GENOME SIZE: A RESEARCH DISCIPLINE IN DEVELOPMENT

Report on the International Botanical Congress official workshop held at the Institute of Botany, University of Vienna 22nd July 2005)

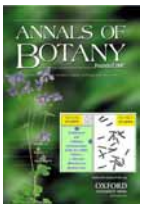
On 22nd July 2005, 40 scientists from 13 countries met at the Institute of Botany, University of Vienna to attend a workshop entitled 'Genome size: a research discipline in development' (co-organized by Johann Greilhuber and Mike Bennett). This represented the first meeting of GSI (Genome size Initiative) - an international group of scientists interested genome size analysis which was launched at the Second Plant Genome Size Workshop (held at the Royal Botanic Gardens, Kew in September 2003).

The aims of the workshop were:

(i) To assess progress in plant genome size knowledge in relation to targets set at the Second Plant Genome Size Workshop

(ii) To discuss new developments in the field which are likely to impinge on future progress and understanding of the significance of genome size in a holistic context.

The special issue of Annals of Botany 'Plant Genome Size' arising from the Second Plant Genome Size meeting was handed out to all participants. It contains 18 papers by leading experts in genome size research covering a wide range of aspects of current research thinking and trends on plant nuclear DNA amount and genome size.



Outline of the workshop:

Session I: Data - progress since the last meeting and targets

A. Review progress since 2003

B. Update of Plant DNA C-values database

Session II: Best practice – the effects of cytosolic compounds

Including;

'Anthocyanins in *Poinsettia'* by S Johnston

'Flow cytometric analysis of nuclear genome size – the problematic effect of cytosolic compounds' by J Loureiro

'Genome size estimation in herbarium vouchers by DAPI flow cytometry' by J Suda

Session III: Genome size and ecology

Session IV: New Technology

Including:

'NdYAG solid state laser in flow cytometry' by M Steinberg

Session V: Genome size and reproductive mode

Including:

'Genome size and reproductive mode' by D Albach

'Reproductive mode and genome size in *Hypochaeris'* by C König

'Genome size and apomixis' by P Ozias-Akins

Session VI: Terminology

Including:

'Identifying any problem(s) with terminology and seeking a solution' by J Greilhuber

Session VII: Conservation and genome size

Including:
'Genome size, conservations genetics – problems and solution?' by M Fay

Session VIII: Evolution, species diversity and molecular mechanisms

Including:
'Evolution of genome size among *Gossypium* species' by C Grover
'Genome size variation in the endemic sagebrushes and their allies (*Artemisia*)' by S Garcia

The future and closing remarks

SESSION 1: 'Data; progress since the last meeting in relation to targets set

A key aim of the session was to review progress since the 2003 workshop in relation to targets set in 2003.

A. Progress since 2003

(i) Angiosperms (reviewed by MD Bennett, Royal Botanic Gardens, Kew, UK)

Within Key recommendation 1 of the Second Plant Genome Size Workshop a target to estimate first C-values for the next 1% of angiosperm species was set, and within this to achieve 75 % familial representation (i.e. an additional c. 114 families) and 10% generic representation (i.e. an additional c. 400 genera).

Analysis of available data showed that

(i) since the 2003 workshop first C-values for > 850 species were now known, and
(ii) the mean number of estimates published or communicated per year was currently at a record level (c. 290/year).

Bennett concluded that at the species level, if output continued at the current rate, the five-year total since 2003 should reach c. 1500 to 1700 first estimates for species, representing c. 60 – 70% of the 2003 target.

Progress towards achieving 75% familial representation by 2008/9 was less good. Available data showed that first C-values for only 25 new families were currently available. At this rate it was predicted that C-values for only 50 additional families would be added representing just 44% (i.e. 50/114) of the target set.

(ii) Gymnosperms (reviewed by BG Murray, University of Auckland, New Zealand)

While no targets were set at the 2003 meeting (as gymnosperms are currently the plant group with the best representation of C-values) Murray reviewed new data available since 2003. In total 85 new measurements were published, of which 39 were first estimates for species. However, he noted that these did not include data for genera not currently represented in the Gymnosperm DNA C-values database so gaps for data remained.

Genera with no C-value data are listed below with the number of species for each genus given in brackets.

CYCADS

- Zamiaceae: *Ceratozamia* (10), *Chigua* (2), *Dioon*, (10), *Lepidozamia*, (2), *Macrozamia* (14), *Microcycas* (1).

CONIFERS

- Cephalotaxaceae: *Amenotaxus* (5).

- Cupressaceae: *Actinostrobus* (3)*, Austrocedrus* (1)*, Diselma* (1)*, Fitzroya* (1), *Neocallitropsis* (14)*, Pilgerodendron* (1)*, Papuacedrus* (2).
- Pinaceae: *Cathaya* (1)*, Hesperopeuce* (1)*, Keteleeria* (3-7)*, Nothotsuga* (1)*, Pseudolarix* (1).
- Podocarpaceae: *Acmopyle* (2)*, Afrocarpus* (3)*, Microcachrys* (1)*, Microstrobos* (2)*, Nageia* (5)*, Parasitaxus* (1) (= parasitic genus)*, Retrophyllum (5), Saxegothaea* (1)*, Sundacarpus* (1).
- Taxaceae: *Austrotaxus* (1), *Pseudotaxus* (1)*, Torreya* (6).

(iii) Pteridophytes (reviewed by IJ Leitch, Royal Botanic Gardens, Kew, UK)

At the 2003 workshop a target of estimating first C-values for 100 pteridophytes was made, with particular emphasis on leptosporangiate ferns (the most diverse group of land plants after angiosperms). However, only two papers containing C-values for just 16 species had been published since 2003 and it was concluded that progress towards the 2003 target had been poor. In helping to reach the target a list of leptosproangiate fern families which are still without a single C-value estimate are given below followed by the number of genera and species in each family.

- **'Lower' leptosporangiate families with no C-value data**
  - Anemiaceae (1/100)
  - Gleicheniaceae (6/125)
  -
- **Higher' leptosporangiate families with no C-value data**
  - Blechnaceae (9/200)
  - Grammitidaceae (20/600)
  - Lindaeaceae (6/200)
  - Thelypteridaceae (5-30/1000)

(iv) Bryophytes (reviewed by J Greilhuber, Institute of Botany, University of Vienna, Vienna)

Greilhuber reported the following progress in bryophyte C-value data.

(i) Since the 2003 meeting a reasonably correct genome size of 1C = 511 Mb for the moss *Physcomitrella patens* (used in plant functional genomic studies), has now been published (Schween *et al.*, 2003).

(ii) His group had recently estimated genome sizes for some liverworts and a hornwort although these are currently unpublished except in abstracts for the 11th Meeting of the Austrian Botanists (Vienna, 2004). For three liverwort species (*Riccia duplex*, *Pellia endiviifolia* and *Plagiochila asplenioides*) both haploid and diploid cytotypes of each species were identified, highlighting the importance of obtaining a reliable chromosome count for each accession studied.

(iii) The range of C-values in bryophytes has been extended from 12-fold to 73-fold following his group's measurements of 1C = 85 Mb in the hornwort *Anthoceros agrestis* (the smallest reported for any bryophyte to date) and 1C = 6,289 Mb for the liverwort *Mylia taylorii* (the largest genome size for any bryophyte so far reported, although no chromosome count has yet been made, its haploid status has been confirmed).

In summary, while the above data contribute to knowledge of genome sizes in bryophytes, progress has generally been poor and C-value data for bryophytes particularly in the liverworts and hornworts remains very low. Further, despite the Key recommendation 'To estimate C-values in species from the tropics, Southern hemisphere and rare taxa in the European flora' there are still no new data for species in these geographical regions.

B: Update of Plant DNA C-values database (reviewed by IJ Leitch, Royal Botanic Gardens, Kew, UK)

Leitch noted that since the 2003 workshop there had been a new release of the Plant DNA C-values database (release 3.0, December 2004) with a 23% increase in amount of data. This included the addition of the Algal DNA C-values database which currently contains data for 253 algal species.

The following table summarizes the progress made.

Group	Second Plant Genome Size Workshop, Sept. 2003	XII International Botanical Congress workshop, July 2005
	Data in release 2.0 of the Plant DNA C-values database	Data in release 3.0 of the Plant DNA C-values database
Algae	0	253
Bryophytes	171	176
Pteridophytes	82	87
Gymnosperms	181	207
Angiosperms	3493	4119
Total	**3927**	**4842**

Future plans for the database were outlined, including the addition of 308 first C-values arising out of the publication of Zonneveld, Leitch and Bennett (2005).

Session II: Best practice – the effects of cytosolic compounds

Including;
'Anthocyanins in *Poinsettia'* by S Johnston (Texas A & M University, Texas, USA)
'Flow cytometric analysis of nuclear genome size – the problematic effect of cytosolic compounds' by J. Loureiro (University of Aveiro, Portugal)
'Genome size estimation in herbarium vouchers by DAPI flow cytometry' by J Suda (Charles University, Prague, Czech Republic)

The second session addressed the effect that certain cytosolic compounds can have on the accuracy of C-value estimates obtained by flow cytometry. Two short talks presented by Johnston and Loureiro highlighted the scope of the problem.

Johnston described the dramatic effect that the anthocyanin, cyanidin-3-rutinoside can have on C-value estimations based on work in poinsettia (*Euphorbia pulcherrima*; Bennett, Price and Johnston, in prep.). The main conclusions arising from the work included:

(i) Avoid use of calibration standard plant material with red coloration
(ii) Cultivate calibration standards in environments expected to minimize pigmentation
(iii) Use plant tissues which produce little pigmentation.

Loureiro outlined the effects of three cytosolic compounds (caffeine, tannic acid and gallic acid) on nuclei isolated from *Pisum sativum* using four of the most popular isolation buffers

(LB01, Galbraith's buffer, Otto butter and Tris-$MgCl_2$). The quality of the flow histograms was shown to be effected by:

(i) The cytosolic compound and its concentration
(ii) The isolation buffer used
(iii) The incubation time of material in isolation buffer.

Clearly these and other studies are essential for clarifying the extent and scope of the problem cytosolic compounds can have on DNA C-value estimations made with flow cytometry so that reliable procedures for analyzing DNA amounts can be determined.

Suda discussed his work investigating the possibility of obtaining genome size and ploidy estimations from herbarium material using DAPI flow cytometry. The effects of different drying methods, storage conditions and age of herbarium vouchers (1-36 months) on the quality of the flow histogram were tested using four cytotypes of *Vaccinium* subgenus *Oxycoccus* (2x, 4x, 5x and 6x) as a model. While the mode of desiccation had little impact on histogram quality, material stored in a deep freezer yielded significantly lower CVs than samples stored at room temperature. Subsequent investigations of 20-month old herbarium vouchers gave reproducible signals in 43 out of 60 vascular plants (pteridophytes, gymnosperms and angiosperms). While the results demonstrated the feasibility of estimating ploidy level from herbarium vouchers, when the fluorochrome propidium iodide was used, the quality of the peaks was too poor to estimate absolute genome size.

The results of this study will be published in the following paper: Suda J and Travnicek P. Reliable DNA ploidy determination in dehydrated tissues of vascular plants by DAPI flow cytometry – new prospects for plant research. Cytometry (in press).

Session III: Genome size and ecology (CK Knight, California Polytechnic State University, USA and B Vilhar, University of Ljubljana, Slovenia)

A discussion of the functional and ecological significance of genome size in ecology was led by Knight and Vilhar, raising the question 'Is genome size one of the attributes that define the fundamental ecological niche of a species?' Vilhar outlined the need for more fieldwork to provide information on genome size in a plant community context.

Knight stressed the need to view genome size data across broad environmental gradients to assess fully the interaction of genome size with various ecological parameters. He also discussed the potential to join up genome size data with plant functional traits using some of the databases on plant growth etc. currently available on the web.

Session IV: New Technology

Including:
'Nd:YAG solid state laser in flow cytometry' by M Steinberg (Partec, Germany)

Steinberg provided an overview of a novel solid state crystal laser (Nd:YAG) developed by Partec and its application to genome size measurements in plants using propidium iodide based flow cytometry. The Nd:YAG 100 mWatt laser has the advantage that it provides light at 532 nm which is close to the optimal excitation wavelength for propium iodide, in contrast with most lasers which emit light at 488 nm (suboptimal for propidium iodide). The resulting laser is thus more sensitive and has lower CVs. Other advantages include the long life (c. 5000 hours) of the laser and its compact size, making it feasible to incorporate it into a small, portable machine (CyFlow®SL – see http://www.partec.de/products/cyflow.html).

Session V: Genome size and reproductive mode

Including:

'Genome size and reproductive mode' by D Albach (University of Mainz, Germany)
'Reproductive mode and genome size in *Hypochaeris'* by C König (Institute of Botany, Vienna)
'Genome size and apomixis' by P Ozias-Akins (University of Georgia, USA)

The complex interrelationship between reproductive mode (selfing versus outbreeding), life cycle type (annual versus perennial) and genome size was outlined by Albach. While studies such as those in *Veronica* (Albach and Greilhuber, 2004), *Microseris* (Price *et al.*, 1981) and *Lathryus* (Rees and Hazarika, 1969) suggested some links, the limited number of examples prevent broad conclusions being drawn. Albach concluded that further case studies are needed which had information on genome size, life cycle type, breeding type, phylogenetic information and dense taxonomic sampling.

Two further talks provided case examples. König presented data on genome size evolution in *Hypochaeris* noting that in South American species the inbreeders had smaller monoploid genomes than outbreeders. Ozian-Akins then discussed the relationship between genome size and apomixis noting that the majority of apomicts were polyploid but that it was currently unclear whether polyploidy was a necessity for or a consequence of apomixis.

Interestingly in a study of apomixes and genome size in *Hypericum* the apomictic species had significantly larger genomes compared with sexuals (Matzk *et al.*, 2003). While in some sections this was solely due to polyploidy (their monoploid genome sizes were similar to sexual species), in one section the monoploid genome sizes were larger than those of sexual species indicating an increase in genome size. Whether this was a consequence or cause of asexual seed formation, if any was unclear. However, it was suggested that the relationship between genome size and apomixis may be a mechanistic one. While expansion of genome size through transposon activity is still an option for apomicts, the lack of meiosis in apomicts prevents loss of DNA through recombination mechanisms, suggesting they have no option but to get bigger.

Session VI: Terminology

Including:
'Identifying any problem(s) with terminology and seeking a solution' by J Greilhuber (Institute of Botany, University of Vienna, Vienna)

Greilhuber outlined some of the exceptional cases where the terms C-value and Cx-value are inadequate and proposed a detailed terminology which was designed to deal with all cases (Greilhuber *et al*. in preparation). In the discussion which followed it was felt that while researchers need to be aware of such exceptions when citing DNA amounts, the proposed terminological approach cannot and should not be mandatory.

Session VII: Conservation and genome size

Including:
Genome size, conservations genetics – problems and solution? by M. Fay (Royal Botanic Gardens, Kew, UK)

The relationship between genome size and threat of extinction was reviewed by Fay including the work of Vinogradov (2003) who noted that species with large genomes appeared to be at a greater risk of extinction compared with small-genomed species. Fay went on to discuss how some of the techniques used to assess genetic diversity in plants at risk of extinction (e.g. microsatellites, AFLPs etc) were sensitive to genome size, with large-genomed species being more difficult to analyse (Fay *et al.*, 2005).

Session VIII: Evolution, species diversity and molecular mechanisms

Including:
'Evolution of genome size among *Gossypium* species' by C. Grover (Iowa State University, USA)
'Genome size variation in the endemic sagebrushes and their allies (*Artemisia*)' by S Garcia (University of Barcelona, Spain)

Two talks discussed mechanisms and patterns of genome size change in particular species.

Comparative sequencing of large homologous regions of genomic DNA from organisms that differ in genome size provides an opportunity to reveal mechanisms of genome size change at the DNA sequence level. Taking this approach Grover presented a comparative analysis of two BACs corresponding to homologous regions of genomic DNA from the A and D genomes comprising polyploid cotton *Gossypium hirsutum* (Grover et al., 2004). Despite parental genome donors differing two-fold in genome size since they speciated c. 5-10 mya, comparative sequence analysis revealed a remarkable conservation of gene and intergenic organization. Grover concluded that the evolutionary forces and molecular mechanisms responsible for rapid intergenic divergence reported in other plant systems do not operate similarly in the regions analyzed of *Gossypium*. The data indicate that mechanisms leading to genome size differences do not affect all genomic constituents equally.

In contrast Garcia reported how limited genome size variation at the inter- and intra-specific level in 30 species of *Artemisia* endemic to North America revealed a recent diversification of this group of the genus, supporting previous morphological, chemical and karyological affinities.

The future and closing remarks

The meeting was concluded by some final remarks by Mike Bennett.

Thanks were given to Johann Greilhuber and Eva Temsch for hosting and organizing the workshop, and for Partec who provided sponsorship.

While no new targets were set as this was an interim meeting, overall it provided a valuable opportunity to take stock of the targets set in 2003. It was clear that while some good progress had been made, renewed efforts would be needed if all the 2003 targets were to be met within the timeframe.

Interest in genome size is clearly substantial and increasing as judged by the number attending the main symposium entitled 'Plant Genome Size – its evolution and significance' (session number 2.5) held on Monday 18th July and the workshop. We look forward to future discussions of the GSI group possibly at RBG Kew to monitor progress against the five year targets set in 2003 and to celebrate Kew's 250th anniversary.

References

Albach DC, Greilhuber J. 2004. Genome size variation and evolution in *Veronica*. *Annals of Botany* **94:** 897-911.

Fay MF, Cowan RS, Leitch IJ. 2005. The effects of nuclear DNA content (C-value) on the quality and utility of AFLP fingerprints. *Annals of Botany* **95:** 237-246.

Grover CE, Kim HR, Wing RA, Paterson AH, Wendel JF. 2004. Incongruent patterns of local and global genome size evolution in cotton. *Genome Research* **14:** 1474-1482.

Matzk F, Hammer K, Schubert I. 2003. Coevolution of apomixis and genome size within the genus *Hypericum*. *Sexual Plant Reproduction* **16:** 51-58.

Price HJ, Chambers KL, Bachmann K. 1981. Geographic and ecological distribution of genomic DNA content variation in *Microseris douglasii* (Asteraceae). *Botanical Gazette* **142:** 415-426.

Rees H, Hazarika MH. 1969. Chromosome evolution in *Lathyrus*. *Chromosomes Today* **2:** 158-165.

Schween G, Gorr G, Hohe A, Reski R. 2003. Unique tissue-specific cell cycle in *Physcomitrella*. *Plant Biology* **5:** 50-58.

Vinogradov AE. 2003. Selfish DNA is maladaptive: evidence from the plant Red List. *Trends in Genetics* **19:** 609-614.

Zonneveld BJM, Leitch IJ, Bennett MD. 2005. First nuclear DNA amounts in more than 300 angiosperms. *Annals of Botany* **96:** 229-244.