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Opinion

From genome size to trait evolution during angiosperm radiation

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Angiosperm diversity arises from trait flexibility and repeated evolutionary radiations, but the role of genomic characters in these radiations remains unclear. In this opinion article, we discuss how genome size can influence angiosperm diversification via its intricate link with cell size, tissue packing, and physiological processes which, in turn, influence the macroevolution of functional traits. We propose that integrating genome size, functional traits, and phylogenetic data across a wide range of lineages allows us to test whether genome size decrease consistently leads to increased trait flexibility, while genome size increase constrains trait evolution. Combining theories from molecular biology, functional ecology and macroevolution, we provide a framework to better understand the role of genome size in trait evolution, evolutionary radiations, and the global distribution of angiosperms.

The enigma of angiosperm evolutionary radiations

Representing nearly 90% of the world's flora with about 330 000 species (www.plantsoftheworldonline.org), angiosperms occupy almost every type of biome and ecosystem on earth with a spectacular variation in growth forms, flowers, fruits and leaves [1]. Many factors have been proposed to have contributed to the ecological and evolutionary success of angiosperms. For example, key innovations (i.e., evolutionary **functional trait** (see Glossary) novelties) may have enabled angiosperms to expand into new environments or 'adaptive zones', leading to increased **diversification rates** [1,2]. Furthermore, **trait flexibility** may have provided opportunities for angiosperms to rapidly adapt, persist, and diversify under environmental and/or ecological changes [2]. Such changes, including the major upheavals at the Cretaceous–Paleogene (K-Pg) boundary [3], may have provided the opportunities under which some of the earliest and most extensive angiosperm **evolutionary radiations** took place. Our understanding of angiosperm diversification and the role of functional traits therein has improved with the increasing availability of phylogenetic data, functional trait data, and model inferences [2,4–6], but the molecular processes underpinning these radiations remain puzzling, despite numerous suggestions (e.g., [7,8]). While many biotic and abiotic factors have been highlighted as contributing towards the success of angiosperms (e.g., [1,2,4–6]), here, we discuss how **genome size** itself may also have played a role in contributing towards their success by influencing angiosperm diversification via the intricate interactions between genome size, phenotype, and trait evolution.

Molecular processes generating genome size diversity in angiosperms

Genome size varies c. 2400-fold in angiosperms (**1C-value** = 0.061 to 148.852 Gbp [9]). This variability arises from the balance between processes leading to increases in DNA (particularly **polyploidization** [whole genome duplication] and/or repeat amplification [including transposable elements and satellite repeats]), and those that eliminate DNA (primarily via recombination pathways including those involved in DNA repair [10,11]). There is clear evidence from the increasing amount of cytogenetic and DNA sequence data that changes in genome size are frequent and

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ongoing in the evolution of angiosperms (e.g., [12–14]), but the underlying processes triggering these changes, and their consequences for the expression and ultimately the evolution of traits, remain unclear.

Based on chromosome count data, it is estimated that c. 33% of extant angiosperm species are recognizable as **polyploids** [15]. In addition, whole-genome sequencing and transcriptomic data have highlighted the prevalence of polyploidy throughout the evolutionary history of angiosperms, with an ever-increasing number of paleopolyploid events being detected in many lineages that have given rise to extant angiosperms [3,16]. Polyploid formation results, at least initially, in an immediate stepwise increase in genome size in a single generation (since the whole genome, including both coding and noncoding regions, is multiplied).

However, polyploidization is often followed by changes at the genomic (e.g., gene subfunctionalization, neofunctionalization, DNA elimination, and chromosome rearrangements), epigenetic (e.g., DNA and histone methylation leading to changes in gene expression and regulation of genetic networks [17]), and proteomic (e.g., nonadditive protein expression [18]) levels [19]. Consequently, over thousands to millions of years, the genome becomes 'diploidized' with reductions in both chromosome number and genome size (i.e., **genome downsizing**) [11,20]. For example, the model plant *Arabidopsis thaliana* is predicted to be 48-ploid based on the identification of multiple paleopolyploid events in its ancestry. Assuming no genome downsizing, the predicted genome size of *A. thaliana* would be ~82 Gbp/1C, and yet it is only 0.157 Gbp/1C [11,20]. Thus, contrary to expectation that polyploids will have larger genomes than diploids, over evolutionary time genome downsizing has resulted in the lack of a proportional relationship between genome size and ploidy level in many species and families (except in the most recently formed polyploids) [11]. Indeed, based on genome size data for 10 770 angiosperm species [21], it becomes apparent that despite the c. 2400-fold range in genome sizes and frequency of polyploidy, most angiosperms have small genomes (i.e., ≤3.43 Gbp/1C, *sensu* Leitch *et al.* [22]), with a mean and modal genome size of 5.02 and 0.60 Gbp/1C, respectively [9].

Genome size increases also arise from the amplification of repeats, independent of polyploidy. In contrast to polyploidization, increases due to repeat amplification typically occur at a slower rate, over multiple generations (e.g., doubling genome size in *Oryza australiensis* over 3 million years [12]). Nevertheless, their impact on the organization, functioning, and evolution of the genome can be considerable (reviewed by [23,24]). The length of time that an active repeat remains in the genome before it is inactivated via mutations, epigenetic silencing, and/or deleted by recombination processes [24] will, of course, influence the extent of their impact on genome size dynamics.

Biophysical effects of genome size on functional traits

Regardless of how genome size changes, it is important to recognize that its impact on phenotypic (including functional) traits arises not only from changes in the DNA sequences, but also directly from the biophysical impact of the bulk DNA. Hence, regardless of the coded information, genome size acts as a first-order constraint that determines the lower limit of both size and rate-related traits (i.e., the **nucleotypic effect** [25]) (Figure 1). At the cellular level, genome size sets the lower limit of growth-related characters, such as the minimum duration of mitosis and meiosis, and hence the time necessary to complete the cell cycle. Genome size also determines the minimum cell size, as you cannot fit a big genome into a small cell [26–29]. The total number of cells that can occupy a given space, and consequently the distance between cell types in a tissue (i.e., cell packing density), is therefore also influenced by genome size [30,31]. This in turn can affect the phenotype and physiological and metabolic functioning of organs and tissues, such as leaves. For example,

(A) Trait evolution in response to genome size increase
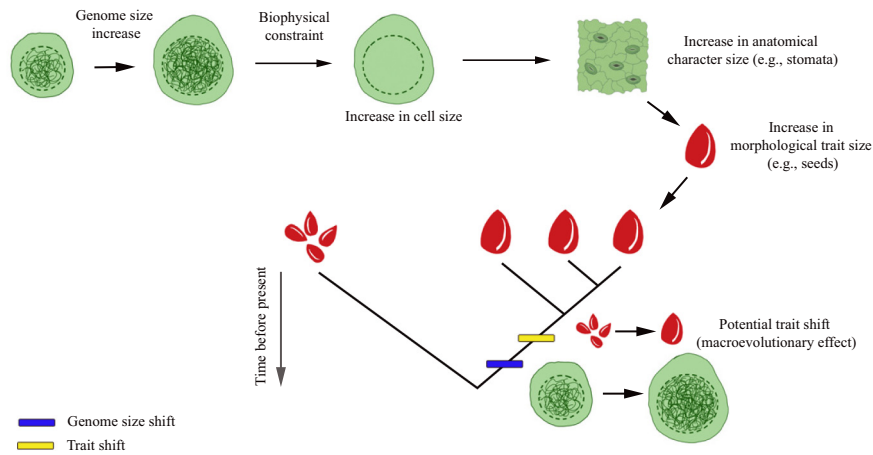
(B) Trait evolution in response to genome size decrease

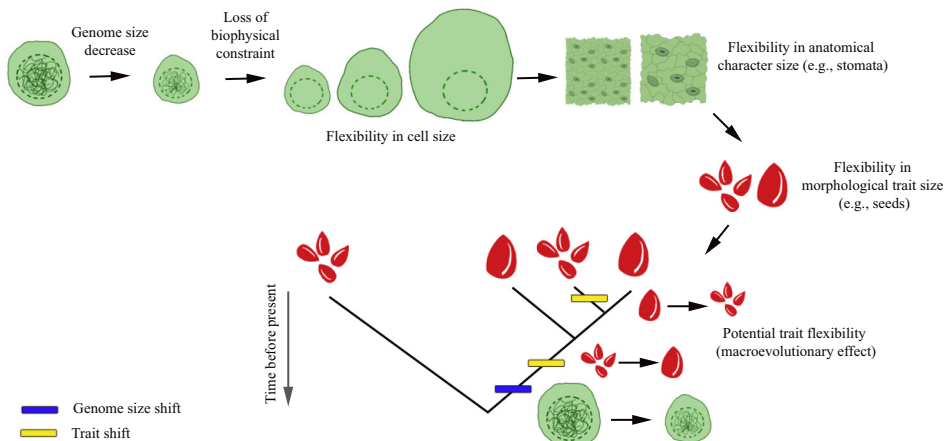Figure 1. Schematic representation illustrating the relationship between genome size change and trait evolution. (A) With increases in genome size (e.g., by polyploidization, repeat amplification), the nucleotypic (biophysical) effect of a large genome constrains minimum cell size and growth-related characters such as the minimum duration of the cell cycle, enforcing an obligatory shift to larger cells that divide more slowly. Large cells can result in larger anatomical characters and morphological traits, potentially leading to a macroevolutionary shift towards large-sized traits (e.g., increased seed size) as detected on a phylogenetic tree. (B) With a decrease in genome size, genome size no longer constrains the size of cells, anatomical characters, and morphological traits. Hence, this may allow trait change, and thus increase trait flexibility over macroevolutionary time scales. N.B. Genome size and associated trait changes and their expression over phylogenetic time scales are dependent on the ancestral state, the historical (ancestral) environment (e.g., nutrient availability, climatic factors), and the prevailing genome sizes and traits of species and lineages over time and space.

increases in genome size will be directly accompanied by increases in the minimum guard cell size, leading to larger stomatal pore sizes and lower stomatal density [30]. These, in turn, can affect the balance between stomatal conductance of CO_2 and water, and hence the potential maximum photosynthetic rate of leaves [30,32]. Furthermore, while water use efficiency is influenced by many factors, it includes stomatal pore size and density, vein density, cell water-storage capacity, and speed of stomatal closure in response to water limitations [33,34], all of which can be influenced by genome size. While other abiotic and genetic factors can also modulate the extent to which genome size influences photosynthesis and water use efficiency, they can only operate

above the minimum size set by the biophysical constraints imposed by genome size [27,35]. Thus, increased genome sizes, accompanied by larger guard cell sizes, may result in increased water loss, desiccation, a decrease in photosynthetic rate, and a potential loss of plant performance in certain environments [27,30,36,37]. Furthermore, genome size correlates with numerous other traits (e.g., see reviews by [38–40]), such as pollen size (in many angiosperm genera [41]) and seed size (e.g., in Cactaceae [42]), with potential impacts on germination success, seedling survival, dispersal and pollination.

Context-dependence in genome size and functional trait evolution

By providing genetic material for natural selection to act on, genome size may also indirectly affect phenotypic (functional) traits and hence trait evolution. For example, increases in genome size (via polyploidy and/or amplification of repeats) may result in changes in genetic variation in genes and epigenetic gene expression networks, which may affect the (evolutionary) development of new traits and functions, and thus impact plant performance and tolerance under environmental changes [23,43]. To illustrate, aridity-driven preferential expression of stress-responsive repeat elements may play a role in plant survival in arid systems, as reported in palms [44] (see also review on the impact of transposable element-derived RNAs and their ability to influence a diversity of stress responses in angiosperms [45]). Furthermore, the insertion/deletion of repeats in flowering-related genes may result in changes in flowering time in angiosperms (e.g., in maize, rice, and *Arabidopsis* [46]).

While we recognize that changes in genome size can indeed result in changes in the expression of genes (see previous paragraph), our focus here is on the biophysical effects of genome size on plant performance, and we note that such effects are context-dependent - i.e., they depend on the environmental setting. Genome size may play a role in influencing the survival of plants in nutrient-limited environments, as building larger genomes requires more nitrogen (N) and phosphorous (P); thus plants with large genomes struggle to grow and compete successfully in low-nutrient conditions (e.g., [47]). Nevertheless, even in environments that are not resource-limited, genome size may still play a role in influencing how species respond to different climatic conditions. In temperate regions, for example, genome size can influence the timing of plant growth, restricting species with larger genomes (e.g., Liliaceae) to dominate plant communities only in early spring [48]. Thus, soil nutrients and climatic factors play a significant role in underpinning the distribution of angiosperms and their genome sizes [49]. Genome size also influences species characteristics such as life form (e.g., annuals are restricted to having smaller genomes [50]) or invasiveness (e.g., invasive angiosperms typically have small genomes [29,51]). Furthermore, while both polyploidy and repeat activity lead to changes in genome size, the contrasting nature of these genomic processes means that their impacts are not necessarily equivalent. For example, while an increase in genome size due to polyploidy results in all components of the genome potentially being impacted, repeat activity typically influences the genome more locally through insertions or deletions, resulting in mutations that can influence, for example, the epigenetic regulation of specific genes, transcription and specific stress responses [17,23,45]. Thus, although similar genome size increases can arise from polyploidy or repetitive DNA activity, the genomic consequences are likely to be different with regard to the types of genetic variation they generate, and hence their potential to create opportunities for the exploration of novel trait space and broadening of ecological niches.

Constraints of large genomes

An important observation based on the previous section is that species with large genomes, due to their biophysical impact, are typically more constrained in their phenotype and functional traits, and thus in the ecological and evolutionary opportunities available to them (i.e. 'the **large**

genome constraint hypothesis' [37]). While ongoing stochastic changes in genome size are likely to be common [9,27], such constraints imposed on species with larger genomes may contribute to explaining why the direction of genome size change over time is typically downwards (e.g., via genome downsizing following polyploidy, and repeat elimination via deletion-biased recombination processes) and why species with larger genomes often fail to compete successfully in certain environments [11,27].

Large genomes may also be more constrained in their ability to generate genetic novelty upon which selection can act, because genome size itself has been shown to play a critical role in determining the persistence of repeats in the genome and hence their influence on genome dynamics. For example, Novák *et al*. [52] showed that the turnover and activity of repeats in species with genomes up to c. 10 Gbp/1C was relatively rapid - i.e., amplification and elimination over thousands to a few million years. In contrast, the dynamics of repeat turnover in species with genomes larger than c. 10 Gbp/1C appeared to be slower. Given these observations, the evolution of large-genomed species may be more constrained than those with smaller genomes, due to their reduced ability to generate genetic novelty, resulting in lower trait flexibility, and potential speciation slow-downs [37].

The macroevolutionary consequences of genome size–trait relationships

The biophysical impact of genome size thus closely links genome size with cell size, which in turn can play a role in influencing phenotypic trait space, trait flexibility, plant function, and ultimately plant performance (Figure 1). However, trait variations, and hence the functioning, performance, distribution and diversification of angiosperms, can vary widely depending on other biotic and abiotic factors [2,30]. Increases in genome size may lead to larger trait values and therefore diversification slow-downs [37] (e.g., large genomes are typically associated with large seeds which may increase dispersal ability and decrease allopatric speciation, for example in vertebrate-dispersed palms [53]). In addition, increases in genome size constrain the variation of trait values (trait flexibility), and thereby constrain diversification [37], whereas a decrease in genome size may lift such constraints, and hence provide the ecological opportunity for diversification rate increases [30]. For example, genome downsizing following polyploidization in angiosperms led to smaller genomes which, in turn, opened up the opportunity for leaves to have higher stomatal and vein densities, and hence elevated photosynthetic rates and faster biomass accumulation [30]. Such opportunities might have been particularly advantageous during the early evolution of angiosperms in the Cretaceous when CO_2 levels were low relative to today, contributing to their competitive advantage over other land plant lineages and enabling their rapid diversification and ecological dominance [30,36]. Although the associations between genome size and phenotype are more established and understood, and how these may have facilitated the evolution of trait novelties and influenced rates of angiosperm diversification [16,30,54,55], a framework to quantitatively test the macroevolutionary links between genome size, trait evolution and trait flexibility in angiosperms is missing.

From a macroevolutionary viewpoint, the close biophysical link between changes in genome size and values of certain phenotypic (including functional) traits may translate into correlated evolution between them [56] and thus enforce 'trait evolutionary shifts' as detected on phylogenetic trees (e.g., [54]) (Figure 1). We hypothesize that lineages with large genomes are constrained towards trait values at the upper end of the scale, thereby restricting trait flexibility, resulting in lower diversification rates. This is because an increase in genome size at the root of a lineage in a phylogenetic tree imposes a corresponding increase in minimum cell size, restricting the range of cell sizes that are possible. If this lineage maintains its larger genome size throughout macroevolutionary time, the descendent lineages will also be constrained by a narrow range of trait

variation, resulting in reduced flexibility of trait evolution over time, and a corresponding lower rate of diversification (Figure 1A). In contrast, we hypothesize that lineages with small genomes will be characterized by higher trait flexibility (i.e., a wider range of possible trait values), and hence greater opportunities for diversification. This is because species with smaller genomes have smaller minimum cell sizes, and hence have the potential to generate a greater range of cell sizes above the minimum set by genome size (e.g., via larger vacuoles). Thus, lineages which have undergone reductions in genome size will have the opportunity to generate greater trait variation, leading to increased flexibility of trait evolution over time, and higher diversification rates [2,26] (Figure 1B). In both scenarios (Figure 1), trait evolution in response to genome size change will depend on the ancestral trait state. For example, an increase in genome size in a species with a small genome but already possessing a large seed may not necessarily be accompanied by a further increase in seed size. Similarly, a decrease in genome size in a large- or small-seeded lineage does not necessarily lead to a phenotypic change, but the lineages which started with a smaller genome will have the opportunity to evolve smaller seeds over time, as trait evolution is no longer constrained by the size of their genomes [37,57].

The scenario presented in Figure 1 may contribute to explaining the ecological and evolutionary success of angiosperms compared to other land plant clades [30], but testing the extent to which it explains variation in trait evolution, trait flexibility and evolutionary radiations within angiosperms [2] requires a lineage-specific approach, and a complementary assessment of the eco-evolutionary context (e.g., past environmental conditions). This could be achieved by integrating well-sampled phylogenies with functional trait, genome size, and paleo-environmental data. By applying phylogenetic comparative methods (e.g., [4–6,56,58]), we can evaluate whether trait evolution and diversification rates differ between lineages with small versus large genomes. Evidently, it is challenging to obtain reliable deep-time estimates of genome size based on data from extant taxa only. Hence, the relationship between stomatal size and genome size has been used to predict ancestral genome sizes from stomatal measurements of fossilized leaves [34,59]. Although past environments were different from those of today, and this could potentially impact the relationship between genome size and stomatal size, studies have shown that the relationship is not necessarily extensively modulated by the environment [60] (as shown for *A. thaliana* [61], but see [32]).

Concluding remarks and future perspectives

Genome size, by fundamentally constraining size and rate-related traits, has played an important role in angiosperm evolution through its impacts on opportunities for trait innovation and trait flexibility. However, interactions between genome size and trait evolution are context-dependent, and may vary depending on the (ancestral) environment, genome size, and functional traits of lineages (see Outstanding questions). The uneven distribution of genome size diversity across angiosperms suggests that large genomes may often not persist over macroevolutionary time (but see [19]), whereas small genomes are typically more resilient (although persistence will also depend on the environmental context). Nevertheless, the overriding trend towards smaller genomes in angiosperms suggests that selection and/or environmental filtering frequently results in species with large genomes failing to thrive (e.g., due to high resource requirement, less stress tolerance), leading to their extinction from the angiosperm tree of life [9,37,50]. Such extinctions may have implications for our understanding of the link between genome size, trait evolution, and angiosperm diversification [5,6]. However, genome size increases arising from duplications of alleles (e.g., from polyploidy) and repeats (from repeat amplification) may also give rise to novel variants of existing genes with new functions or expression patterns, that may facilitate trait evolution in some cases (e.g., novel function leading to innovation) and diversification [26,27,38,39,54,62]. Overall, such multidimensional effects of genome size on plant traits,

Outstanding questions

How does the relative contribution of repeat activity versus polyploidy impact the ability of a species to explore trait space? To what extent do external factors (e.g., climatic factors) constrain the molecular processes that generate genome size diversity and thus trait flexibility?

Is there a tipping point in genome size beyond which a lineage's capacity to generate genetic novelties and trait diversity decreases, such that larger genomes become a hindrance to evolution?

Which angiosperm radiations are the result of decreases in genome size allowing greater trait flexibility, and which are not? Are there differences in the molecular mechanisms underpinning changes in genome size across evolutionary radiations?

Does the relationship between genome size and trait flexibility vary across life forms (e.g., herbs vs. woody trees), growth forms (e.g., annuals vs. perennials), and geographical regions (e.g., temperate vs. tropical) due to differences in molecular, diversification, and trait evolution rates?

Given the vast diversity of angiosperm genome sizes (~2400-fold), frequency and levels of polyploidy (up to 30-ploid), and variations in chromosome number (2n = 4 to ~640) in angiosperms, how do genome size, chromosome number, and polyploidy interact to impact the generation of genetic diversity, and hence the extent of trait flexibility a lineage can exhibit?

To what extent can the relationship between genome size and trait flexibility observed in angiosperms be extended to other plant lineages, such as gymnosperms, monilophytes, and bryophytes, and even to animal lineages with variable genome sizes and frequent polyploidy?

performance, and diversification make the design of a simple predictive model to explain the role of genome size in angiosperm trait evolution and diversification challenging. We suggest that a synthesis of genome size and trait evolution across a wide range of angiosperm lineages in a comparative phylogenetic framework will provide the opportunity to assess the conditions under which genome size and angiosperm trait flexibility have affected angiosperm radiations, and under which conditions they did not. The growing availability of whole genome sequences will further allow to dissect the genes, pathways and networks involved in genome size change and how this impacts the evolution of relevant traits and diversification.

Declaration of interests

The authors declare no competing interests.

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