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.

Key words: Genome size, C-value, intraspecific variation, DNA amounts, genome evolution, holistic genomics, algae, genetic fingerprinting.
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.rbgkew.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 monopoloid 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 Cx-value and C-value, plus a numerical prefix such as 1C, 1Cx, 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.
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 techniques 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 (Oberrmayer et al., 2002) and bryophytes (Voglmaier, 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). 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.

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

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

**LITERATURE CITED**


Bennett MD, Leitch IJ, Price HJ, Johnston JS. 2003. Comparisons with *Caenorhabditis* (~100 Mb) and *Drosophila* (~175 Mb) using flow cytometry show genome size in *Arabidopsis* to be ~157 Mb and thus 25 % larger than the *Arabidopsis* genome initiative estimate of ~125 Mb. *Annals of Botany* 91: 547–537.


