

Genome size is a strong predictor of cell size and stomatal density in angiosperms

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Summary

- Across eukaryotes phenotypic correlations with genome size are thought to scale from genome size effects on cell size. However, for plants the genome/cell size link has only been thoroughly documented within ploidy series and small subsets of herbaceous species.
- Here, the first large-scale comparative analysis is made of the relationship between genome size and cell size across 101 species of angiosperms of varying growth forms. Guard cell length and epidermal cell area were used as two metrics of cell size and, in addition, stomatal density was measured.
- There was a significant positive relationship between genome size and both guard cell length and epidermal cell area and a negative relationship with stomatal density. Independent contrast analyses revealed that these traits are undergoing correlated evolution with genome size. However, the relationship was growth form dependent (nonsignificant results within trees/shrubs), although trees had the smallest genome/cell sizes and the highest stomatal density.
- These results confirm the generality of the genome size/cell size relationship. The results also suggest that changes in genome size, with concomitant influences on stomatal size and density, may influence physiology, and perhaps play an important genetic role in determining the ecological and life-history strategy of a species.

Key words: cell size, genome size, independent contrasts, stomata, stomatal density.

Introduction

Eukaryotic genome size (nuclear DNA amount) ranges nearly five orders of magnitude. Early observations of genome size variation noted various correlations at the cellular level, including a positive correlation with nuclear volume (Baetcke *et al.*, 1967; Jovtchev *et al.*, 2006) and cell volume (Mirsky & Ris, 1951; Commoner, 1964; Darlington, 1965; Bennett, 1972; Price *et al.*, 1973), and a negative correlation with the duration of the cell cycle (Van't Hof & Sparrow, 1963; Evans *et al.*, 1972; Van't Hof, 1974). For the genome size/cell size relationship, a broad sampling of the animal kingdom has consistently reported a strong positive relationship (i.e. Horner & Macgregor, 1983; Hardie & Hebert, 2003; Organ *et al.*, 2007). For plants, many studies have relied on within-species

comparisons across varying ploidy series (i.e. Mowforth & Grime, 1989; Melaragno *et al.*, 1993; Kudo & Kimura, 2002). From these studies it is apparent that polyploid cells are significantly larger than their diploid progenitors. However, comparisons across large taxonomically diverse species assemblages are sparse and the results reported in the literature are not consistent, with correlations ranging from 1.0 (Price *et al.*, 1973) to -0.48 (Grime *et al.*, 1997). Moreover, all studies of the plant genome size/cell size relationship have been carried out using limited samples of herbaceous angiosperm species. Nevertheless, the relationship between genome size and cell size is often casually assumed for plants and serves as the basis for testing genome size-dependent variation in higher phenotypic scales (Bennett, 1972, 1987; Knight *et al.*, 2005; Beaulieu *et al.*, 2007a,b). This paper examines to what extent the relationship

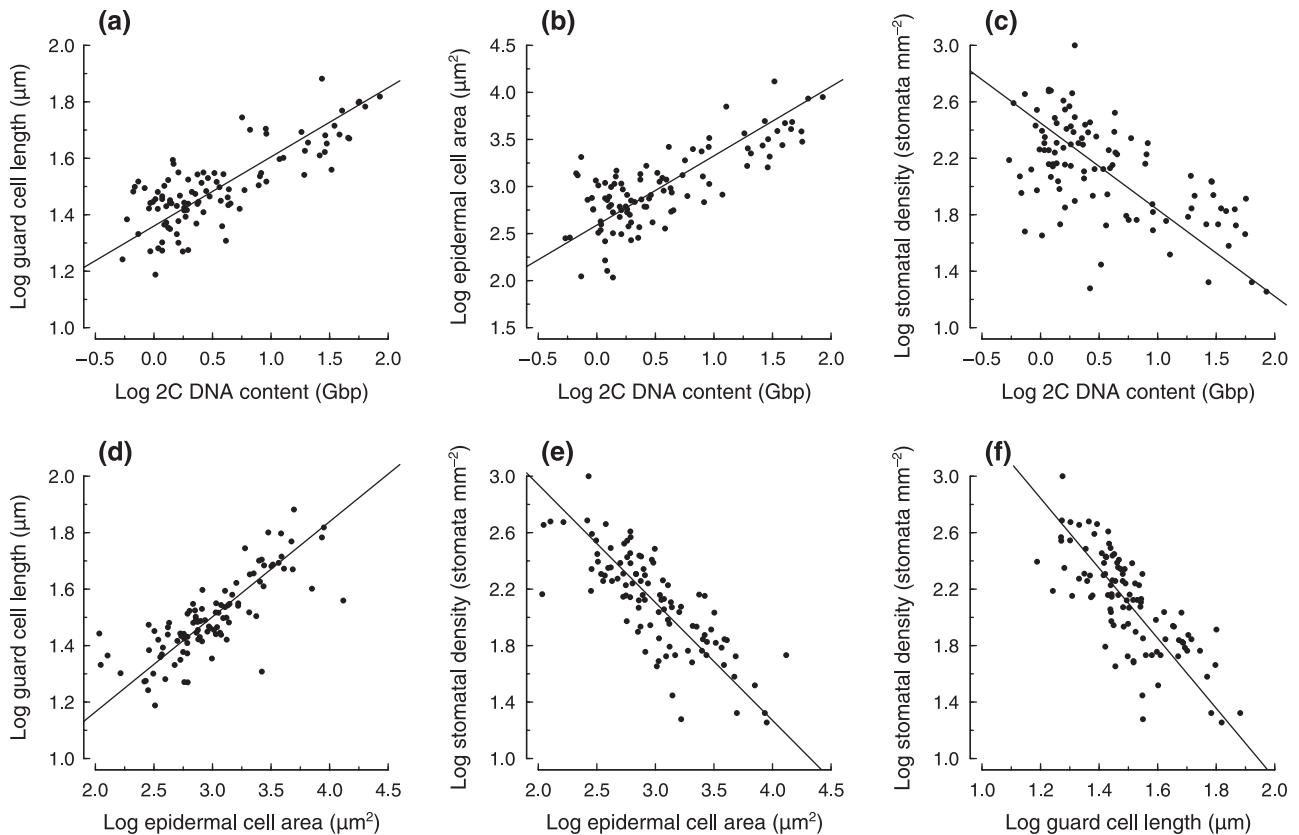


Fig. 2 Scatter plot of the significant cross-species relationships (without considering phylogeny) between all pair-wise trait combinations (see Table 1). All slope estimates are the standardized major axis (SMA; model II regression) describing the best-fit line from minimizing residuals in both dependent and independent variables.

lower trait values. When analyses were partitioned across growth forms, herbaceous species showed comparable slope estimates to those calculated across all species (Table 1). However, interestingly, relationships within shrubs and trees were not significant for 2C DNA content and guard cell length, epidermal cell area, and stomatal density. In all the above cases, results for 1Cx were very similar (but slightly weaker) when compared with results for 2C DNA content.

Relationships among leaf traits were all highly significant (Table 1, Fig. 2d–f). For example, both epidermal cell area and guard cell length were negatively associated with stomatal density (Table 1, Fig. 2e,f). The slope estimates for all pair-wise leaf cell trait comparisons were similar and not significantly different between monocots and eudicots, or between growth forms (Table 1).

Phylogenetic signal and independent contrasts

Closely related species were more similar than would be expected by chance, indicating there is phylogenetic signal for all traits. 2C DNA content exhibited a stronger degree of phylogenetic signal ($K = 0.959$) than did guard cell length ($K = 0.685$), epidermal cell area ($K = 0.630$), or stomatal density ($K = 0.540$). Therefore,

because of the phylogenetic signal in our data set, we used independent contrasts for further analyses.

The slope estimates obtained from independent contrasts analyses were significantly greater in magnitude but had a lower r^2 when compared with cross-species results (slope = 1.10; 95% confidence interval (CI) 1.05–1.15). Partitioning the analyses for eudicots, for monocots, or within each growth form did not lead to differences in r^2 or magnitude (Table 1). However, independent contrasts for trees and shrubs still did not uncover any significant relationships between 2C DNA content and leaf cell traits, but there were significant relationships between leaf cell traits (excluding 2C DNA content; Table 1). All pair-wise trait relationships within herbaceous species were significant.

Trait differences

Monocots had a greater mean genome size (both 2C and 1Cx DNA), guard cell length, and epidermal cell area when compared with eudicots. Mean stomatal density was also significantly lower in monocots compared with eudicots. However, phylogenetically corrected ANOVA suggested that the mean values for monocots and eudicots were significantly

Source	Conventional ANOVA		Monte Carlo simulation	
	Observed F	P	Critical value	P
Log_{10} 2C DNA				
Clade	18.9	< 0.001	79.8	0.351
Growth form	48.9	< 0.001	18.6	< 0.001
Log_{10} guard cell length				
Clade	10.5	0.001	76.3	0.458
Growth form	31.1	< 0.001	19.3	0.009
Log_{10} epidermal cell area				
Clade	24.8	< 0.001	137.8	0.282
Growth form	68.5	< 0.001	24.8	< 0.001
Log_{10} stomatal density				
Clade	31.2	< 0.001	149.7	0.223
Growth form	43.7	< 0.001	26.5	0.002

The critical value is the 95th percentile obtained from a distribution of 1000 Monte Carlo simulated F -statistics assuming a gradual model of Brownian motion evolution. P -values from Monte Carlo simulations are the proportion of simulated F -statistics that are greater than the observed F -statistic using conventional ANOVA. Monte Carlo simulations were carried out using log_{10} -transformed branch lengths for epidermal cell area and stomatal density (see text).

Table 2 Results from a conventional analysis of variance (ANOVA) and Monte Carlo simulation to test for significant trait differences between monocots and eudicots (clade), and among trees, shrubs, and herbs (growth form), relative to those expected based on random Brownian motion evolution

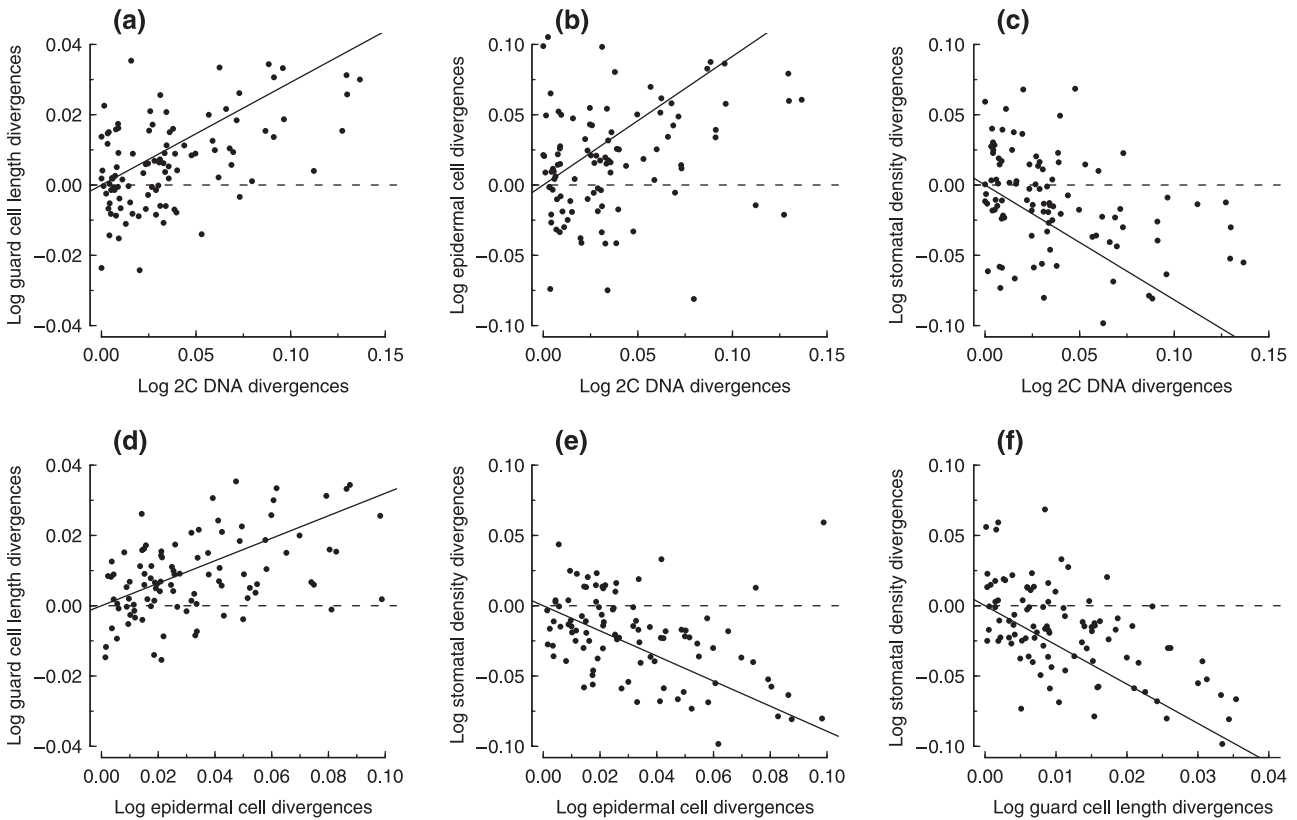


Fig. 3 Contrast plots depicting the significant relationships for all pair-wise trait combinations (see Table 1). All slope estimates are the standardized major axis (SMA; model II regression) describing the best-fit line from minimizing residuals in both dependent and independent variables. All SMA lines were forced through the origin (Garland *et al.*, 1992).

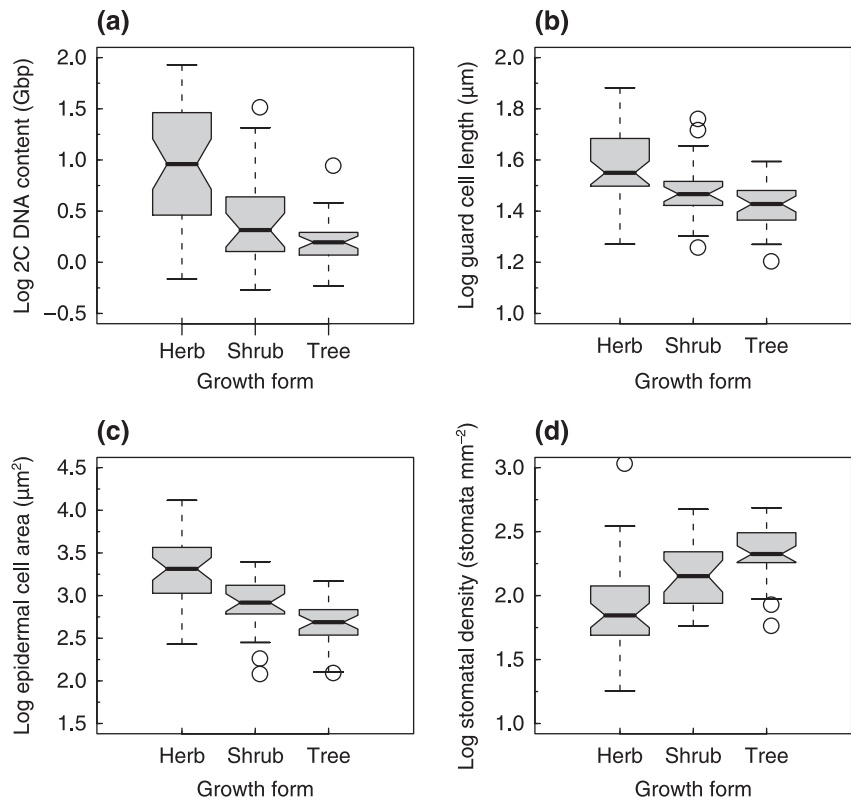


Fig. 4 The relationship between growth form and (a) 2C DNA content, (b) guard cell length, (c) epidermal cell area, and (d) stomatal density. The box plots represent the median (central line), first and third quartiles (gray box), and outliers. Median line notches that do not overlap indicate significant differences between growth forms. There are significant evolutionary differences among growth forms for all four traits (see Table 2), where trees and shrubs have smaller 2C DNA values, smaller cells (i.e. guard and epidermal cells) and higher stomatal densities than do herbaceous growth forms.

different but not more different than would be expected given a model of Brownian motion evolution (Table 2). In other words, the difference observed between the two clades could have arisen by chance. Among growth forms, trees and shrubs had significantly smaller genome sizes, smaller cells (guard and epidermal cells), and higher stomatal density than herbaceous species (Fig. 4). In addition, after incorporating both chance and phylogeny in the ANOVA, we found that all trait values among growth forms varied significantly more than expected given a random model of Brownian motion (Table 2, Fig. 4).

Discussion

The main purpose of this study was to re-examine the relationship between genome size and cell size within angiosperms using a large species set and a comparative approach. Across 101 species of varying growth forms, one of the most striking results is the remarkable linearity (on a log-transformed scale) in the relationship between genome size and cell size. There is a steady progression of species with larger genomes with increasingly larger cells (Figs 1, 2a,b). Moreover, we found that across all species genome size explains nearly 60% of the total variation in both guard cell length and epidermal cell area. Tests of phylogenetic signal indicated that this pattern was not independent of ancestry; however, even after incorporating phylogenetic history, slope estimates were similar to those found using conventional statistics (Table 1).

Thus, we found not only a strong association across extant species (regression results) but also strong correlated evolution (independent contrasts results) between genome size and cell size. The strength of the relationship was growth form dependent. Despite nonsignificant associations between genome size and cell size within trees, trees were characterized by having small genome sizes and cell sizes with decreased variance within the group compared with other growth forms (Fig. 4). Therefore, our results provide support for the general assumption that genome size evolution (whether towards smaller or larger size) is a strong predictor of the *minimum* size of any given cell type (Bennett, 1972; Gregory, 2001). Additional factors such as the influence of individual genes (e.g. *Too Many Mouths* (*TMM*); Nadeau & Sack, 2002) and environmental conditions must also play an important role in determining cell size, but perhaps only by modulating the final cell size from the minimum set by DNA content. A specific model clarifying the mechanism for this relationship is needed.

Among stomatal traits, there was also a general congruency between cross-species and independent contrasts results for all pair-wise comparisons. Moreover, these slope estimates for leaf cell traits were also congruent within each of the three growth forms, despite significant evolutionary differences in stomatal traits among trees, shrubs, and herbs (Table 1, Fig 4). These results may signal general functional constraints coordinating the evolution of stomatal traits (Hetherington & Woodward, 2003; Kerckhoff *et al.*, 2006). The number and

subsequent expansion of epidermal cells influence stomatal density through compensatory mechanisms associated with cell size and cell number (Salisbury, 1927; Beerling & Chaloner, 1993; Weijschedé *et al.*, 2008). The coordination of the size and frequency of stomata is thought to signify an optimal balance of carbon fixation per unit of water lost across many different environments. Large and significant changes to genome size could alter the water use efficiency. For example, within herbaceous species the evolution of larger genome sizes and larger cell sizes (guard cell length and epidermal cell area) was associated with a decrease in stomatal density. If genome size sets the minimum size of both guard cells and epidermal cells, the resulting change in stomatal density may predispose a species to a particular ecological and life-history strategy. In dry environments, smaller stomata allow a rapid response to water stress, while high densities allow maximization of CO₂ diffusion during optimal photosynthetic conditions (Aasamaa *et al.*, 2001; Hetherington & Woodward, 2003). Large genomes are never associated with this trait combination and therefore may be limited in their response to water stress and high temperature. Knight & Ackerly (2002) have shown that large-genome species are less frequent in environments characterized by low precipitation and high temperatures.

Trees tend to have small genome size and small, dense stomata. Interestingly, within the tree sample, there is no significant relationship between genome size and any of these cell traits (Table 1). However, when these data are superimposed on the entire data set, significant relationships emerge (Figs 2a–c, 3a–c). The small cells and generally high stomatal density found in trees may have adaptive significance. Increased stomatal density is associated with greater stomatal conductances and transpiration rates, which are thought to be necessary for moving water and nutrients through longer xylem pathways (Woodward, 1998). In addition, smaller stomata allow greater stomatal resistance and stomatal control during water stress conditions (Aasamaa *et al.*, 2001; Hetherington & Woodward, 2003). Thus, we expect that large and significant increases in DNA content might negatively impact trees by decreasing stomatal control of water loss, which may represent another ecological constraint on large-genome species (Knight *et al.*, 2005). Consistent with this hypothesis, polyploidy is rare among angiosperm trees (Stebbins, 1938; Ancel Meyers & Levin, 2006). Conversely, genome size evolution may also be generally slower in angiosperm trees because of longer generation times.

Leaf cell traits, including cell size, exhibited less phylogenetic signal than did genome size. That is, closely related species were less similar in their stomatal trait values than expected under a random model of Brownian motion evolution. Deviations from the expected phylogenetic signal (i.e. $K = 1$) can be a result of an adaptive response to selection and/or the inclusion of several sources of error, such as tree topology, branch length information, or species measurements (Blomberg & Garland,

2002; Blomberg *et al.*, 2003; Ives *et al.*, 2007). There are certainly potential errors in our phylogeny given that it is mostly resolved to family level and aged using interpolated branch lengths from a small sample of divergence time estimates (Wikström *et al.*, 2001). However, errors attributed to phylogeny should generally reduce phylogenetic signal among all traits (Rezende *et al.*, 2004). Yet, consistent with studies reported for various clades of angiosperms (Albach & Greilhuber, 2005; Weiss-Schneeweiss *et al.*, 2005; Leitch *et al.*, 2007), our genome size sample showed phylogenetic signal very near the expectation assuming random Brownian motion ($K = 0.959$).

While we do not discount the presence of various forms of error, selection may also contribute to the reduction in phylogenetic signal exhibited by stomatal traits (Blomberg & Garland, 2002; Blomberg *et al.*, 2003). There is a recognized functional link between stomatal density and atmospheric CO₂ (McElwain & Chaloner, 1995; Beerling & Woodward, 1997; Beerling *et al.*, 2001). The steady decline in atmospheric CO₂ over the last 200 Myr (Crowley & Berner, 2001) has been associated with an overall increase in stomatal density, which from our results implies declining guard cell length and epidermal cell area (Table 1, Figs 2, 3). Moreover, the stomatal response to environmental change can also be rapid, occurring on 100-yr timescales (Royer, 2001). Thus, the large discrepancy in the degree of phylogenetic signal between genome size and stomatal traits may have biological significance. Perhaps environmental factors that influence stomata do not directly influence genome size variation. Instead, genome size may generally evolve stochastically (i.e. Oliver *et al.*, 2007; Leitch *et al.*, 2007) but can impose a limit to the response of stomata to environmental factors (Knight & Ackerly, 2002; Knight *et al.*, 2005). While this is intriguing, more work is needed to examine whether it is true for all cell types.

Taken together, results from animals and plants suggest that the relationship between genome size and cell size is a universal phenomenon. The robustness of the relationship will make it possible to infer genome size from fossil plant specimens, just as Organ *et al.* (2007) used osteocyte cell size in fossil dinosaurs to infer that the small genome size of birds was a pre-existing trait within the saurischian dinosaur lineage. Leaf impression fossils with well-defined guard cells are common in the fossil record for plants, and therefore, based on the results presented here, we suggest ancestral genome sizes could be inferred for early land plants (Leitch, 2007). Extending this work further could examine how genome size responds to climatic catastrophes (e.g. the KT extinction event). This type of analysis will provide further insight into the tempo of genome size evolution.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Raw data for genome size and leaf cell traits for the 101 species of angiosperms used in this study

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