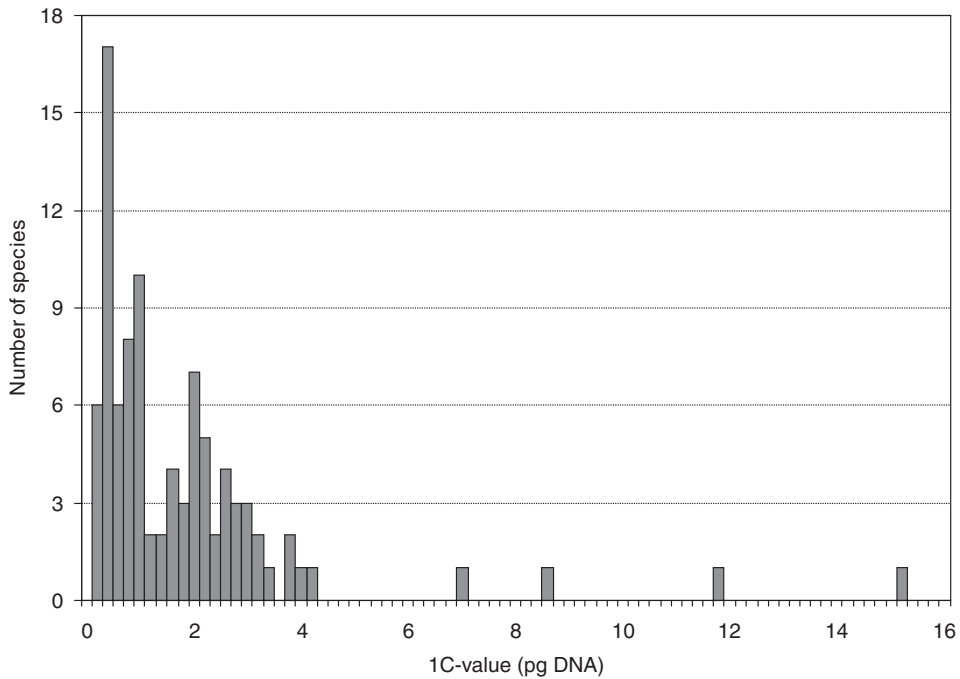
Fig. 2. – Distribution of 1C-values (pg means) in 93 naturalized alien species occurring in the Czech Republic.

Table 2. – Contingency table on observed and expected counts of genome size categories according to Leitch et al. (1998) in naturalized plants of the Czech flora (n = 93) and non-invading species (n = 4148) taken from the Plant DNA C-values database (Bennett & Leitch 2005). Genome size categories "intermediate", "large" and "very large" were pooled together due to small sample sizes in these categories for naturalized species. Statistics are given in the text.

Genome size category	Naturalized species		Non-invading species	
	observed	expected	observed	expected
Very small (1C ≤ 1.40 pg)	49	30.0	1287	1306.7
Small (1C = 1.41–3.50 pg)	36	22.1	958	972.2
Intermediate to very large (1C ≥ 3.51 pg)	8	40.9	1903	1869.1

Genome size in naturalized alien species vs their non-invading relatives

Naturalized aliens had significantly smaller holoploid and monoploid genome sizes than their non-invading congeners (two-sided paired t-tests: 2C-values t = 2.161, df = 45, P = 0.04; Cx-values t = 2.70, df = 44, P = 0.01), and the same held for 2C-values on confamilials (t = 3.161, df = 30, P = 0.004). Visual inspection of data indicates that naturalized aliens had smaller genomes in 19 of 31 families considered (Fig. 3).

Table 1. – List of analyzed species, with their family affiliation, life history, LH (an – annual; mono – monocarpic perennial; per – polycarpic perennial), invasion status, moisture score (see Methods for calculation) and genome size characteristics: mean holoploid genome sizes (2C-values with standard deviation, and 1C-values in picograms of DNA and megabase pairs; 1 pg = 978 Mbp), ploidy levels, monoploid genome sizes (1Cx-values), and internal reference standard used (B – *Bellis perennis*; G – *Glycine max*; P – *Pisum sativum*; S – *Solanum lycopersicum*; V – *Vicia faba*; Z – *Zea mays*). Invasion status was taken from Pyšek et al. (2002); species marked with asterisk are classified differently to better reflect situation in sampled localities. Species analyzed for the first time are shown in bold; those used for comparison with non-invading congeners are designated by "+" after species name. Empty cells – data not available.

Species	Family	Life history	Invasion status	Moisture score	2C-value (pg DNA)	S.D.	1C-value (pg DNA)	1C-value (Mbp)	Ploidy level	1Cx-value (pg DNA)	Internal standard
Abutilon theophrasti	*Malvaceae*	an	naturalized*	4.0	2.17	0.02	1.09	1061.1	6	0.36	B
Amaranthus albus +	*Amaranthaceae*	an	naturalized	2.0	1.11	0.00	0.55	540.8	4	0.28	S
Amaranthus blitoides +	*Amaranthaceae*	an	naturalized	2.3	1.13	0.00	0.56	552.1	4	0.28	G
Amaranthus powellii +	*Amaranthaceae*	an	invasive	4.0	1.02	0.00	0.51	496.8	4	0.25	B
Amaranthus retroflexus +	*Amaranthaceae*	an	invasive	2.2	1.12	0.01	0.56	546.2	4	0.28	S
Ambrosia artemisiifolia	*Asteraceae*	an	invasive	3.0	2.32	0.03	1.16	1134.5	4	0.58	B
Ambrosia trifida	*Asteraceae*	an	casual	3.7	3.82	0.03	1.91	1865.5	2	1.91	P
Angelica archangelica	*Apiaceae*	mono	invasive	5.0	6.46	0.10	3.23	3158.0	2	3.23	P
Antirrhinum majus	*Plantaginaceae*	mono	naturalized	1.0	1.17	0.01	0.58	570.7	2	0.58	S
Arabis alpina	*Brassicaceae*	per	naturalized	2.0	0.83	0.02	0.42	406.8	2	0.42	S
Asclepias syriaca	*Apocynaceae*	per	naturalized	3.0	0.84	0.03	0.42	409.8	2	0.42	S
Aster lanceolatus +	*Asteraceae*	per	invasive	4.0	5.41	0.01	2.71	2647.0	8	0.68	P
Bidens connata	*Asteraceae*	an	casual	5.0	6.44	0.06	3.22	3150.6	4	1.61	Z
Bidens frondosa	*Asteraceae*	an	invasive	5.0	3.20	0.02	1.60	1566.3	4	0.80	P
Bunias orientalis +	*Brassicaceae*	mono	invasive	2.1	5.40	0.02	2.70	2641.6	2	2.70	P
Cannabis ruderalis +	*Cannabaceae*	an	naturalized*	2.5	1.81	0.01	0.91	885.1	2	0.91	S
Cardamine chelidonia +	*Brassicaceae*	mono	naturalized	3.0	0.71	0.00	0.36	349.1	4	0.18	S
Claytonia alsinoides	*Portulacaceae*	an	naturalized	4.0	6.91	0.05	3.45	3377.0			P
Collomia grandiflora	*Polemoniaceae*	an	naturalized	3.0	4.15	0.04	2.08	2030.3	2	2.08	B
Consolida orientalis	*Ranunculaceae*	an	naturalized	2.4	4.57	0.04	2.28	2233.3	2	2.28	P
Conyza canadensis	*Asteraceae*	an	invasive	1.8	0.91	0.01	0.45	443.5	2	0.45	S
Corydalis lutea	*Papaveraceae*	per	naturalized	1.0	1.26	0.01	0.63	616.1	4	0.32	S
Cuscuta campestris	*Convolvulaceae*	an	invasive	3.0	0.96	0.00	0.48	469.4	8	0.12	S
Datura stramonium +	*Solanaceae*	an	naturalized	2.0	4.19	0.05	2.09	2047.4	2	2.09	Z
Digitalis purpurea	*Plantaginaceae*	mono	invasive	2.4	1.86	0.01	0.93	910.0	8	0.23	G
Duchesnea indica	*Rosaceae*	per	naturalized	3.0	3.45	0.01	1.72	1686.6	12	0.29	P
Echinocystis lobata	*Cucurbitaceae*	an	invasive	3.0	1.49	0.01	0.74	727.6	4	0.37	S
Echinops sphaerocephalus	*Asteraceae*	per	invasive	2.5	8.02	0.11	4.01	3923.7	4	2.01	Z
Epilobium ciliatum +	*Onagraceae*	per	invasive	4.0	0.82	0.03	0.41	401.0	4	0.21	S
Epilobium dodonaei +	*Onagraceae*	per	naturalized	5.0	1.17	0.01	0.59	573.1	4	0.29	S
Erigeron annuus	*Asteraceae*	mono	invasive	2.0	4.33	0.05	2.17	2118.8	3	1.44	Z
Erucastrum gallicum	*Brassicaceae*	mono	naturalized	2.0	2.08	0.01	1.04	1018.6	4	0.52	B
Galega officinalis	*Fabaceae*	per	naturalized	3.0	4.42	0.04	2.21	2161.4	2	2.21	G
Galinsoga parviflora	*Asteraceae*	an	invasive	3.0	1.47	0.01	0.74	720.3	4	1.01	S
Galinsoga quadriradiata	*Asteraceae*	an	invasive	2.0	4.03	0.04	2.02	1972.6	2	0.74	Z
Geranium pyrenaicum	*Geraniaceae*	per	invasive	3.0	2.87	0.02	1.44	1403.9	2	1.44	G
Helianthus tuberosus +	*Asteraceae*	per	invasive	4.0	23.89	0.09	11.95	11682.7	12	1.99	V
Heracleum mantegazzianum +	*Apiaceae*	mono	invasive	3.0	3.56	0.03	1.78	1740.8	2	1.78	Z
Hesperis matronalis	*Brassicaceae*	per	naturalized	3.4	7.61	0.07	3.80	3719.3	4	1.90	P
Hordeum jubatum +	*Poaceae*	an	naturalized	2.0	17.38	0.09	8.69	8499.8	4	4.35	P
Chenopodium pumilio +	*Amaranthaceae*	an	naturalized	1.0	0.73	0.02	0.37	357.0	2	0.37	P
Chenopodium strictum +	*Amaranthaceae*	an	naturalized	3.1	1.60	0.01	0.80	782.4	4	0.40	P

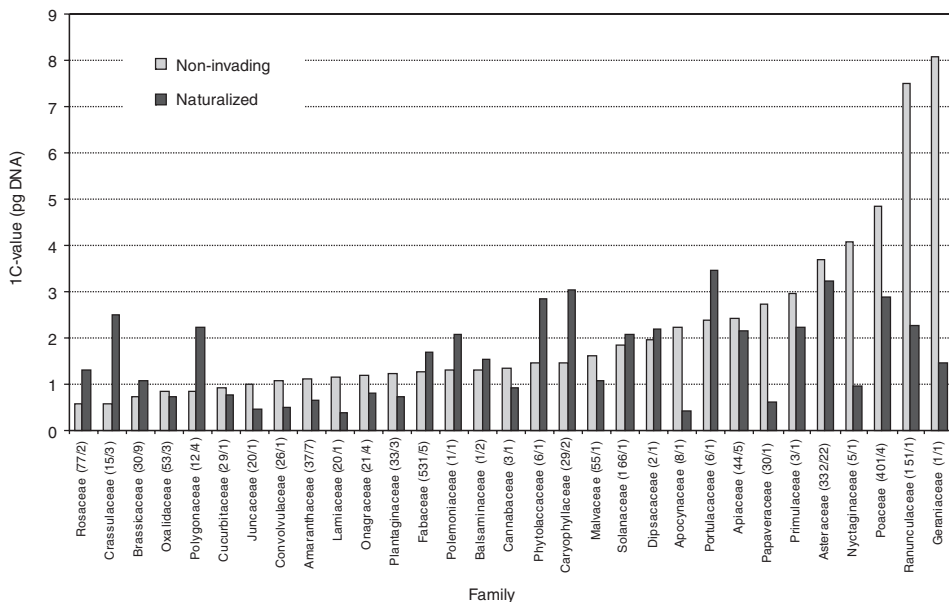| Species	Family	Life history	Invasion status	Moisture score	2C-value (pg DNA)	S.D.	1C-value (pg DNA)	1C-value (Mbp)	Ploidy level	1Cx-value (pg DNA)	Internal standard
Impatiens glandulifera +	*Balsaminaceae*	an	invasive	3.3	1.90	0.01	0.95	927.6	2	0.95	B
Impatiens parviflora +	*Balsaminaceae*	an	invasive	3.0	4.26	0.04	2.13	2083.6	2	2.13	Z
Imperatoria ostruthium	*Apiaceae*	per	invasive	3.0	3.89	0.08	1.95	1904.2	2	1.95	P
Inula helenium +	*Asteraceae*	mono	naturalized	3.0	4.53	0.01	2.26	2214.2	2	2.26	P
Iva xanthiifolia	*Asteraceae*	an	naturalized	2.0	6.34	0.07	3.17	3098.8	4	1.58	Z
Juncus tenuis +	*Juncaceae*	per	invasive	3.0	0.92	0.01	0.46	450.9	8	0.12	S
Kochia scoparia	*Amaranthaceae*	an	invasive	2.3	2.23	0.01	1.12	1090.5	2	1.12	G
Lepidium densiflorum +	*Brassicaceae*	mono	naturalized	2.0	0.66	0.03	0.33	322.7	4	0.17	S
Lupinus polyphyllus +	*Fabaceae*	per	invasive	3.0	1.60	0.02	0.80	783.4	4	0.40	S
Lychnis coronaria	*Caryophyllaceae*	mono	naturalized	2.0	6.30	0.17	3.15	3078.7	2	3.15	Z
Lysimachia punctata	*Primulaceae*	per	naturalized	5.0	4.43	0.02	2.21	2165.8	2	2.21	P
Matricaria discoidea +	*Asteraceae*	an	invasive	3.0	4.70	0.01	2.35	2298.3	2	2.35	B
Medicago sativa +	*Fabaceae*	per	naturalized*	3.0	3.49	0.03	1.74	1706.1	4	0.87	S
Mimulus guttatus	*Phrymaceae*	per	invasive	5.0	0.73	0.03	0.37	357.0	4	0.18	S
Myrrhis odorata	*Apiaceae*	per	invasive	5.0	1.81	0.01	0.90	883.1	2	0.90	G
Oenothera biennis +	*Onagraceae*	mono	invasive	2.0	2.30	0.02	1.15	1124.7	2	1.15	S
Oenothera glazoviana +	*Onagraceae*	mono	naturalized	3.0	2.30	0.01	1.15	1122.7	2	1.15	S
Oxalis corniculata subsp. repens +	*Oxalidaceae*	mono	naturalized*	2.0	2.11	0.02	1.05	1031.3	8	0.26	S
Oxalis dillenii +	*Oxalidaceae*	mono	naturalized	2.0	1.01	0.01	0.50	491.9	4	0.25	G
Oxalis fontana +	*Oxalidaceae*	mono	naturalized	3.0	1.22	0.01	0.61	594.1	4	0.30	G
Oxybaphus nyctagineus	*Nyctaginaceae*	per	naturalized		1.89	0.01	0.95	924.2	6	0.32	B
Panicum capillare +	*Poaceae*	an	naturalized	2.0	0.91	0.01	0.45	443.5	2	0.45	B
Panicum miliaceum +	*Poaceae*	an	casual	4.3	2.09	0.04	1.04	1020.5	4	0.52	B
Phytolacca esculenta +	*Phytolaccaceae*	per	naturalized	3.0	5.68	0.10	2.84	2778.5	8	0.71	P
Potentila intermedia +	*Rosaceae*	mono	naturalized	1.0	1.80	0.01	0.90	880.2	4	0.45	S
Rudbeckia hirta	*Asteraceae*	per	naturalized	2.6	14.33	0.09	7.17	7008.3	4	3.58	P
Rudbeckia laciniata	*Asteraceae*	per	invasive	4.0	30.54	0.12	15.27	14935.5	8	3.82	P
Rumex alpinus +	*Polygonaceae*	per	invasive	3.0	0.96	0.01	0.48	467.0	2	0.48	S
Rumex longifolius +	*Polygonaceae*	per	invasive	2.0	3.99	0.07	2.00	1951.6	6	0.67	B
Rumex patientia +	*Polygonaceae*	per	naturalized	2.0	4.87	0.02	2.43	2380.5	6	0.81	Z
Rumex thyrsiflorus +	*Polygonaceae*	per	invasive	1.0	7.81	0.05	3.90	3817.1	2	3.90	P
Scutellaria altissima	*Lamiaceae*	per	naturalized	2.0	0.79	0.03	0.40	386.3	4	0.20	S
Sedum hispanicum	*Crassulaceae*	per	invasive	1.0	5.39	0.04	2.70	2636.2			S
Sedum rupestre	*Crassulaceae*	per	naturalized	3.0	5.41	0.09	2.70	2643.0	4	1.35	P
Sedum spurium +	*Crassulaceae*	per	naturalized	1.0	4.16	0.03	2.08	2034.2	2	2.08	P
Senecio inaequidens +	*Asteraceae*	per	naturalized*	3.0	2.90	0.01	1.45	1419.6	4	0.73	B
Senecio vernalis +	*Asteraceae*	an	naturalized	1.7	2.33	0.01	1.16	1138.4	2	1.16	B
Setaria faberi +	*Poaceae*	an	naturalized	3.0	2.56	0.02	1.28	1253.8	4	0.64	B
Silene dichotoma +	*Caryophyllaceae*	mono	naturalized	3.0	5.89	0.02	2.94	2878.7	2	2.94	P
Sisymbrium altissimum	*Brassicaceae*	an	naturalized	2.3	0.52	0.00	0.26	255.7	2	0.26	S
Sisymbrium loeselii	*Brassicaceae*	an	invasive	3.5	0.48	0.00	0.24	233.3	2	0.24	S
Sisymbrium strictissimum	*Brassicaceae*	per	naturalized	4.0	1.39	0.01	0.70	680.2	4	0.35	S
Smyrnium perfoliatum	*Apiaceae*	mono	naturalized	3.0	5.64	0.05	2.82	2758.0	2	2.82	P
Solidago canadensis	*Asteraceae*	per	invasive	3.0	2.04	0.01	1.02	999.5	2	1.02	G
Solidago gigantea	*Asteraceae*	per	invasive	4.0	3.65	0.03	1.82	1782.9	4	0.91	Z
Telekia speciosa	*Asteraceae*	per	invasive	3.0	2.57	0.01	1.29	1258.2	2	1.29	S
Trifolium hybridum +	*Fabaceae*	mono	invasive	2.0	1.09	0.01	0.54	532.0	2	0.54	S
Veronica persica +	*Plantaginaceae*	an	invasive	4.0	1.38	0.01	0.69	672.9	4	0.34	S
Vicia grandiflora +	*Fabaceae*	an	naturalized	3.0	6.23	0.07	3.11	3046.0	2	3.11	P
Virga strigosa	*Dipsacaceae*	mono	invasive	3.0	8.76	0.05	4.38	4283.2	2	4.38	Z
Xanthium albinum	*Asteraceae*	an	naturalized	5.0	5.18	0.09	2.59	2531.6	4	1.29	P

Fig. 3. – Comparison of median genome sizes (1C-values) of naturalized aliens with those of their non-invading confamilials in 31 plant families. Genome sizes for non-invading species were taken from the Plant DNA C-values database (Bennet & Leitch 2005), those known to be naturalized or invasive in any part of the world were excluded. Plant families are sorted according to the genome size of non-invading species. Numbers of species (non-invading/naturalized) are shown in parentheses.

Discussion

Genome size variation

We determined nuclear DNA amounts in a representative set of naturalized plant species in the flora of the Czech Republic and compared their values with genome sizes of non-invading species taken from the Plant DNA C-values database (Bennett & Leitch 2005). Out of 93 naturalized species included, 66 (= 71%) were analyzed for the first time. In addition, the first record was obtained for the family *Phrymaceae*, which had a very small genome (*Mimulus guttatus*; 1C = 0.37 pg).

A comparison of genome size values for the same species as determined in our study with those extracted from the database (Bennett & Leitch 2005) revealed some discrepancies. Disregarding variation caused by potential differences in ploidy level, the average absolute difference in C-values was 28%. While about one third of the species (nine out of 26) showed differences below 10% (which is within the acceptable between-laboratory limit as suggested by Doležel et al. 1998), four species differed more than 1.5-fold (*Amaranthus retroflexus*, *Solidago canadensis*, *Galinsoga parviflora* and *Antirrhinum majus*). It should, however, be noted that the difference largely depended on the methodology used. The best congruency between our data and the database values was observed for measurements performed using propidium iodide flow cytometry (absolute difference <

10%, n = 4), which is generally recommended as the most reliable technique for genome size estimation in plants (Greilhuber et al. 2007, Temsch et al. 2010). Species analyzed either by Feulgen densitometry (which is much more sensitive to working conditions; Greilhuber 2005) or flow cytometry with base-selective fluorochromes (Doležel et al. 1992) showed higher differences (30%, n = 21 and 54%, n = 1, respectively). Whereas small differences in determined genome sizes can be explained by minor variation in adopted protocols between laboratories (use of different buffers, different internal reference standards, etc.), it is possible that more serious methodological flaws were involved in other cases and such data should be treated with caution.

Very small and small genomes (in the sense of the classification of genome sizes defined by Leitch et al. 1998) clearly prevailed in our data set, accounting for 53 and 39% of the species total, respectively. This is a dramatically different frequency of individual genome size categories as compared to non-invading species (Table 2). For example, while intermediate genomes (1C = 3.51–14.0 pg) are as common as the very small ones (1C ≤ 1.4 pg) in non-invading plants, their ratio drops down to only one seventh of the frequency of very small genomes in naturalized aliens. Significantly smaller genomes in naturalized plants as compared to their phylogenetically related non-invading counterparts were also confirmed at both taxonomic levels tested, i.e., the rank of genus and family. The same pattern of genome size variation as in naturalized plants (i.e. predominance of species with low nuclear DNA amounts and the lack of DNA-obese species) was observed, for instance, in weedy plants (Bennett et al. 1998) or in endemics on oceanic islands (Suda et al. 2005). Selection for rapid development, fast growth, and production of many light and easily dispersible seeds are plausible evolutionary forces that constrain the genome size in these plant groups.

The presence of species with different life histories allowed us to test the potential association between genome size and life history (Bennett 1972). Although basic descriptive statistics for 36 polycarpic perennial plants (mean and median 1C-values 2.44 pg and 1.73 pg, respectively) somehow differed from corresponding values for both 36 annuals (mean = 1.58 pg, median = 1.10 pg) and 21 monocarpic species (mean = 1.65 pg, median = 1.15 pg), the differences were not statistically significant.

Relationship between genome size and invasion success

By using the data set analyzed in this study, we were unable to detect the effect of any species traits examined on the genome size. However, it should be noted that the low test power of the model does not allow us to conclude that such effects do not exist. The analysis was carried out with individual species as random independent data points. This can inflate degrees of freedom, because the species can be mutually dependent due to their phylogenetic relatedness (e.g. Harvey & Pagel 1991). However, removing phylogenetic effects from the nonsignificant general linear model would require methods based on eigenvector filtering (Diniz-Filho et al. 1998) and repeating the analysis after this correction could thus only further decrease the statistical significance of the results. That genome size is associated with invasion success is clearly demonstrated by the comparison of naturalized aliens in the Czech flora with the reference global set of non-invading congeners. In this analysis the effect of phylogenetic relatedness, which was shown to bias the effect of traits on species' invasion success (e.g. Crawley et al. 1996, Pyšek 1997, Goodwin et al.

1999, Grotkopp et al. 2004, Hamilton et al. 2005, Lloret et al. 2005, Cadotte et al. 2006) was suppressed by the congeneric comparison. This is a convenient approach to studying the role of species traits in plant invasions (Pyšek & Richardson 2007, Perglová et al. 2009).

Our results therefore provide robust evidence, based on a large number of species across a wide range of plant families, that alien species that successfully naturalize have smaller genomes than those that do not reach the stage of naturalization. It should be, however, noted that sample size for some families was rather limited (e.g. only one naturalized and/or non-invading species was available for 18 out of 31 families used for comparison), which may limit the generality of our conclusions and calls for further comparative studies. The association of small genome size with invasiveness was previously suggested in a number of studies (Rejmánek 1996, 2000, Grotkopp et al. 2002, Rejmánek et al. 2005, Garcia et al. 2008). Small genome size seems to be a result of selection for short minimum generation time. It is also associated with small seed size, high leaf area ratio and high relative growth rate of seedlings (Grotkopp et al. 2002), and as such may be an ultimate determinant of plant species invasiveness in disturbed habitats (Rejmánek 1996, Bennett et al. 1998, Grotkopp et al. 1998, Rejmánek 2000).

However, studies that addressed the role of genome size in plant species' invasiveness usually compared invasive and non-invasive species and did not distinguish between species at different stages of the invasion process. In our data there was no difference in the genome size of invasive species compared to naturalized but non-invasive species. This indicates that the small genome size may provide alien plants with an advantage already at the stage of naturalization and need not necessarily play a role during the follow-up step, transition from naturalized to invasive species. It also points to the importance of distinguishing the stages of invasion in such studies since the determinants of invasion success may differ between stages (Williamson 2006, Pyšek et al. 2008, 2009a, b).

See http://www.preslia.cz for Electronic Appendix 1.

Souhrn

Velikost genomu bývá považována za vlastnost ovlivňující invazivnost rostlinných druhů. V článku je tato souvislost testována na souboru 93 nepůvodních naturalizovaných druhů české flóry ze 32 čeledí, u nichž byl změřen obsah jaderné DNA metodou průtokové cytometrie. Hodnoty získané pro naturalizované druhy byly srovnány s hodnotami udávanými v databázi velikosti genomu rostlin pro druhy ze stejných rodů a čeledí, o nichž není známo, že by byly někde ve světě invazní. Ukázalo se, že druhy naturalizované v České republice mají statisticky průkazně menší genom než neinvazní druhy ze stejných rodů. Tento trend potvrdilo i srovnání naturalizovaných druhů s druhy ze stejných čeledí; zde byl zjištěn menší genom u 19 z 31 analyzovaných čeledí. Nepůvodní naturalizo-

vané druhy oproti druhům neinvazním navíc vykazovaly i zcela odlišné zastoupení jednotlivých kategorií velikosti genomu, nápadná je zejména výrazná převaha velmi malých genomů a velice nízký podíl druhů s velkými genomy. Tyto výsledky jsou prvním kvantitativním potvrzením založeném na velkém počtu druhů, že malé genomy přispívají k invazivnosti rostlinných druhů. Přitom invazní druhy v analyzovaném souboru se nelišily od druhů naturalizovaných, leč neinvazních. To ukazuje, že působení malého genomu jakožto vlastnosti výhodné pro invazi je spíše spojeno se stádiem naturalizace, zatímco při přechodu do stádia vlastní invazi již tak významné být nemusí.

References

Bennett M. D. (1972): Nuclear DNA content and minimum generation time in herbaceous plants. – Proc. R. Soc. London B 181: 109–135.

Bennett M. D. (1987): Variation in genomic form in plants and its ecological implications. – New Phytol. 106 (Suppl.): 177–200.

Bennett M. D. & Leitch I. J. (2005): Plant DNA *C*-values database (release 4.0, Oct. 2005). – URL: [http://www.kew.org/cvalues/].

Bennett M. D., Leitch I. J. & Hanson L. (1998): DNA amounts in two samples of angiosperm weeds. – Ann. Bot. 82: 121–134.

Binimelis R., Born W., Monterroso I. & Rodríguez-Labajos B. (2007): Socio-economic impacts and assessment of biological invasions. – In: Nentwig N. (ed.), Biological invasions, p. 331–347, Springer, Berlin.

Blackburn T. M., Lockwood J. L. & Cassey P. (2009): Avian invaders: the ecology and evolution of exotic birds. – Oxford University Press, Oxford.

Cadotte M. W., Murray B. R. & Lovett-Doust J. (2006): Evolutionary and ecological influences of plant invader success in the flora of Ontario. – Ecoscience 13: 388–395.

Chytrý M., Maskell L. C., Pino J., Pyšek P., Vilá M., Font X. & Smart S. M. (2008): Habitat invasions by alien plants: a quantitative comparison among Mediterranean, subcontinental and oceanic regions of Europe. – J. Appl. Ecol. 45: 448–458.

Chytrý M., Pyšek P., Tichý L., Knollová I. & Danihelka J. (2005): Invasions by alien plants in the Czech Republic: a quantitative assessment across habitats. – Preslia 77: 339–354.

Chytrý M., Wild J., Pyšek P., Tichý L., Danihelka J. & Knollová I. (2009): Maps of the level of invasion of the Czech Republic by alien plants. – Preslia 81: 187–207.

Crall A. W., Meyerson L. A., Stohlgren T. J., Jarnevich C. S., Newman G. J. & Graham J. (2006): Show me the numbers: what data currently exist for non-native species in the USA? – Front. Ecol. Environ. 4: 414–418.

Crawley M. J. (1993): GLIM for Ecologists. – Blackwell, Oxford.

Crawley M. J. (2002): Statistical computing: an introduction to data analysis using S-Plus. – Wiley, Chichester.

Crawley M. J., Harvey P. H. & Purvis A. (1996): Comparative ecology of the native and alien floras of the British Isles. – Biol. Trans. R. Soc. B 351: 1251–1259.

DAISIE (2009): Handbook of alien species in Europe. – Springer, Berlin.

Davis M. A. (2009): Invasion biology. – Oxford University Press, Oxford.

Davis M. A., Grime J. P. & Thompson K. (2000): Fluctuating resources in plant communities: a general theory of invasibility. – J. Ecol. 88: 528–534.

Diniz-Filho J. A. F., De Santana C. E. R. & Bini L. M. (1998): An eigenvector method for estimating phylogenetic inertia. – Evolution 52: 1247–1262.

Doležel J., Greilhuber J., Lucretti S., Meister A., Lysák M. A., Nardi L. & Obermayer R. (1998): Plant genome size estimation by flow cytometry: inter-laboratory comparison. – Ann. Bot. 82 (Suppl. A): 17–26.

Doležel J., Greilhuber J. & Suda J. (2007): Estimation of nuclear DNA content in plants using flow cytometry. – Nature Protoc. 2: 2233-2244.

Doležel J., Sgorbati S. & Lucretti S. (1992): Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. – Physiol. Plant. 85: 625–631.

Ekrt T., Trávníček P., Jarolímová V., Vít P. & Urfus T. (2009): Genome size and morphology of the *Dryopteris affinis* group in Central Europe. – Preslia 81: 261–280.

Ellenberg H., Weber H. E., Düll R., Wirth W., Werner W. & Paulißen D. (1992): Zeigerwerte von Pflanzen in Mitteleuropa. Ed. 2. – Scripta Geobotanica 18: 1–258.

Essl F., Dullinger D. & Kleinbauer I. (2009): Changes in the spatio-temporal patterns and habitat preferences of *Ambrosia artemisiifolia* during the invasion of Austria. – Preslia 81: 119–133.

Gaertner M., Breeyen A. D., Hui C. & Richardson D. M. (2009): Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. – Progr. Phys. Geogr. 33: 319–338.

Garcia S., Canela M. A., Garnatje T., McArthur E. D., Pellicer J., Sanderson S. C. & Vallés J. (2008): Evolutionary and ecological implications of genome size in the North American endemic sagebrushes and allies (*Artemisia*, *Asteraceae*). – Biol. J. Linn. Soc. 94: 631–649.

Goldblatt P. & Johnson D. E. (eds) (1979 onwards): Index to plant chromosome numbers. – Missouri Botanical Garden, St. Louis, URL: [http://mobot.mobot.org/W3T/Search/ipcn.html].

Goodwin B. J., McAllister A. J. & Fahrig L. (1999): Predicting invasiveness of plant species based on biological information. – Conserv. Biol. 13: 422–426.

Gregory T. R. (2005): The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. – Ann. Bot. 95: 133-146.

Greilhuber J. (2005): Intraspecific variation in genome size in angiosperms: identifying its existence. – Ann. Bot. 95: 91–98.

Greilhuber J., Doležel J., Lysák M. & Bennett M. D. (2005): The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. – Ann. Bot. 95: 255–260.

Greilhuber J., Temsch E. M. & Loureiro J. C. M. (2007): Nuclear DNA content measurement. – In: Doležel J., Greilhuber J. & Suda J. (eds), Flow cytometry with plant cells, p. 67–101, Wiley-VCH, Weinheim.

Grotkopp E., Rejmánek M. & Rost T. L. (2002): Toward a causal explanation of plant invasiveness: seedling growth and life-history strategies of 29 pine (*Pinus*) species. – Am. Nat. 159: 396–419.

Grottkop E., Rejmánek M., Sanderson M. J. & Rost T. L. (2004): Evolution of genome size in pines (*Pinus*) and its life-history correlates: supertree analyses. – Evolution 58: 1705–1729.

Grotkopp E., Stoltenberg R., Rejmánek M. & Rost T. L. (1998): The effect of genome size on invasiveness. – Am. J. Bot. 85 (Suppl.): 34.

Hamilton M. A., Murray B. R., Cadotte M. W., Hose G. C., Baker A. C., Harris C. J. & Licari D. (2005): Life-history correlates of plant invasiveness at regional and continental scales. – Ecol. Lett. 8: 1066–1074.

Harvey P. H. & Pagel M. D. (1991): The comparative method in evolutionary biology. – Oxford University Press, Oxford.

Hejda M., Pyšek P. & Jarošík V. (2009a): Impact of invasive plants on the species richness, diversity and composition of invaded communities. – J. Ecol. 97: 393–403.

Hejda M., Pyšek P., Pergl J., Sádlo J., Chytrý M. & Jarošík V. (2009b): Invasion success of alien plants: do habitats affinities in the native distribution range matter? – Glob. Ecol. Biogeogr. 18: 372–382.

Hejný S. & Slavík B. (eds) (1988–1992): Květena ČR [Flora of the Czech Republic]. Vol. 1 (1988), 2 (1990), 3 (1992). – Academia, Praha.

Hulme P. E., Bacher S., Kenis M., Klotz S., Kühn I., Minchin D., Nentwig W., Olenin S., Panov V., Pergl J., Pyšek P., Roques A., Sol D., Solarz W. & Vilà M. (2008): Grasping at the routes of biological invasions: a framework for integrating pathways into policy. – J. Appl. Ecol. 45: 403–414.

Hulme P. E., Nentwig W., Pyšek P. & Vilà M. (2009a): Common market, shared problems: time for a coordinated response to biological invasions in Europe? – Neobiota 8: 3–19.

Hulme P., Pyšek P., Nentwig W. & Vilà M. (2009b): Will threat of biological invasions unite the European Union? – Science 324: 40–41.

Keller R., Lodge D. M. & Finnoff D. (2007): Risk assessment for invasive species produces net bioeconomic benefits. – Proc. Natl Acad. Sci. USA 104: 203–207.

Kettunen M., Genovesi P., Gollasch S., Pagad S., Starfinger U., ten Brink P. & Shine C. (2009): Technical support to EU strategy on invasive species (IAS): assessment of the impacts of IAS in Europe and the EU (final module report for the European Commission). – Institute for European Environmental Policy, Brussels.

Knight C. A., Molinari N. A. & Petrov D. A. (2005): The large genome constraint hypothesis: evolution, ecology and phenotype. – Ann. Bot. 95: 177–190.

Kron P., Suda J. & Husband B. C. (2007): Applications of flow cytometry to evolutionary and population biology. – Annu. Rev. Ecol. Evol. Syst. 38: 847–876.

Lambdon P. W., Pyšek P., Basnou C., Hejda M., Arianoutsou M., Essl F., Jarošík V., Pergl J., Winter M., Anastasiu P., Andriopoulos P., Bazos I., Brundu G., Celesti-Grapow L., Chassot P., Delipetrou P., Josefsson M., Kark S., Klotz S., Kokkoris Y., Kühn I., Marchante H., Perglová I., Pino J., Vilà M., Zikos A., Roy D. & Hulme P. E. (2008): Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. – Preslia 80: 101–149.

Lawrence M. E. (1985): *Senecio* L. (*Asteraceae*) in Australia: nuclear DNA amounts. – Aust. J. Bot. 33: 221–232.

Leitch, I. J. & Bennett M. D. (2007). Genome size and its uses: the impact of flow cytometry. – In: Doležel J., Greilhuber J. & Suda J. (eds), Flow cytometry with plant cells, p. 153–176, Wiley-VCH, Weinheim.

Leitch I. J., Chase M. W. & Bennett M. D. (1998): Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. – Ann. Bot. 82 (Suppl. A): 85–94.

Levine J. M., Vilà M., D'Antonio C. M., Dukes J. S., Grigulis K. & Lavorel S. (2003): Mechanisms underlying the impacts of exotic plant invasions. – Proc. R. Soc. London B 270: 775–781.

Lloret F., Médail F., Brundu G., Camarda I., Moragues E., Rita J., Lambdon P. & Hulme P. E. (2005): Species attributes and invasion success by alien plants on Mediterranean islands. – J. Ecol. 93: 512–520.

Loureiro J., Trávníček P., Rauchová J., Urfus T., Vít P., Štech M., Castro S. & Suda J. (2010): The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. – Preslia 82: 3–21.

Marhold K., Mártonfi P., Mereďa jun. P., Mráz P., Hodálová I., Kolník M., Kučera J., Lihová J., Mrázová V., Perný M. & Valko I. (2007): Karyologická databáza paprađorastov a semenných rastlín Slovenska [Karyological database of the ferns and flowering plants of Slovakia], ver. 1. – URL: [http://www.chromosomes.sav.sk].

McGeoch M. A., Butchart S. H. M., Spear D., Marais E., Kleynhans E. J., Symes A., Chanson J. & Hoffmann M. (2010): Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. – Diversity Distrib. 16: 95–108.

Meyerson L. A. & Mooney H. A. (2007): Invasive alien species in an era of globalization. – Front. Ecol. Environ. 5: 199–208.

Mukherjee S. & Sharma A. K. (1990): Mitotic cycle duration and its relationship with nuclear DNA content, chromosome number and size and rate of growth in species of *Acacia* growing under stress. – Ind. J. Exp. Biol. 28: 508–510.

Nentwig W., Kühnel E. & Bacher S. (2010): A generic impact-scoring system applied to alien mammals in Europe. – Cons. Biol. 204: 302–311.

Otto F. (1990): DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. – In: Crissman H. A. & Darzynkiewicz Z. (eds), Methods in cell biology 33: 105–110, Academic Press, New York.

Perglová I., Pergl J., Skálová H., Moravcová L., Jarošík V. & Pyšek P. (2009): Differences in germination and seedling establishment of alien and native *Impatiens* species. – Preslia 81: 357–375.

Pyšek P. (1997): Clonality and plant invasions: can a trait make a difference? – In: de Kroon H. & van Groenendael J. (eds), The ecology and evolution of clonal plants, p. 405–427, Backhuys Publishers, Leiden.

Pyšek P., Jarošík V. , Pergl J., Randall R., Chytrý M., Kühn I., Tichý L., Danihelka J., Chrtek jun. J. & Sádlo J. (2009a): The global invasion success of Central European plants is related to distribution characteristics in their native range and species traits. – Diversity Distrib. 15: 891–903.

Pyšek P., Křivánek M. & Jarošík V. (2009b): Planting intensity, residence time, and species traits determine invasion success of alien woody species. – Ecology 90: 2734–2744.

Pyšek P. & Richardson D. M. (2007): Traits associated with invasiveness in alien plants: where do we stand? – In: Nentwig W. (ed.), Biological invasions, p. 97–125, Springer-Verlag, Berlin & Heidelberg.

Pyšek P., Richardson D. M. & Jarošík V. (2006): Who cites who in the invasion zoo: insights from an analysis of the most highly cited papers in invasion ecology. – Preslia 78: 437–468.

Pyšek P., Richardson D. M., Pergl J., Jarošík V., Sixtová Z. & Weber E. (2008): Geographical and taxonomic biases in invasion ecology. – Trends Ecol. Evol. 23: 237–244.

Pyšek P., Richardson D. M., Rejmánek M., Webster G., Williamson M. & Kirschner J. (2004): Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. – Taxon 53: 131–143.

Pyšek P., Sádlo J. & Mandák B. (2002): Catalogue of alien plants of the Czech Republic. – Preslia 74: 97–186.

Rejmánek M. (1996): A theory of seed plant invasiveness: the first sketch. – Biol. Cons. 78: 171–181.

Rejmánek M. (2000): Invasive plants: approaches and predictions. – Austral Ecol. 25: 497–506.

Rejmánek M., Richardson D. M. & Pyšek P. (2005): Plant invasions and invasibility of plant communities. – In: Van der Maarel E. (ed.), Vegetation ecology, p. 332–355, Blackwell Science, Oxford.

Ricciardi A. & MacIsaac H. J. (2008): The book that began invasion ecology. – Nature 452: 34.

Richardson D. M., Holmes P. M., Esler K. J., Galatowitsch S. M., Stromberg J. C., Kirkman S. P., Pyšek P. & Hobbs R. J. (2007): Riparian vegetation: degradation, alien plant invasions, and restoration prospects. – Diversity Distrib. 13: 126–139.

Richardson D. M. & Pyšek P. (2008): Fifty years of invasion ecology: the legacy of Charles Elton. – Diversity Distrib. 14: 161–168.

Richardson D. M., Pyšek P., Rejmánek M., Barbour M. G., Panetta F. D. & West C. J. (2000): Naturalization and invasion of alien plants: concepts and definitions. – Diversity Distrib. 6: 93–107.

Simberloff D. (2009): We can eliminate invasions or live with them: successful management projects. – Biol. Inv. 11: 149–157.

Slavík B. (ed.) (1995–2000): Květena ČR [Flora of the Czech Republic]. Vol. 4 (1995), 5 (1997), 6 (2000). – Academia, Praha.

Slavík B. & Štěpánková J. (eds) (2004): Květena ČR 8 [Flora of the Czech Republic. Vol. 8]. – Academia, Praha.

Sokal R. R. & Rohlf F. J. (1995): Biometry. Ed. 3. – Freeman, New York.

Štajerová K., Šmilauerová M. & Šmilauer P. (2009): Arbuscular mycorrhizal symbiosis of herbaceous invasive neophytes in the Czech Republic. – Preslia 81: 341–355.

Steidel R. J. & Thomas L. (2001): Power analysis and experimental design. – In: Scheiner S. M. & Gurevitch J. (eds), Design and analysis of ecological experiments, Ed. 2., p. 14–36, Oxford University Press, Oxford.

Stevens P. F. (2001 onwards): Angiosperm Phylogeny Website. Version 9, June 2008. – URL: [http://www.mobot.org/MOBOT/Research/APweb/welcome.html].

Suda J., Kyncl T. & Jarolímová V. (2005): Nuclear DNA amounts in Macaronesian angiosperms: forty percent of Canarian endemic flora completed. – Plant Syst. Evol. 252: 215–238.

Temsch E. M., Greilhuber J. & Krisai R. (2010): Genome size in liverworts. – Preslia 82: 63–80.

Vidic T., Greilhuber J., Vilhar B. & Dermastia M. (2009): Selective significance of genome size in a plant community with heavy metal pollution. – Ecol. Appl. 19: 1515–1521.

Vilà M., Basnou C., Pyšek P., Josefsson M., Genovesi P., Gollasch S., Nentwig W., Olenin S., Roques A., Roy D., Hulme P. E. & DAISIE partners (2010): How well do we understand the impacts of alien species on ecological services? A pan-European cross-taxa assessment. – Front. Ecol. Environ. 8: 135–144.

Wakamiya I., Newton R. J., Johnston J. S. & Price H. J. (1993): Genome size and environmental factors in the genus *Pinus*. – Am. J. Bot. 80:1235–1241.

Weber E. (2003): Invasive plant species of the world: a reference guide to environmental weeds. – CAB International Publishing, Wallingford.

Williamson M. (2006): Explaining and predicting the success of invading species at different stages of invasion. – Biol. Inv. 8: 1561–1568.