

Ecological effects of cell-level processes: genome size, functional traits and regional abundance of herbaceous plant species

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- **Background and Aims** Genome size is known to be correlated with a number of phenotypic traits associated with cell sizes and cell-division rates. Genome size was therefore used as a proxy for them in order to assess how common plant traits such as height, specific leaf area and seed size/number predict species regional abundance. In this study it is hypothesized that if there is residual correlation between genome size and abundance after these traits are partialled out, there must be additional ecological effects of cell size and/or cell-division rate.
- **Methods** Variation in genome size, plant traits and regional abundance were examined in 436 herbaceous species of central European flora, and relationships were sought for among these variables by correlation and path analysis.
- **Key Results** Species regional abundance was weakly but significantly correlated with genome size; the relationship was stronger for annuals ($R^2 = 0.145$) than for perennials ($R^2 = 0.027$). In annuals, genome size was linked to abundance via its effect on seed size, which constrains seed number and hence population growth rate. In perennials, it weakly affected (via height and specific leaf area) competitive ability. These relationships did not change qualitatively after phylogenetic correction. In both annuals and perennials there was an unresolved effect of genome size on abundance.
- **Conclusions** The findings indicate that additional predictors of regional abundance should be sought among variables that are linked to cell size and cell-division rate. Signals of these cell-level processes remain identifiable even at the landscape scale, and show deep differences between perennials and annuals. Plant population biology could thus possibly benefit from more systematic use of indicators of cell-level processes.

Key words: Annuals, C-value, functional traits, genome size, LEDA traitbase, native herbaceous plant species, path analysis, phylogenetic correction, perennials.

INTRODUCTION

One of the ultimate goals of ecology is to explain patterns of species abundance, both within communities and across regions. While determinants of abundance are complex and poorly known, the abundance patterns are ultimately due, through a chain of mediating processes, to plant functional traits, such as dispersal or growth traits (Suding *et al.*, 2003). For example, plant regional abundance and/or species range size have been shown to be affected by seed size (Thompson *et al.*, 1999; Murray *et al.*, 2002; Kolb *et al.*, 2006; van der Veken *et al.*, 2007; Gove *et al.*, 2009); likelihood of decrease in fragmented landscape is also affected by dispersal traits (Ozinga *et al.*, 2009). Growth and competition traits such as specific leaf area (SLA) (as a proxy for growth rate) also affect local or regional abundance (Grotkopp and Rejmánek, 2007; Cornwell and Ackerly, 2010; van Kleunen *et al.*, 2010).

In principle, all these traits can be traced down to cell-level processes such as cell size, number or cell-division rate. However, very little is known about the linkages that are

involved, because cell-level processes are rather difficult to study over larger sets of species. Recently, there has been increasing evidence that these cell-level processes are in close relationship with the size of the nuclear genome (Francis *et al.*, 2008; Šimová and Herben, 2012). Sizes of plant nuclear genomes vary over three orders of magnitude (from about 0.065 pg/1C to 152.23 pg/1C; Greilhuber *et al.*, 2006; Pellicer *et al.*, 2010). Although its full functional meaning is yet unknown, there is a strong evidence that genome size acts as a phenotypic trait and is correlated both with cell sizes (Bennett, 1987; Knight and Beaulieu, 2008; Hodgson *et al.*, 2010) and cell division/tissue growth rate (Bennett, 1987; Šimová and Herben, 2012). Because of these effects, genome size has been suggested to be one of the fundamental attributes that constrain plant fitness in the ecological time (Knight *et al.*, 2005, see also Grime, 1998). Because it can be measured easily, genome size can be a promising proxy variable for cell- and tissue-level processes such as cell size and division rate (Knight and Beaulieu, 2008).

On the other hand, cell size and division rate are correlated with a number of functional traits, leading to correlation between genome size and these traits (Knight and Beaulieu, 2008). For example, genome size has been shown to be correlated with specific leaf area (SLA), although the sign and intensity of the correlation varies across plant groups (mainly negative in gymnosperms, and mainly positive in angiosperms; Grotkopp *et al.*, 2004; Knight *et al.*, 2005; Morgan and Westoby, 2005; Beaulieu *et al.*, 2007a). Existing empirical evidence largely supports the negative effect of genome size on seedling relative growth rate (Grotkopp *et al.*, 2004; Knight *et al.*, 2005) and on SLA (Knight and Beaulieu, 2008), which is one of the key traits affecting individual growth rate (Reich *et al.*, 1992, 1998). Consequently, there is a positive relationship between generation time and genome size (Leitch and Bennett, 2007; but see Grotkopp *et al.*, 2004); because of this, plants with larger genomes are always perennials (Knight *et al.*, 2005). Further, genome size is correlated with seed mass (Thompson, 1990; Grotkopp *et al.*, 2004; Knight *et al.*, 2005; Knight and Beaulieu, 2008; for a deeper analysis, see Beaulieu *et al.*, 2007b) and consequently also seed number (Grotkopp *et al.*, 2004). In contrast, there is no unequivocal evidence on the relationship of genome size with plant height and/or overall biomass (Knight and Beaulieu, 2008; Gallagher *et al.*, 2011) with the exception of smaller genomes in woody species (Knight and Beaulieu, 2008).

It is thus apparent that genome size can be an important surrogate of species functional traits, but also of a broader range of ecological processes that are determined by cell size and division rate. Surprisingly, only little attention has been paid to this approach so far. In this paper, we therefore use the power of genome size to proxy cell-level processes as a tool to examine the role of these processes for species abundance. We address (a) whether indeed landscape-level phenomena such as species regional abundance can be traced down to cell- and tissue-level processes, and (b) whether current predictions of abundance by several key traits capture most of the variation due to these processes. Specifically, we examine seed size and number, plant height and SLA, which are known to be correlated with species abundance (Thompson *et al.*, 1999; Murray *et al.*, 2002; Kolb *et al.*, 2006; Grotkopp and Rejmánek, 2007; van der Veken *et al.*, 2007; Gove *et al.*, 2009; Cornwell and Ackerly, 2010) and, with a possible exception of plant size, are correlated with genome size.

Based on these findings, we hypothesize that there should be a correlation between genome size and abundance. However, if the investigated traits capture most of the variation due to cell- and tissue-level processes, this correlation should disappear after effects of these traits are taken into account. Any residual correlation remaining when these traits are partialled out would indicate that cell/tissue level processes affect abundance through other ways not captured by these traits.

It has indeed been demonstrated that genome size does correlate with some indicators of species abundance or size of distribution range. Northern latitudinal range limit tends to be negatively correlated with genome size (Bennett, 1987; Grotkopp *et al.*, 2004; Knight *et al.*, 2005). Successful invasive plants (i.e. those with a potential to attain high abundance) tend to have smaller genomes (Grotkopp *et al.*, 2004;

Kubešová *et al.*, 2010; Lavergne *et al.*, 2010). Vinogradov (2003) showed that plant species classified as endangered (i.e. typically rare) tend to have larger genomes than plants of no conservation concern (see also Zalewski and Ciuzycki (2010) for similar data on chrysomelid and coccinellid beetles).

However, no study has examined this relationship more closely. We therefore assembled data on species abundance from compiled database data on vegetation and flora of the Czech Republic and link them with genome size data from the angiosperm DNA C-values database (Bennett and Leitch, 2010) and plant traits from the LEDA database (Kleyer *et al.*, 2008). We examine these data by simple correlation analysis between genome size and abundance, between traits and abundance, and between genome size and traits. Consequently, we examine different types of partial relationships in the data using path analysis to find out how successful the traits are in mediating the effects of genome size. We work on the assumption that relationships of genome size and/or traits to abundance can be viewed as unidirectional, with abundance being a causally affected variable. In contrast, relationships between genome size and traits are more complex and potentially bidirectional (see Hodgson *et al.*, 2010). While it is likely that genome size acts as a primary factor in developmental/ecological time, on evolutionary time scales there should be a selective feedback from traits to genome size (e.g. see Suda *et al.*, 2005). To account for phylogenetic non-independence, we analyse both raw data and data corrected for phylogenetic relationships of species. As genome size has a fundamental relationship with generation time, we analyse these relationships separately for annual and perennial plants.

METHODS

Species data

Genome size data (C-values) together with chromosome number, ploidy level and estimation method were taken from the angiosperm DNA C-values database (Bennett and Leitch, 2010). This database contains 6287 species from the whole world; only a subset of herbaceous species native to the Czech Republic was taken (Table 1). Data on woodiness were taken from LEDA (Kleyer *et al.*, 2008) and Kubát *et al.* (2002), data on native/alien status from Pyšek *et al.* (2002). Floating water plants (such as *Wolffia arrhiza*) and parasitic plants were excluded. If more than one readout of C-values was available for one species, the prime estimate (Bennett and Leitch, 2010) was chosen. When results were questionable, flow cytometry data were preferred over those obtained by other estimation methods. In species with more cytotypes, only ploidies previously reported from the Czech Republic (according to the internal karyological database of plants of the Czech Republic, held at the Institute of Botany AS CR, Průhonice) or likely to occur there (based on records from neighbouring countries) were considered. Most species known to have several cytotypes in the Czech Republic were excluded unless one of the cytotypes is known to be considerably more common than the other(s). In case of doubt the species was excluded from the analysis.

Whenever possible (i.e. when chromosome counts as direct estimates of the ploidy level were available), monoplloid

TABLE 1. Pearson correlation coefficients of two abundance variables and summed abundance with genome size and plant-trait variables

	All plants					Annual plants					Perennial plants				
	CNFQ	FLDOKQ	Summed abundance	Summed abundance PC	Summed abundance PC	CNFQ	FLDOKQ	Summed abundance	Summed abundance PC	Summed abundance PC	CNFQ	FLDOKQ	Summed abundance	Summed abundance PC	Summed abundance PC
Cx-value	-0.211***	-0.173***	-0.194***	-0.166**	-0.381***	-0.381***	-0.355***	-0.381***	-0.273*	-0.179**	-0.137*	-0.164**	-0.111 n.s.		
N	411	411	411	325	120	120	120	120	74	291	291	291	233		
C-value	-0.157***	-0.108*	-0.129**	-0.07 n.s.	-0.271**	-0.206*	-0.256**	-0.144 n.s.	-0.152**	-0.102	-0.102	-0.126*	-0.037 n.s.		
N	436	436	436	325	126	126	126	74	310	n.s.	n.s.	310	233		
SLA	0.119*	0.145**	0.12*	0.141*	0.141 n.s.	0.124 n.s.	0.146 n.s.	0.105 n.s.	0.161*	0.18**	0.18**	0.161*	0.23**		
N	358	358	358	274	108	108	108	66	250	250	250	250	190		
Height	0.122*	0.197***	0.148**	0.094 n.s.	0.017 n.s.	0.068 n.s.	0.027 n.s.	-0.033 n.s.	0.162**	0.237***	0.237***	0.19***	0.131*		
N	432	432	432	339	125	125	125	76	307	307	307	307	245		
Seed mass	-0.09 n.s.	-0.067	-0.081 n.s.	-0.052 n.s.	-0.148	-0.171	-0.169 n.s.	-0.135 n.s.	-0.071	-0.034	-0.034	-0.053 n.s.	0.034 n.s.		
N	353	353	353	275	105	105	105	63	n.s.	n.s.	n.s.	248	194		
Seed no.	0.116*	0.13*	0.109 n.s.	0.055 n.s.	0.305**	0.305**	0.311**	0.264*	0.088 n.s.	0.114 n.s.	0.114 n.s.	0.086 n.s.	0.039 n.s.		
N	295	295	295	222	103	103	103	60	192	192	192	192	144		

All variables except summed abundance (which is already in the log scale) were log-transformed before the analysis. The numbers of observations (N) are indicated below each correlation coefficient. CNFQ, Number of grid squares containing phytosociological records with the species; FLDOKQ, number of FLDOK grid squares containing the species; PC, partial correlation with phylogenetic relatedness removed.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values significant at $P < 0.05$ are indicated in bold.

genome sizes (Cx-values; Greilhuber *et al.*, 2005) were calculated by dividing the 2C-value by the ploidy level. Ploidy levels were taken from Bennett and Leitch (2010); corrections were done in some cases such as when different ploidy levels had originally been assigned to related species with the same number of chromosomes. A search was made for additional ploidy data in Goldblatt and Johnson (1979 onward) but only used if ploidy homogeneity was found. In particular, we assigned the same ploidy level to all infrageneric taxa (species) with the same number of chromosomes, irrespective of the original source. To estimate ploidy level, we used cytogenetic criteria (basic number of chromosomes) despite the fact that recent molecular data often support higher (poly)ploidy (cf. Soltis *et al.*, 2009).

This yielded 436 species with available C-values and 411 species with available Cx-values. Both values were highly correlated ($R = 0.923$, $n = 411$, $P < 0.001$; $R^2 = 0.852$ for log-transformed data) indicating that variation in the holoploid genome size is primarily due to variation in the monoploid genome size and not to the ploidy level.

Trait data were taken from the LEDA traitbase (Kleyer *et al.*, 2008) and Kubát *et al.* (2002). Three traits were taken only from LEDA (number of species for which values were available in parentheses): SLA (358), seed mass (353) and number of seeds per ramet/individual (295). If several records were available for one species, the simple (unweighted) arithmetic mean value was used. Data on height (432; data for tall non-woody lianas were not used and were treated as a missing value) and life span (436) were taken from LEDA and supplemented by data from Kubát *et al.* (2002). Life span was classified into two categories only: annual (including winter annuals and strict biennials, 126 species) and perennial (including long-lived monocarps, 310 species).

Two sources of abundance data were used. (1) The Czech National Phytosociological Database (CNF; Chytrý and Rafajová, 2003) was used to get (a) the number of records of a given species in a stratified set of 20 468 vegetation samples used by Chytrý *et al.* (2005), and (b) number of mapping grid fields (3' × 5', i.e. approx. 6 × 5.5 km at 50°N latitude; see Niklfeld, 1999) where the species was recorded in this set. (2) The floristic database of the Institute of Botany, Academy of Sciences of the Czech Republic (FLDOK; see <http://www.ibot.cas.cz/index.php?p=databaze&site=default>) was used to determine the number of mapping grid fields (Niklfeld, 1999) in which the species was recorded. All three abundance measures were highly correlated (CNF records vs. CNF fields: $R^2 = 0.938$, $P < 0.001$; CNF records vs. FLDOK fields: $R^2 = 0.890$, $P < 0.001$; CNF fields vs. FLDOK fields: $R^2 = 0.898$, $P < 0.001$ for (log + 1)-transformed data, $n = 436$ in all cases).

Phylogenetic data

All species in the list were checked in the GenBank via the NCBI web site (<http://www.ncbi.nlm.nih.gov>) and available sequence data were downloaded to make taxonomic coverage at the family level as comprehensive as possible; if multiple records were present, those having longest overlap with the rest of the dataset were chosen, and eventual unalignable flanks were trimmed (for the list of species and accession

numbers, see Table 2). If no data were available for a given species in GenBank, data from congeneric species were taken; species for which no sufficient data were available at GenBank and which had no congeneric species with sufficient data were excluded from the phylogenetic analysis. This yielded independent sequence information for 145 species. For genera with more than one species in the database, only one species was used (typically one with the best data available in GenBank). As a result, all congeneric species had zero phylogenetic distance by definition. Taking congeners into account, phylogenetic information was available for 360 species.

To evaluate phylogenetic relatedness of the taxa, a cladogram with branch lengths was needed. We employed a combined methodological attitude working with Bayesian inference tree, a majority-rule consensus parsimony tree and cladograms taken from the Angiosperm Phylogeny Website (<http://www.mobot.org/MOBOT/research/APWeb>).

The starting tree was computed from downloaded sequences using the MrBayes software (<http://mrbayes.csit.fsu.edu/>). Maximum likelihood settings corresponding to the GTR + I model with assumption of some invariable sites were employed (nst = 6, rates = invgamma) and the default priors were used. Two simultaneous independent runs were performed starting from different random trees to assess stationarity of analysis (the average standard deviation of split frequencies had to fall below 0.01 before the end of the computation). Each run comprised six chains (one cold and five heated) which were sampled every 1000th generation for a total of 10 000 000 generations. The first 2500 samples from each run were discarded as burn-in and the remaining were pooled to produce one 50 % majority rule consensus tree.

As this tree showed polytomies at its backbone and a few of inconsistent (moreover weakly supported) groupings in the terminal-most section, topology presented at APWeb was used to manually clarify these ambiguities (using the Mesquite software; <http://mesquiteproject.org/mesquite/mesquite.html>). Finally, the constrained maximum-parsimony search with majority-rule consensus was conducted in PAUP* (Swofford, 2003) to get rid of the remaining polytomies and to obtain branch lengths. Patristic software (Fourment and Gibbs, 2006) was used to convert branch lengths to a patristic matrix, applicable to further statistics operating with the phylogenetic proximity of taxa.

Data analysis

First, data were analysed by means of simple and partial correlations of individual trait variables and abundances. All quantitative variables were log-transformed. Correlations were calculated both for the whole dataset, and for annual and perennial plants separately. To correct for phylogenetic relatedness we used the approach of Diniz-Filho *et al.* (1998; see also Desvignes *et al.*, 2003), i.e. removing species relatedness by using partial correlations with all 16 phylogenetic axes as covariates. The matrix of patristic distances was summarized using non-standardized principal co-ordinates analysis (PCoA) using the ADE4 package for R (Dray and Dufour, 2007). Scores at the first 16 PCoA axes (accounting for 90.0 % of the total phylogenetic variation) were used to capture

TABLE 2. Pearson correlation coefficients of genome size variables and plant-trait variables

	All plants						Annuals						Perennials					
	Simple		PC		Simple		PC		Simple		PC		Simple		PC			
	Cx-value	C-value	Cx-value	C-value	Cx-value	C-value	Cx-value	C-value	Cx-value	C-value	Cx-value	C-value	Cx-value	C-value	Cx-value	C-value		
SLA	-0.065 n.s.	-0.071 n.s.	0.021 n.s.	0.004 n.s.	-0.2*	-0.188 n.s.	-0.179 n.s.	-0.099 n.s.	-0.01 n.s.	-0.012 n.s.	0.037 n.s.	0.008 n.s.	0.037 n.s.	0.037 n.s.	0.037 n.s.	0.008 n.s.		
N	336	358	259	259	104	108	64	64	232	250	177	177	177	177	177	177		
Height	0.129**	0.128**	0.098 n.s.	0.101 n.s.	0.227*	0.259**	0.278*	0.348**	0.093 n.s.	0.085 n.s.	0.033 n.s.	0.025 n.s.	0.033 n.s.	0.033 n.s.	0.033 n.s.	0.025 n.s.		
N	407	432	322	322	119	125	73	73	288	307	231	231	231	231	231	231		
Seed mass	0.459***	0.419***	0.354***	0.338***	0.442***	0.435***	0.408**	0.476***	0.47***	0.416***	0.31***	0.265***	0.31***	0.31***	0.265***	0.265***		
N	331	353	260	260	100	105	60	60	231	248	182	182	182	182	182	182		
Seed no.	-0.265***	-0.243***	-0.234***	-0.268***	-0.147 n.s.	-0.074 n.s.	-0.167 n.s.	-0.163 n.s.	-0.277***	-0.268***	-0.256**	-0.292***	-0.256**	-0.256**	-0.292***	-0.292***		
N	277	295	209	209	98	103	57	57	179	192	134	134	134	134	134	134		

All variables were log-transformed before the analysis. The numbers of observations (N) indicated below each correlation coefficient. PC: Partial correlation with phylogenetic relatedness removed. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values significant at $P < 0.05$ are indicated in bold.

phylogenetic relatedness of the taxa. Species with no phylogenetic information were treated as missing in all analyses that employed phylogenetic information. Univariate statistical calculations were done in SPSS ver. 19 (2010, IBM SPSS Inc., Chicago, IL).

Relationships of genome size variables, plant functional traits and individual measures of species abundance were analysed by means of path analysis (Grace, 2006). The initial path model was formulated based on relationships known from published sources. For the current analysis we assumed the chain of effects to run from cell-level phenomena such as genome size up to organ and plant levels. We therefore took the genome size variables to be independent (exogenous), i.e. having capacity to constrain values of other (endogenous) variables; this treatment should not be understood as taking the genome size necessarily as the true causative agent, at least not in evolutionary time. Further, neither seed size nor seed number per ramet was assumed to be the primary relative of each other; therefore both were taken as endogenous, and constrained by covariance between their respective error variables. Finally, height and SLA were assumed to be exogenous. We also examined possible structures with either or both of them as endogenous (dependent), but these never yielded a better fit. This basic structure was successively refined by adding/removing further relationships between variables until the best possible fit was obtained. Only relationships justifiable on theoretical grounds or past knowledge were considered for adding. Unidirectional (regression) relationships between endogenous variables were used if there was a theoretical justification for them. We tested compatibility of predictions of individual path models with the covariance structure of the data using the χ^2 criterion and the root mean square error of approximation (RMSEA). We accepted models with RMSEA below 0.05 and $\chi^2/\text{d.f.}$ below 2; (non-)significance of the model was used as an additional criterion.

After satisfactory fit was obtained for the model with the genome size and trait variables, regional abundance was

added as another endogenous variable and all possible combinations of its predictors were examined until best fit was obtained. Only significant relationships in the path diagram were retained. To simplify the analysis, the three abundance measures were summarized by taking the first axis from standardized principal component analysis of log-transformed values of the three abundance estimates. The first PCA axis (further referred to as 'summed abundance') accounted for 93.9% of the total variation; all three abundance estimates had loadings greater than 0.95.

Path analysis was performed using Amos ver. 19 (Arbuckle, 2010). Separate models were built for annual and perennial plants.

RESULTS

Species abundance was weakly but significantly correlated with its genome size ($R^2 = 0.017$ for the C-value and $R^2 = 0.038$ for the Cx-value; 95% confidence interval 0.013–0.091; see also Table 1 and Fig. 1). The correlations became weaker after phylogenetic correction, but the correlation with the Cx-value remained significant (Table 1). Annual and perennial plants showed markedly different patterns in the correlation of genome size and abundance: while the correlations were rather low in perennial plants ($R^2 = 0.016$ for C-value and $R^2 = 0.027$ for Cx-value; 95% confidence interval 0.004–0.083), they were much stronger in annual plants ($R^2 = 0.066$ for C-value and $R^2 = 0.145$ for Cx-value; 95% confidence interval 0.047–0.275).

Out of the trait variables, species summed abundance was weakly correlated only with SLA ($R^2 = 0.014$) and plant height ($R^2 = 0.022$; Table 1). The correlation with SLA remained essentially unchanged after phylogenetic correction, while the correlation with height decreased (Table 1). Other predictor variables examined had no effect on species summed abundance; seed number had a weak effect on CNF quadrats and FLDOK quadrats. Again, there were strong

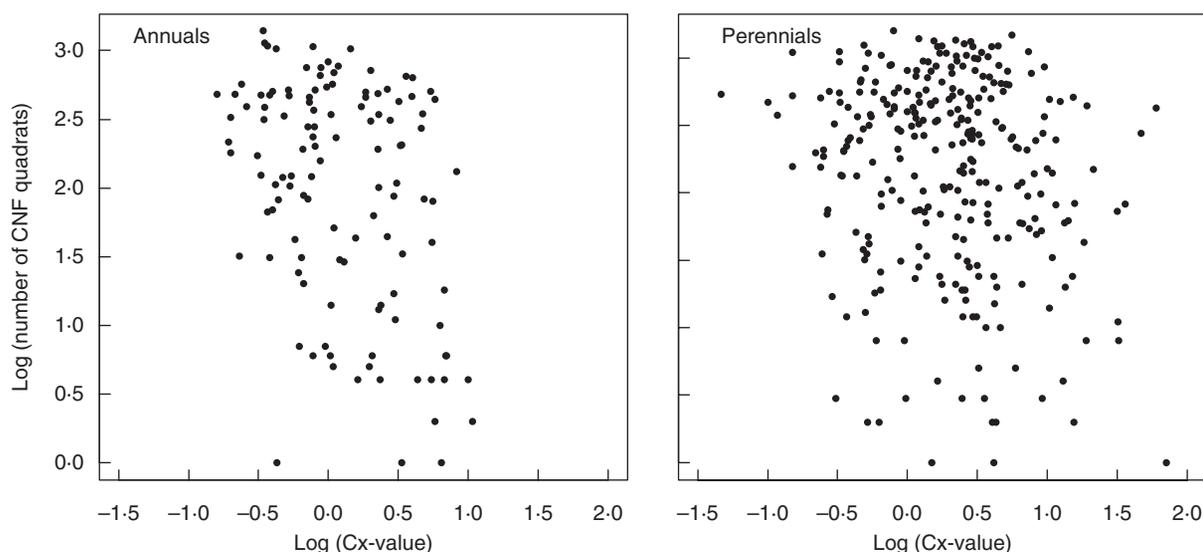


FIG. 1. Relationship between monoploid genome size (Cx-value) and species summed abundance in annuals and in perennials. Annuals: $R^2 = 0.145$, $n = 120$, $P < 0.001$; perennials: $R^2 = 0.027$, $n = 291$, $P < 0.01$.

differences between annuals and perennials. Abundance of annuals was affected by seed number ($R^2 = 0.097$), abundance of perennials was weakly affected by height ($R^2 = 0.036$) and SLA ($R^2 = 0.026$).

Genome size variables were positively correlated with seed mass and (weakly) plant height and negatively with seed number (Table 2). Seed mass and size correlations are essentially stable under phylogenetic correction; in contrast, correlation with height decreased after phylogenetic correction. While correlations with seed size are similar in annuals and perennials, other trait variables differ. In annuals, genome size is correlated rather strongly with height, and weakly also with SLA; in perennials, there is no correlation with either height or SLA, but there is a rather strong correlation with seed number (much weaker in annuals).

Path analysis revealed rather complex relationships of individual trait variables to genome size with annual and perennial plants showing very different structures (Figs 2 and 3). In annual plants, genome size (either Cx- or C-values) had a significant relationship with all four trait variables (height, SLA, seed size and seed number; Fig. 2). In addition to the effect of genome size, seed size and number were affected by an independent source of variation associated with plant height; combined effects of genome size and height explained 41% of variation in seed size and 7% of variation in seed number per ramet. Abundance of annuals was affected by genome size both directly and indirectly through seed number; the

latter effect was still composed of two separate chains, i.e. genome size \rightarrow height \rightarrow seed number \rightarrow abundance and genome size \rightarrow seed size \rightarrow seed number \rightarrow abundance. All these effects explained altogether 19% of the total variation in abundance. SLA, while itself correlated by genome size, had no effect either on seed variables or (directly or indirectly) on abundance. While the effect of SLA on abundance in annual plants is not significant, its magnitude is numerically similar to that in perennial plants; since both sets differ in size, differences in significance must be interpreted with caution. However, path model of annuals with SLA \rightarrow abundance link included does not have a significantly better fit than without it.

In perennial plants the basic structure was very different (Fig. 3). Links between genome size variables and seed variables were strong, but other effects were either much weaker (on height) or absent (on SLA). Abundance of perennial plants was completely independent of seed reproduction (seed size and seed number). Effects of height on seed variables remained, but the effect on seed size was in perennials much weaker in contrast to annual plants. Abundance of perennials was affected by three sources: direct effect of genome size, effect of SLA (independent of the genome-size effect) and effect of height (which had a weak contribution of genome size but otherwise represented an independent source of variation). The total variation in abundance explained by the model (10%) was lower than that in annual plants.

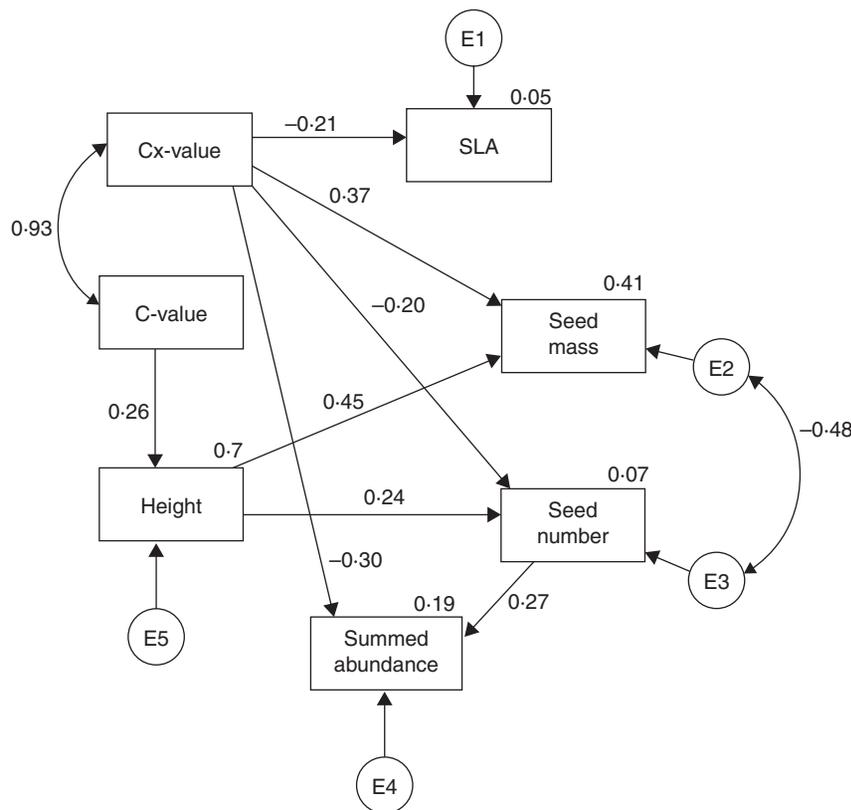


FIG. 2. Path analysis of relationships between genome-size variables, plant traits and summed abundance in annuals. Directional arrows indicate causal effects, bidirectional arrows unresolved correlations. Numbers close to the individual lines are standardized path coefficients; numbers close to the upper-right corners of boxes are squared multiple correlation coefficients. E1 to E5 are unique error variables. The overall fit is $\chi^2 = 11.0$, d.f. = 11, $P = 0.445$, RMSEA < 0.001.

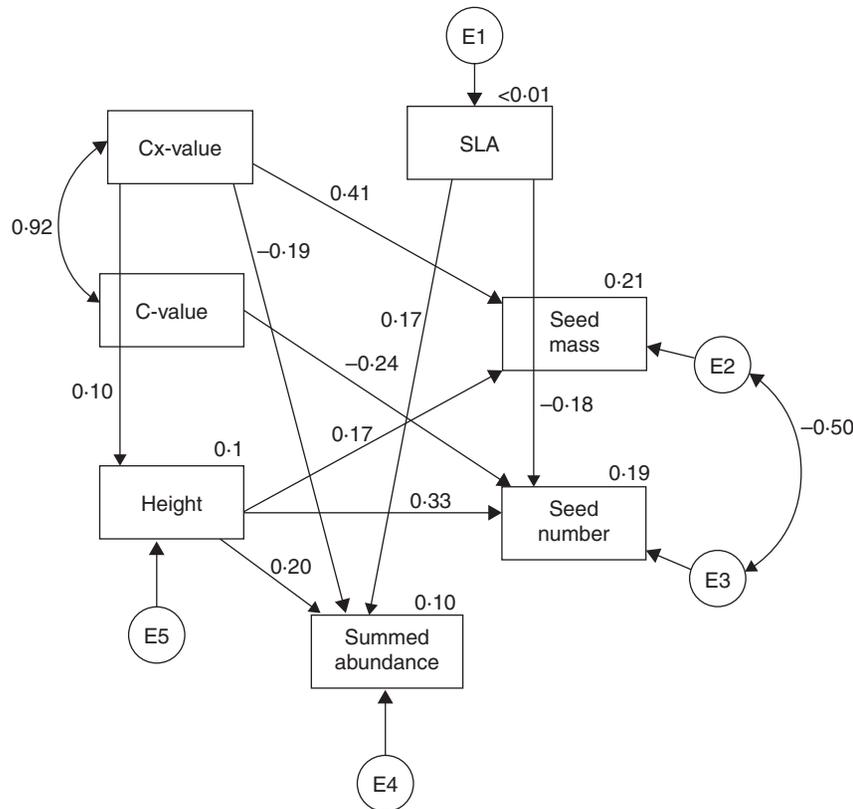


FIG. 3. Path analysis of relationships between genome-size variables, plant traits and summed abundance in perennials. Directional arrows indicate causal effects, bidirectional arrows unresolved correlations. Numbers close to the individual lines are standardized path coefficients; numbers close to the upper-right corners of boxes are squared multiple correlation coefficients. E1 to E5 are unique error variables. The overall fit is $\chi^2 = 6.8$, d.f. = 10, $P = 0.748$, RMSEA < 0.001.

DISCUSSION

The results show that regional abundance of species is related to genome size (namely the Cx-value). Plants with bigger genomes tend to be rare in comparison with plants with smaller genomes and this relationship is stronger in annual plants. This is, to our knowledge, the first attempt to link genome size directly to species frequency or abundance for such a large set of species at a regional level. While the predictive power of the relationships is weak, abundance is rather difficult to predict by more direct traits (e.g. see Kolb *et al.*, 2006; van der Veken *et al.*, 2007). This means that we do not understand well the processes that underlie abundance, but also that within the part of its variation that can be predicted, predictive power of the genome size (and hence role of cell-level processes) is rather important. Use of path analysis made it possible to disentangle the role of individual traits in these relationships. As expected, a large part of this relationship disappeared if plant traits were taken into account. Out of the traits examined, SLA, plant height and seed number had significant effects on plant abundance; there seems to be a fundamental difference in these relationships between annual and perennial plants.

Prediction of abundance in annuals

In annual plants, large part of the genome-size effect disappears in the path model, as abundance is strongly predicted by

seed number per individual, which is known to be a good proxy for population growth rate in annuals (Silvertown *et al.*, 1993; Metcalf *et al.*, 2003). Seed number is negatively correlated with seed size, which is in turn correlated with genome size in most datasets (e.g. see Beaulieu *et al.*, 2007b; Knight and Beaulieu, 2008). Existing published reports on seed size/number and abundance indeed report positive relationships [Thompson *et al.* (1999) in phylogenetically corrected data; Kolb *et al.*, 2006; Ozinga *et al.*, 2009], but there are also studies showing no relationships (Eriksson and Jakobsson, 1998), making it clear that the relationship is specific for some plant groups and/or habitat types. It should be noted here that annuals have a smaller range of genome sizes in comparison with perennials; large genomes (>25 pg/1C) are clearly not compatible with annual life strategy (Knight *et al.*, 2005; Leitch and Bennett, 2007). The present data show that the key limitation may be due to the number of seeds they are capable of producing.

Hence while part of the genome-size effect on abundance in annuals is due to relationships between seed number per individual and seed size, and seed size and genome size, path analysis revealed a rather strong additional unexplained effect of genome size on abundance. This indicates that in annuals there is a source of variation in abundance that is independent of seed size/number, but that is still due to processes associated either with cell size or (more probably) with cell division and generation time, both of which are known to be correlated with

genome size (Bennett, 1987; Francis *et al.*, 2008; Šímová and Herben, 2012).

Prediction of abundance in perennials

In contrast to annuals, explained variation in abundance of perennials was lower. Moreover, path analysis showed that most of the genome-size effect disappeared if two traits, i.e. SLA and height, were taken into account. SLA is a good proxy for individual growth rate (see Reich *et al.*, 1992, 1998; Wright and Westoby, 1999) and is known to predict, for example, invasive status (van Kleunen *et al.*, 2010) and local abundance (Cornwell and Ackerly, 2010) of species. Height may be thought of as a proxy for competitive ability in productive environments; it has been demonstrated to affect local and regional abundance (Pyšek *et al.*, 2009; Cornwell and Ackerly, 2010), although its effect is typically not very strong (Eriksson and Jakobsson, 1998).

While both these traits contribute to perennial species abundance in our dataset, none of them is strongly correlated with genome size in perennials. Indeed, published data show that SLA is weakly related to genome size due to effects of genome size on cell division and tissue growth rate (Bennett, 1987; Knight *et al.*, 2005; Knight and Beaulieu, 2008; Francis *et al.*, 2008). The relationship of height to genome size is even less straightforward: both positive, negative and no relationships have been reported, indicating an array of mechanisms that possibly link genome size to plant stature (Knight and Beaulieu, 2008; Gallagher *et al.*, 2011).

Path analysis shows that SLA and height are unrelated to each other, both in their relationship to genome size, and in their effect on abundance. This means that in the current dataset there is little support for the effect of genome size through plant growth rate on plant ultimate size and hence on its competitive potential (Knight and Beaulieu, 2008). In contrast, both growth rate (as proxied by SLA) and height variables constitute separate components determining plant abundance. These relationships taken together are the most important identifiable components of species abundance in perennials. In addition to them, there is a small residual variation in abundance due to genome size, but it is smaller than in annuals. Again, its existence indicates the ecological effects of cell size and/or division rate that are not mediated by the examined traits.

Regional abundance and population biology of plants

Regional abundance of a species is an outcome of many ecological processes, ranging from differential breadth of habitat niche of species through competitive ability to biogeographic/dispersal constraints, which cannot be separated in the current dataset. It is always a function of two elements, i.e. abundance of suitable habitats and commonness within such habitats. High abundance may then be due to either high relative frequency of habitats favourable for a given species linked with the capacity of the species to disperse, or to the capacity of the species to become highly abundant even if its habitats are not very common. While high abundance due to habitat suitability is due to a suite of traits that underlie species ecological specialization (see Thompson *et al.*, 1999; Fridley

et al., 2007; Zelený, 2008), population biology traits determine both within-habitat abundance (Eriksson and Jakobsson, 1998; Cornwell and Ackerly, 2010), and capacity to disperse between habitats (Ozinga *et al.*, 2009).

In the current set of species, abundance of perennials seems to be affected more by processes that operate within habitats: both by growth rate as such (as proxied by SLA) and by competitive ability due to taller stature (see also Craine *et al.*, 2001; Aarssen *et al.*, 2006; Gross *et al.*, 2009). In contrast, annuals are affected by a trait (seed number per individual) that determines both local growth rate and dispersal among habitats. As annual life strategy is typically based on fast population growth (Metcalf *et al.*, 2003), large numbers of seeds are indispensable for population growth and hence regional abundance. Further, small and more numerous seeds also persist longer in the seed bank (Thompson *et al.*, 1993).

The better prediction of regional abundance of annuals in contrast to perennials shows that processes that determine it are simple and more easily captured by simple traits such as seed size or number. In contrast, the processes that account for regional abundance of perennials are much less straightforward. Growth and competition traits (SLA and height) play some role, but their direct effect on abundance is much weaker. However, in contrast to annuals, perennials span a very large range of habitat types, from productive habitats where success is largely determined by fast growth and competition for light, to low-productivity habitats where very different traits are likely to be important. In addition, in perennials reproductive insurance is due to clonal growth and therefore selection for cell generation time is likely to be much lower.

Both in annuals and in perennials, there is also an unresolved effect of genome size on abundance which is independent of the examined traits. There are several candidate mechanisms that could account for this relationship. First, the traits we worked with so far do not constitute the complete set of possible ecological determinants of success and many other traits may be included (e.g. parameters of vegetative growth). Secondly, genome size is known to be related to the reproductive mode of plants, including auto/allogamy and proportion of selfed progeny (Whitney *et al.*, 2010), which is likely to affect plant fitness, although the relationship is not straightforward. In addition, high abundance may be due to a capacity for fast adaptive evolution which is also known to correlate with genome size (e.g. see Lavergne *et al.*, 2010).

Genome size and polyploidy

Our study systematically used and compared both holoploid genome size (the C-value) and monoploid genome size (the Cx-value) in a representative set of central European herbaceous plant species. Holoploid genome size is, in addition to the monoploid genome size, determined also by the ploidy level. Polyploids are known to be larger and often more successful in more extreme or stressful habitats (Brochmann *et al.*, 2004). However, neither correlation analysis nor path models supports the hypothesis that polyploidy would be an important factor determining ecological success of a species. This indicates that the key effect of genome size on species abundance is due to the correlation of the monoploid genome size with seed number (in annuals) or

with other growth-related traits (in perennials), and not to positive effects of polyploidy. Analysis of a path model with the ploidy level as a separate variable used instead of the C-value also failed to find any direct effect of polyploidy on abundance (results not shown).

However, the weaker effect of the holoploid genome size on abundance in contrast to monoploid genome size is clearly due to the role of polyploidy (as polyploids, at least in some groups, are more common). In polyploids, the duration of meiosis/mitosis is reduced compared with diploids despite their proportionally increased nuclear DNA content (e.g. in diploid wheat meiosis at 20 °C takes 42 h while in hexaploids only 24 h; [Bennett and Smith, 1972](#)). The mechanism behind this phenomenon is unknown but might be related to the increased number of replicons in polyploids. The cell-cycle division rate is therefore primarily governed by Cx-value, not the total DNA amount. However, these effects are bound to be small as the Cx-value determines an overwhelming proportion of variation in the holoploid genome size (85 %).

Phylogenetic relationships

Taking phylogenetic relatedness into account showed no major change in the relationships. Correlation of SLA (and to some extent, seed size, namely in perennials) with genome size decreased with phylogenetic correction. In contrast, plant height (in annuals) showed the opposite pattern and became more positively correlated (albeit still weakly) with genome size in phylogenetically corrected data, indicating recent divergence in this trait associated with genome size. In their analysis of genome size and plant height, [Knight and Beaulieu \(2008\)](#) also showed a strong change in the relationship when phylogenetic correction was used; in their case, however, there was negative correlation at the species level which disappeared after phylogenetic correction. This is presumably due to the fact that their dataset included both herbs and trees – the inclusion of trees accounted for the large part of the negative correlation between genome size and overall plant height (e.g. see [Knight and Beaulieu, 2008](#)) which disappeared for phylogenetically corrected data. No relationship between genome size and plant height at infra-generic level was reported by [Gallagher et al. \(2011\)](#).

Limitations of the approach

We limited our analysis to native herbaceous plants. It is likely that the relationships examined will be different for both woody species ([Knight and Beaulieu, 2008](#)) and for alien species ([Kubešová et al., 2010](#)), but we were not able to obtain sufficiently large datasets for either of these groups to allow for sufficiently robust model fitting. For herbaceous plants, we do have a reasonable number of species for good model fitting, but this high number of species also means that significance values in such a set are of limited importance due to large sample sizes. However, effect magnitudes are often big enough to warrant a real signal in the data.

The quality of all analyses is, among other things, determined by the quality of the abundance data coming from the databases used. These data have not been collected in any systematic fashion – frequency of a species in a database is due to

many factors in addition to the true abundance of the species. Species records may be biased, e.g. by paying more attention to conspicuous, rare or otherwise attractive species. Although using numbers of mapping grid fields may alleviate the effect of more attention being paid to rare species, the issue of species conspicuousness remains. Indeed, there may be an indirect indication of this effect in the data, the FLDOK abundance shows higher correlation with height than CNF abundance. As FLDOK is based on floristic records, visibility (as proxied by height) may play a bigger role here than in CNF, which is based on plot data that guarantee better recording of even inconspicuous species. In spite of this, a very good correlation between these two sets (and using variation common to them) provides good assurance that the overall differences in abundance are real and reasonably estimated.

Genome size data (C- and Cx-values) can also be burdened with inaccuracy. First, the Angiosperm DNA C-values database ([Bennett and Leitch, 2010](#)) harbours genome size values estimated using different densitometric and/or fluorometric techniques; while data obtained by flow cytometry can generally be considered reliable, more caution should be exercised for older estimates done, among others, by Feulgen densitometry. Another potential difficulty is due to the determination of ploidy level and, consequently, monoploid genome size (note that Cx-value was found to have a much stronger effect on almost all variables examined than C-value). To alleviate this problem, we compared several existing sources to determine the most likely candidate ploidy level, but a certain degree of uncertainty remains (see also [Soltis et al., 2009](#)).

Finally, it must be noted that while we analyse directional relationships between traits and abundance, the approach should not be taken to imply a causal relationship between genome size and abundance. Path analysis is a tool that allows assumptions to be made about causality, and to evaluate alternatives within that assumed causal framework, but it cannot evaluate whether the assumed causal framework is true. While abundance is unlikely to be a fully independent variable, selection can act on any plant traits (including genome size); causality may thus differ depending on whether the question is framed in physiological/ecological or evolutionary time scales which may differ (see also [Hodgson et al., 2010](#)).

Conclusions

The analysis shows that prediction of abundance by the commonly used traits (height, SLA and seed size/number), although significant, does not capture all existing effects of cell- and tissue-level processes as proxied by genome size. Both in annuals and in perennials, there was an unexplained effect of genome size on plant regional abundance, which indicates other (i.e. not mediated by these traits) ecological effects of cell size or cell-division rate, or both. Therefore additional predictors of regional abundance should be sought among traits/proxy variables that express variation in them.

These unresolved effects of the genome size mean that cell size and cell-division rate should be paid more attention in plant population biology. In particular, links between cell- and tissue-level parameters to key components of population

biology (e.g. to capacity for clonal growth vs. capacity for fast seed reproduction) remain entirely unknown. Fundamental differences between annual and perennial plant life strategies (e.g. see Aarssen *et al.*, 2006) may at least partly be governed by different effects of cell size processes on seeds and clonal growth organs.

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