

## OVERVIEW

# Plant Genome Size Research: A Field In Focus

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This Special Issue contains 18 papers arising from presentations at the Second Plant Genome Size Workshop and Discussion Meeting (hosted by the Royal Botanic Gardens, Kew, 8–12 September, 2003). This preface provides an overview of these papers, setting their key contents in the broad framework of this highly active field. It also highlights a few overarching issues with wide biological impact or interest, including (1) the need to unify terminology relating to C-value and genome size, (2) the ongoing quest for accurate gold standards for accurate plant genome size estimation, (3) how knowledge of species' DNA amounts has increased in recent years, (4) the existence, causes and significance of intraspecific variation, (5) recent progress in understanding the mechanisms and evolutionary patterns of genome size change, and (6) the impact of genome size knowledge on related biological activities such as genetic fingerprinting and quantitative genetics. The paper offers a vision of how increased knowledge and understanding of genome size will contribute to holistic genomic studies in both plants and animals in the next decade.

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**Key words:** Genome size, C-value, intraspecific variation, DNA amounts, genome evolution, holistic genomics, algae, genetic fingerprinting.

Nuclear DNA amount and genome size (C-value) are important biodiversity characters, whose study provides a strong unifying element in biology with practical and predictive uses. Recognizing the importance of the field, and the growing interest in such data, the *Annals of Botany* Company sponsored the first 'Angiosperm Genome Size Workshop and Discussion Meeting' (probably the first international meeting devoted exclusively to this specialized topic), hosted at the Royal Botanic Gardens, Kew in September 1997. A collection of 14 papers arising from that meeting formed a special issue published in December 1998 (*Annals of Botany* Supplement A, 1998). Together they addressed the major needs, problems and opportunities for research in the field, and made key recommendations for addressing them (<http://www.kew.org/cval/conference.html#outline>). Paramount was a need for improved representation of the global flora. Second was a need to ensure easy access to plant genome size information. Third was a need to ensure and improve data quality. For example, the workshop agreed to identify the major gaps (systematic, regional and plant type) in our knowledge of plant DNA amounts, and set goals to fill them while measuring 2500 species by international collaboration. The need for follow-up was also recognized, so another meeting to monitor progress and set new goals was planned for about five years later.

This special issue of *Annals of Botany* crystallizes the fulfilment of that plan. Thus, in September (8–12th) 2003, over 70 scientists from 16 countries attended the Second Plant Genome Size Workshop and Discussion Meeting, hosted at the Royal Botanic Gardens, Kew, and again sponsored by the *Annals of Botany* Company. This was exactly six years after the first meeting in 1997 and timed to mark the 60th birthday of Professor Michael Bennett. It was also intended to contribute to the special year celebrating the

central role of DNA in biological research, as April 2003 was the 50th anniversary of the discovery of the double helix structure of DNA and its significance, counting from the publication in *Nature* of the seminal paper by Watson and Crick (1953). However, its main purpose was to review progress in the field, especially against the five-year targets recommended in 1997 and to look ahead. This special issue is a collection of 18 papers based on lectures and discussions by an international spectrum of leading experts in genome size research. Together they cover a wide range of aspects of current research, thinking and trends on plant nuclear DNA amount and genome size, and provide an up-to-date overview of this highly active field. Many are reviews, or include review material. Consequently, the present work is a review of reviews, setting their key contents in the broad framework of the field. It is also highly selective, highlighting a few overarching issues with wide biological impact or interest for the non-specialist, noting important trends and interpreting their significance.

In the last decade *Annals of Botany* (AoB) has played a significant and growing role as a vehicle for communicating estimates of nuclear DNA amounts (genome sizes) in plants. In 1995 a first supplementary list of nuclear DNA C-values (for 899 angiosperm species from 106 sources), assembled primarily for reference purposes, was published in AoB (Bennett and Leitch, 1995). Three further supplementary lists have followed (Bennett and Leitch, 1997; Bennett *et al.*, 2000), including the review with DNA C-values for 804 angiosperm species from 88 sources in this Special Issue (Bennett and Leitch, 2005a). AoB has rapidly become the journal of choice for first publication of such estimates. Only two of the 106 sources (<2 %) cited in Bennett and Leitch (1995) were originally published in AoB, compared with 15 of the 88 original sources (17 %) cited in Bennett and Leitch (2005a). AoB now has the highest proportion of

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papers containing C-value estimates of any plant research journal. Moreover, such data are clearly much used, as the lists published in 1995 and 1997 have received over 270 citations in the Web of Science (by August 2004).

The 2003 Discussion meeting was preceded by a Plant Genome Size workshop, which reviewed progress against key targets set in 1997 and developed new targets for the next five years. The report of this workshop (<http://www.rbgekew.org.uk/cval/workshopreport.html>) can be consulted for a fuller account. Meanwhile, four key actions or aims are noted here:

- (1) Ambiguities in the current uses of the terms ‘genome size’ and ‘C-value’ were noted, and a sub-group was set up to clarify these problems and propose solutions.
- (2) Given the lack of an absolute C-value based securely on complete genome sequencing, work is needed to link to the genome of the nematode worm *Caenorhabditis elegans* and establish a set of ‘gold standards’ for plants.
- (3) A target of at least an additional 1 % for angiosperms (approx. 2500 species) was thought essential, and within this to achieve 75 % familial and 10 % generic coverage by 2008/9.
- (4) The workshop concluded with a proposal to formally constitute an international group for genome size analysis, which after consultation was named GESI (*Genome Size Initiative*). It was also agreed to meet again in about five years, possibly in Texas, and to offer a symposium on ‘Plant Genome Size—its evolution and significance’ (now accepted) for the XVII International Botanical Congress at Vienna, Austria in July 2005.

#### DEFINING ‘C-VALUE’ AND ‘GENOME SIZE’

Under best practice the workshop discussed the definitions of the terms ‘C-value’ and ‘genome’, whose common usage is subject to evolution driven by public opinion. For example, it was recently suggested that the ‘C’ of C-value indicates ‘class’, since Hewson Swift, who coined the term, did not define it (Swift, 1950). This point was easily resolved, as when researching for Bennett and Smith (1976), the first author wrote in a letter to Swift in 1975:

‘. . . My reason for writing to you is therefore to ask whether you were responsible for the origin of the term C-value; and also, to ask what C-stands for? Opinion in Cambridge among my colleagues is that it must stand for ‘complement’ . . .’

In a letter Swift replied:

‘. . . I think my PNAS 1950 paper included the first use of ‘C-value’. I merely wanted to avoid confusion with chromosome number, N, since clearly a diploid cell entering prophase appeared to have the same DNA content as a tetraploid nucleus in early interphase. I am afraid the letter C stood for nothing more glamorous than ‘constant’, i.e. the amount of DNA that was characteristic of a particular genotype.’

[N.B. Copies of the original correspondence are available from the present first author.]

The original meaning of ‘genome’ by Winkler (1920), who coined the term, applied to one monoploid

chromosome set ( $x$ ), and its use was restricted to this for half a century. However, genome has acquired a second meaning, now in common use, as ‘all the nuclear DNA in the chromosome complement ( $n$ ) of a eukaryote’. The latter use of genome is synonymous with C-value for all diploid and polyploid taxa, unlike the former. Difficulty in knowing which meaning is intended can arise, especially when authors use both, without definitions, in one paper. For example, Devos and Gale (1997) used ‘wheat genome’ to refer to the entire complement of nuclear DNA in hexaploid wheat, yet elsewhere in the paper they discussed the ‘three genomes’ of wheat, referring to the individual A, B and D genomes.

Greilhuber *et al.* (2005) review these problems, and propose a unified terminology to stabilize the way in which DNA amounts for taxa are described by authors, and reduce confusion for non-specialists. It accepts that in the future genome size will be used and viewed mainly as a general covering term. The necessary distinction of the two meanings of genome is made by the adjectives ‘monoploid’ and the neology ‘holoploid’ and abbreviated terms for monoploid and holoploid genome size are C<sub>x</sub>-value and C-value, plus a numerical prefix such as 1C, 1C<sub>x</sub>, 2C, etc. to indicate the C-level of all quantitative data on genome size.

#### GOLD STANDARDS FOR PLANT GENOME SIZE ESTIMATION

A main concern of the 1997 workshop was the need to ensure and improve data quality, and this is an ongoing preoccupation. C-values estimated by most methods are subject to technical and other errors, unlike those obtained from a fully sequenced genome. It is clearly important to have a precise C-value as a standard, as without this it is impossible to calibrate all other species accurately. In this connection, the 2003 workshop discussed the possibility of using the current plant genome sequencing data to obtain an absolute standard. It was confirmed that the *Arabidopsis* Genome Initiative’s C-value for *Arabidopsis thaliana* (125 Mb) was a gross underestimate (Bennett *et al.*, 2003) and an exact C-value based on genome sequencing alone is unlikely to be obtained soon for any multicellular plant. Whilst animal standards are still not generally recommended for plant genome size estimations, a need to link plant and animal standards was recognized. As the C-value for *Caenorhabditis elegans* (~100 Mb) does reflect virtually complete genome sequencing, the best link from animals to plants is probably *C. elegans*–*Arabidopsis thaliana*. Consequently, this should be used to establish a set of ‘gold standards’ for plants.

The plant genome size community is serious in its quest for accurate genome size data, active in improving best practice and transparent in weeding out erroneous C-values. Comparing results at the recent workshop provided a striking example of this process. For years the smallest 1C-value estimate listed for an angiosperm was 0.055 pg for *Cardamine amara*. As this seemed suspiciously low, three groups checked it independently.

TABLE 1. 1Cx DNA estimates for *Cardamine amara* by three research groups reported at the second Plant Genome Size Workshop (September 2003)

Research group	Material	Ploidy level	1Cx DNA content (pg)	Reference
Botanical Institute, University of Vienna	Upper Austria	$2n = 4x = 32$	0.242	Greilhuber (pers. comm.)
Royal Botanic Gardens, Kew, UK	Sheffield, UK	$2n = 2x = 16$	0.243	Bennett and Leitch (2005)
Texas A & M University, USA	Krosno, Poland	$2n = 2x = 16$	0.225	Johnston <i>et al.</i> (2005)

We measured UK diploid material in 2003 using flow cytometry (Bennett and Leitch, 2005a), unaware that both Johnston *et al.* (2005) in North America had measured diploid material and that Greilhuber (pers. comm.) had measured tetraploid material from Upper Austria using Feulgen microdensitometry. The estimates of 1Cx genome size (0.225 pg and 0.243 pg, respectively, in the diploids and 0.242 pg in the tetraploid) all show 0.055 pg to be an error (Table 1) and follow the pattern of agreement within 10 % (often much closer) between these laboratories (Doležel *et al.*, 1998). This confirms that C-values estimated by experienced operators using best practice methods can generally be viewed with confidence.

#### IMPROVED KNOWLEDGE OF SPECIES' DNA AMOUNTS

The workshop noted major improvements in the numbers of species with known DNA amounts that are now available in the Plant DNA C-values Database (up 63 % since 1997), including significant advances for several non-angiosperm groups.

The 1997 workshop reviewed most non-angiosperm plant groups, but ignored algae. They were not seen as unimportant, but the gaps identified then for several other groups seemed daunting enough. Once first compilations of DNA C-value estimates for gymnosperms (Murray, 1998), pteridophytes (Obermayer *et al.*, 2002) and bryophytes (Voglmayr, 2000) were available, we noted that no similar database was available for algae. This major gap is now addressed, as Kapraun (2005) gives the first compilation of genome size estimates for 247 species of red, green and brown algae and reviews the considerable diversity in this character and its possible evolutionary significance.

Bennett and Leitch (2005a) review improvements in the representation of angiosperm species' DNA amounts since 1997 and conclude that 1998–2002 saw striking progress in our knowledge, as at least 1700 first estimates for species were measured (the most in any five year period), whilst familial representation rose from 30 % to 50 %.

#### INTRASPECIFIC VARIATION—IDENTIFYING ITS EXISTENCE, CAUSES AND SIGNIFICANCE

Variation in DNA amount between species begins with changes within species, yet intraspecific variation remains one of the most controversial topics in the study of plant genome size. Whilst variation in DNA amount can arise

from chromosome polymorphisms, or is due to taxonomic heterogeneity, robust examples of detectable intraspecific genome size variation are so far few. Critical assessment of claimed examples at the 1997 Plant Genome size meeting led Greilhuber (1998) to conclude that most were due to technical shortcomings. Further, workshop discussions resulted in several key recommendations regarding best practice techniques for estimating DNA amounts as a way to minimize such errors (see [www.kew.org/cval/conference.html#outline](http://www.kew.org/cval/conference.html#outline)). Subsequently, intraspecific variation has continued to receive active research attention. Further discussions at the second workshop led to additional recommendations regarding best practice for Feulgen staining or flow cytometry (see <http://www.rbgkew.org.uk/cval/workshopreport.html>), whilst some of the other key areas of progress are reviewed in this volume.

Greilhuber (2005) revisits the question of whether intraspecific variability of C-values is real or artefact by reviewing several recent studies from his laboratory that have refuted previously claimed examples. He also summarizes the results of recent investigations into critical steps of the quantitative Feulgen procedure in order to minimize the generation of artefactual genome size variation.

Whilst Doležel *et al.* (1998) and Vilhar *et al.* (2001) have shown that Feulgen and flow cytometry can give comparable results when used properly, technical problems arising during genome size estimations by flow cytometry have also resulted in artefactual data and false evidence of intraspecific variation. Doležel and Bartos (2005) review the use of flow cytometry for estimating genome size in plants, highlighting how to optimize data quality and pointing out potential methodological pitfalls. The presence of cytosolic compounds that can interfere with the binding of the fluorochrome to DNA is one such problem, which has been extensively researched by Noirot *et al.* and others (e.g. see Noirot *et al.*, 2000, 2002, 2003; Price *et al.*, 2000). In the present volume Noirot *et al.* (2005) extend their studies by showing how the temperature of the nuclear extract can also contribute to variation in the genome size estimate obtained. Such studies highlight the potential for generating pseudo-intraspecific variation and may explain why many reports are technical artefacts. However, genuine examples obtained using appropriate standards and controls are published, and here questions as to their biological significance need to be addressed. Murray (2005) considers the possible role of intraspecific variation in plant taxonomy, citing several examples where it may have adaptive consequences and/or represent incipient speciation. He concludes that intraspecific variation is most significant for taxonomy as an indicator of taxonomic heterogeneity.

### MECHANISMS AND EVOLUTIONARY PATTERNS OF GENOME SIZE CHANGE

Since the first Angiosperm Genome Size meeting there have been huge advances in our understanding of the mechanisms and forces driving genome size evolution. In 1997 the pervading view was that plants appeared to have a 'one way ticket to genome obesity' through polyploidy and transposon amplification (Bennetzen and Kellogg, 1997). However, it is now clear that mechanisms for genome downsizing also exist. An overview of the mechanisms operating in plants is outlined by Bennetzen *et al.* (2005), while those common to both plants and animals are discussed by Gregory (2005), who also makes the important plea for a more unified approach to genome size research in these different kingdoms.

The evolutionary forces that might be driving changes in genome size are still poorly understood. Cavalier-Smith, who edited an important book on genome size evolution in 1985, has now written his first major review of the topic in 20 years (Cavalier-Smith, 2005). In this he revisits his idea that non-genic DNA plays a skeletal role—'The skeletal DNA theory'—and explains how recent advances in understanding cell cycle control offer a breakthrough in the log-jam of distinguishing between causality and correlation with respect to genome size and cell volume correlations. At a different level, Knight *et al.* (2005) outline their 'large genome constraint' hypothesis, suggesting that the possession of a large genome imposes both ecological and evolutionary constraints. They present evidence to explain why species with large genomes may be trimmed from the evolutionary tree and have restricted ecological distributions. Thus the possession of a large genome may itself act as an evolutionary force. Chase *et al.* (2005) add to this by investigating what role life history traits may play in directing or determining selection for a particular genome size. They examine genome sizes in Oncidiinae, a large subtribe of Orchidaceae, which displays a diversity of life history strategies, including a species that can complete its entire life cycle growing on the ephemeral leaf of another plant.

There is still much to be learned concerning which DNA sequences are involved in changes in DNA amount. Recently a few studies have reported differences in amounts of specific DNA sequences between species differing in DNA amounts (e.g. Vicent *et al.*, 1999; Zhang and Wessler, 2004). Such studies have sometimes led to the assumption (but without complete experimental evidence) that changes in copy number of certain DNA sequences are responsible for the changes in DNA amount. While it is clear that differences in DNA amount between species are predominantly associated with differences in the amounts of repetitive sequences, it has yet to be clearly demonstrated that amplification of a specific DNA sequence is directly responsible for increase in DNA amount. Nevertheless Cullis, who has studied various DNA sequences in flax that can alter in copy number in response to particular environmental conditions, provides an interesting insight into the dynamics and fluidity of the flax genome (Cullis, 2005). Whether or not the changes reported are responsible

for the gross changes in DNA amount is unclear as the molecular work reported here has been uncoupled from the genome size work (Evans *et al.*, 1966).

Another component of understanding genome size evolution is to see where changes in size have taken place from a phylogenetic perspective. The availability of robust phylogenetic trees on which to superimpose genome size data has expanded greatly since the last meeting, and are now available not only at a broad level looking at relationships between different land plant groups (i.e. bryophytes, lycophytes, monilophytes, gymnosperms and angiosperms), but also for many families and genera. This exponential growth in increasingly robust phylogenetic data has enabled Leitch *et al.* (2005) to look for broad patterns of genome size evolution across all land plants, while Johnston *et al.* (2005) and Price *et al.* (2005) have used similar approaches to examine genome size evolution in the angiosperm family Brassicaceae, and the genus *Sorghum* (Poaceae). From such approaches it is clear that genome size evolution is dynamic, with evidence that both increases and decreases have taken place at all taxonomic levels during plant evolution.

### CONSEQUENCES OF GENOME SIZE VARIATION

It is increasingly clear that genome size impacts on other areas of research and that knowledge of it can be important when framing questions or planning research. The small genome size of *Arabidopsis thaliana* undoubtedly played a major role in its selection as the first plant to have its genome sequenced (NSF, 1990; Somerville and Somerville, 1999) and the proposal that poplar (*Populus*) should be the first tree to be sequenced has been based in part on its 'modest' genome size (Brunner *et al.*, 2004). In this issue, two further examples are discussed where knowledge of genome size may be important; namely DNA fingerprinting and quantitative genetics.

Microsatellites are used widely for DNA fingerprinting in population genetic studies analysing population structure, gene flow, genetic diversity, etc. and yet their successful analysis has been shown in part to be determined by genome size. Garner (2002) reported that there was a highly significant positive correlation between genome size and the successful amplification of microsatellites in nine metazoans with 1C-values ranging from 0.791 to 25.62 pg. Similar studies have not been reported in plants, but Fay *et al.* (2005) report here that the use of a related DNA fingerprinting technique, amplified fragment length polymorphisms (AFLPs), is similarly affected by genome size. They conducted AFLP analyses on plant species ranging in C-value from 0.2 to 32.25 pg and found that knowledge of genome size and ploidy level were important for determining what protocol was most likely to yield informative data for population genetic analyses.

Genome size is now starting to be recognized as potentially important in the field of quantitative genetics, which aims to analyse and understand the genetic basis of characters showing continuous variation. With the advent of

the genomics era, the search began for quantitative genetic loci (QTL), genomic regions that affect the variation in specific quantitative characters. Originally it was envisaged that these would ultimately result in the identification of particular structural genes. However, in recent years the failure to understand the mechanisms operating at the genetic level to bring about the phenotypic variation observed has led some researchers to extend the search for QTL to include regulatory genes (e.g. see review by Remington and Purugganan, 2003). In this issue, Meagher *et al.* (2005) suggest that 'in addition to structural genes, some QTL may be made of localized variation in repetitive DNA content, which in turn is effecting quantitative variation through impact of patterns of gene regulation'. They studied QTL influencing flower size in *Silene latifolia* and showed they are correlated with QTL for DNA content. They conclude that future studies searching for the genetic basis of QTL should be extended to include overall aspects of genome regulation including variation in DNA content.

#### 2010 VISION

Interest in the origin, extent and significance of genome size variation has increased greatly in the last decade. This volume is necessarily focussed on genome size in plants, but parallel research in other kingdoms is developing rapidly (see Gregory, 2005*b*). Publication of the special issue of *Genetica* entitled 'Evolution of the Genome size' (edited by Petrov, 2002), and of a new book on this topic (edited by Gregory, 2005*a*) shows that pace in the field is moving up and how many questions transcend taxonomic boundaries and impact on our view of life. Owing to 'complete' genome sequencing, comparative genomics and the ability of modern computers to display and compare such information, the topic is a prime focus of modern attention in the rapidly developing new field of holistic plant and animal genomics. This trend is likely to accelerate in the next decade, as the quality, quantity and availability of genome size data improve greatly. The prospect still beckons of more exact C-values based on truly complete DNA sequencing for more organisms (including the first for multicellular plants), suitable as basal calibration standards. The total number and rate of increase of robust new DNA C-values and the amount of information about genome size(s) available electronically are targeted to improve dramatically. Indeed, the next decade may be the last to focus on estimating C-values. Thereafter, the sample should be sufficiently representative of plant life on earth to allow many big questions to be answered using available databases. Experts in the field now expect imminent advances in our understanding of the role(s) of genome size in ecology, the ecology of genomes and of how these fields may interrelate (Dermitzakis *et al.*, 2003). We have strong clues that DNA C-value plays critical roles in determining the probability of species' becoming extinct, withstanding pollution by heavy metals, surviving ionizing radiation and displaying invasive behaviour typical of the world's worst weeds (see review in Bennett and Leitch, 2005*b*). It is time to explore whether these characters link to predictive trends

in the types and organization of repeated DNA sequences and are related components of a unifying system of plant genome form, function and phylogeny. This volume represents a launch pad for such ideas, but the next ten years and future papers in *Annals of Botany* will record how well these expectations are met.

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