First genome size estimations for some eudicot families and genera

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Abstract
First genome size estimations for some eudicot families and genera.- Genome size diversity in angiosperms varies roughly 2400-fold, although approximately 45% of angiosperm families lack a single genome size estimation, and therefore, this range could be enlarged. To contribute completing family and genera representation, DNA C-Values are here provided for 19 species from 16 eudicot families, including first values for 6 families, 14 genera and 17 species. The sample of species studied is very diverse, including herbs, weeds, vines, shrubs and trees. Data are discussed regarding previous genome size estimates of closely related species or genera, if any, their chromosome number, growth form or invasive behaviour. The present research contributes approximately 1.5% new values for previously unreported angiosperm families, being the current coverage around 55% of angiosperm families, according to the Plant DNA C-Values Database.

Key words: angiosperm families; C-value; eudicots; flow cytometry; genome size; nuclear DNA amounts.

Resumen
Primeras estimaciones del tamaño del genoma para algunas familias y géneros eudicotiledóneos.- La diversidad del tamaño del genoma en angiospermas es muy amplia, siendo el valor más elevado aproximadamente unas 2400 veces superior al más pequeño. Sin embargo, cerca del 45% de las familias no presentan ni una sola estimación, por lo que el rango real podría ser ampliado. Para contribuir a completar la representación de familias y géneros de angiospermas, este estudio contribuye con valores C para 19 especies de 16 familias de eudicotiledóneas, incluyendo los primeros valores para 6 familias, 14 géneros y 17 especies. La muestra estudiada es muy diversa, e incluye hierbas, malezas, enredaderas, arbustos y árboles. Se discuten los resultados en función de estimaciones previas del tamaño del genoma de especies o géneros estrechamente relacionados, del número de cromosomas, la forma de crecimiento o el comportamiento invasor de las especies analizadas. El presente estudio contribuye aproximadamente en un 1,5% de nuevos valores para familias de angiospermas no estudiadas previamente, de las que actualmente existe información para el 55%, según la base de datos de valores C en plantas.

Palabras clave: cantidades de ADN nuclear; citometría de flujo; eudicotiledóneas; familias de angiospermas; tamaño del genoma; valor C.
INTRODUCTION

The C-Value, i.e. the amount of DNA in the unrepli-
cated genome of an individual (Swift, 1950) is an
essential biological character, considered constant
(hence the “C” of the term) for a particular species.
Genome size is of interest in many research fields
and relationships between C-Values and cytological
traits, ecology, life cycle or distribution, among
others, have been discussed in hundreds of scienti-
ﬁc works, most of them from the last two decades
(Ohri et al., 2004; Ohri 2005; Leitch et al., 2010).

Understanding the variation of nuclear DNA
amounts between plant species is an essential step
toward a better knowledge of their evolution and
diversiﬁcation. Genome size in angiosperms varies
~2400-fold, ranging from the carnivorous plant
Genlisea margaretae Hutch. (Lentibulariaceae)
with 1C = 0.0648 pg of DNA (Greilhuber et al.,
2006; Chase et al., 2009) to the monocot Paris
japonica (Franch. & Sav.) Franch. (Melanthiaceae,
1C = 152.23 pg; Pellicer et al., 2010). This huge
variation has interested many researchers, and it
is usually attributed to changes in the proportion
of non-coding DNA such as repetitive sequences,
transposable elements or due to the extent of genome
duplication processes – polyploidization (Leitch &
Hanson, 2002). This large angiosperm genome size
range is also contrasting with the limited 16-fold of
the gymnosperms or the narrow 12-fold variation of
bryophytes (Plant DNA C-values database).

According to Leitch et al. (1998) and Soltis et al.
(2003), genome sizes can be assigned to a series of
distinct categories: “very small” (1C-Values ≤1.4 pg),
“small” (> 1.4 to ≤ 3.5 pg), “intermediate” (> 3.5 to
< 14.0 pg), “large” (≥ 14 to < 35 pg) and “very large”
(≥ 35 pg). Nevertheless, the vast majority of angios-
perm species assessed up to now present “small”
or “very small” values, and “very large” ones are
exceptional. Regarding small genome sizes, Leitch
et al. (2005), superimposing the available C-Value
data onto an angiosperm phylogeny, concluded that
the ancestral genome size of all angiosperms was
reconstructed as “very small” as well as that of most
major clades within angiosperms.

Obtaining C-Value data

Given the apparent interest that data on nuclear
DNA amounts raises, particularly in plant research,
efforts have been done to compile C-Values, ei-
ther in the form of species lists (Marie & Brown,
1993; Bennett & Leitch, 1995, 1997; Bennett et al.,
2000; Zonneveld et al. 2005; Siljak-Yakovlev
et al., 2010) or databases. Several databases are
currently freely available on the internet. They
pool published plant genome size estimations (the
“Plant DNA C-Value Database”, Bennett & Leitch,
2010), focused on ﬂow cytometric measurements of
C-Values (Loureiro et al., 2010) or on a parti-
cular plant family (the upcoming “Genome Size in
the Asteraceae Database”, Garnatje et al., 2010).

Also, scientiﬁc meetings (ﬁrst and second Plant
Genome Size Workshop and Discussion Meetings,
held at the Royal Botanic Gardens, Kew, in 1997
and 2003 respectively) and the workshop “Geno-
me size: a research discipline in development”
(XVII International Botanical Congress, 2005,
Vienna) have been useful in identifying major
gaps and recommending priorities in genome size
assessments. One of these targets was to produce
ﬁrst C-Values for an additional 1% of angiosperm
species, achieving with this at least 75% familial
representation by 2009 (Bennett & Leitch, 2005),
although updates in this sense are yet to come.

Following these guidelines, a subsequent paper
(Hanson et al., 2005) contributed with ﬁrst data for
20 previously unrepresented families, increasing
angiosperm familial representation to 55%.

The present paper addresses some of the key
recommendations exposed at the last discussion
meetings concerning angiosperm families and ge-
nera representation, by increasing the number of
families reported and complementing other scarcely
previously reported ones, according to available
databases and other published research.

MATERIALS AND METHODS

Plant material

Table 1 lists the 19 species from different fa-
milies and genera not or scarcely represented
in the Plant DNA C-Values Database (Bennett
& Leitch, 2010), together with their geographic
origin, growth form, life cycle, chromosome num-
ber (when available), genome size with standard
deviation and calibration standard used for ﬂow
cytometric assessments. Plants were obtained

Table 1. Family, geographical region of origin, habit, life cycle, chromosome number (2n), nuclear DNA amounts (2C) in pg and standard deviation (SD), 2C in Mbp (978 Mbp corresponding to 1pg; Doležel et al., 2003), 1C in pg and calibration standard used to estimate DNA C-Values, for 19 species studied in the present work. (*) First genome size values for these families; (†) previous genome size estimations available for these genera and species. Data on chromosome numbers from the Index to Plant Chromosome Numbers (IPCN) from the Missouri Botanical Garden (Goldblatt & Johnson, 2010) and from Hanson et al. (2005).

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Species</th>
<th>Origin</th>
<th>Habit</th>
<th>Life cycle</th>
<th>2n</th>
<th>C(pg) (SD)</th>
<th>2C (Mbp)</th>
<th>1C (pg)</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asterids</td>
<td>Martyniaceae</td>
<td>Proboscidea louisiana (Mill.) Wooton &amp; Standl.</td>
<td>North America</td>
<td>herbaceous</td>
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<td>0.97 (0.03)</td>
<td>948.66</td>
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<td>Paulowniaceae</td>
<td>Paulownia tomentosa (Thunb.) Steud.</td>
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<td>perennial</td>
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<td>1.2 (0.00)</td>
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<td>Escalloniaceae</td>
<td>Escallonia† pulverulenta Pers.</td>
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<td>perennial/evergreen</td>
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<td>1.13 (0.02)</td>
<td>1105.1</td>
<td>0.57</td>
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<td>Basellaceae</td>
<td>Anredera cordifolia (Ten.) Steenis</td>
<td>South America</td>
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<td>Molluginaceae</td>
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<td>herbaceous</td>
<td>annual or biennial</td>
<td>54</td>
<td>0.98 (0.03)</td>
<td>958.44</td>
<td>0.49</td>
<td>Pisum</td>
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<td>perennial/deciduous</td>
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<td>perennial/deciduous</td>
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<td>Origin</td>
<td>Habit</td>
<td>Life cycle</td>
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<td>2C (Mbp)</td>
<td>1C (pg)</td>
<td>Standard</td>
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<td>Brassicales</td>
<td>Gyrostemonaceae</td>
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<td>0.75</td>
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<td></td>
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<td>Distylium myricoides</td>
<td>East and South Asia</td>
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<td>perennial/evergreen</td>
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<td>2.12 (0.04)</td>
<td>2073.4</td>
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<td>perennial/deciduous</td>
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<td>perennial/deciduous</td>
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<td>1.53 (0.06)</td>
<td>1496.3</td>
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<td>Corylopsis coreana</td>
<td>Buthan to Japan</td>
<td>woody/shrub</td>
<td>perennial/deciduous</td>
<td>-</td>
<td>3.93 (0.02)</td>
<td>3843.5</td>
<td>1.97</td>
<td>Pism</td>
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<td>Corylopsis sinensis</td>
<td>Buthan to Japan</td>
<td>woody/shrub</td>
<td>perennial/deciduous</td>
<td>-</td>
<td>4.62 (0.04)</td>
<td>4518.4</td>
<td>2.31</td>
<td>Petunia</td>
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</table>
from the Jardí Botànic de Barcelona or through Index Seminum of different international institutions, and some of them were grown at the greenhouses of the Institut Botànic de Barcelona and of the Facultat de Farmàcia de la Universitat de Barcelona. Young leaves used for flow cytometry assays were taken from plants cultivated in pots. Seeds of Pisum sativum L. ‘Express Long’ and an adult Petunia hybrid L. ‘PxC6’, both used as internal standards for flow cytometric measurements (Marie & Brown, 1993), were obtained from the Institut des Sciences du Végétal (CNRS, Gif-sur-Yvette, France).

The selected sample is very diverse (Fig. 1), comprising from annuals to perennials, from herbs to trees, of a wide geographical range and with a variety of uses. There are plants with economic importance, as some with medicinal properties (Thymelaea hirsuta Endl., Quillaja saponaria Poir., or Corrigiola littoralis L.), others used as ornamental (the deciduous trees Cercidiphyllum japonicum Siebold & Zucc., Paulownia tomentosa (Thunb.) Steud. and Tamarix L. species), and some ornamentals exhibiting an invasive behaviour (such as Anredera cordifolia (Ten.) Steenis, Ochna serrulata Walp. and the previously mentioned Paulownia).

Flow cytometry

The DNA 2C values of the tested species were estimated using flow cytometry. Young healthy leaf tissue of five individuals for each studied species was chopped in 600 µl of Galbraith’s isolation buffer (Galbraith et al., 1983) with a razor blade, together with the chosen internal standard and supplemented with 100 µg/ml 1 ribonuclease A (RNase A; Boehringer, Meylan, France); two samples per individual were independently extracted. The suspension of nuclei in the isolation buffer was filtered through a nylon mesh with a pore size of 70 µm and stained for 20 min with 36 µl of propidium iodide 1 mg/ml (Sigma-Aldrich Química, Alcobendas, Madrid) to a final concentration of 60 µg/ml. Tubes were kept on ice during staining and then left at room temperature until measurement. The flow cytometer used was an Epics XL (Coulter Corporation, Hialeah, Florida, USA.), at the Serveis Cientificotècnics of the Universitat de Barcelona. More details about the method followed are described in Garcia et al. (2008).

RESULTS AND DISCUSSION

The present study contributes data for 19 species from 16 eudicot families. Genome size estimations are new for families Cercidiphyllaceae, Elaeagnaceae, Gyrostemonaceae, Martyniaceae, Molluginaceae and Paulowniaceae. Besides, genome sizes for 14 new genera and 17 new species are here reported for the first time. Table 1 lists nuclear DNA amounts in the 19 taxa studied. Values range approximately 6-fold: the lowest 1C value in the plants studied is for the tree Quillaja saponaria (0.42 pg) and the highest for the creeper Hibbertia scandens (Willd.) Dryand. (2.64 pg). Most genome sizes of the species analysed (65%) fall within the category “very small”, and the remaining are considered “small” (following Leitch et al., 1998 and Soltis et al., 2003). Although, as mentioned, genome size range across angiosperms is very large, a histogram based on Plant DNA C-Values data extracted from the Plant DNA C-Values Database (Bennett & Leitch, 2010) and including last results from Pellicer et al. (2010) shows the distribution of angiosperm 1C values (Fig. 2), revealing that most species have very small genomes (the histogram is strongly skewed to left). However, while 99.5% of all eudicots have genomes smaller than 25 pg, there is a 10% of monocots with genomes larger than this cipher (Leitch et al., 2010). Lineages with such large genomes are phylogenetically restricted not only to the monocots but also to the Santalales, pointing that very large genomes have independently evolved different times during the evolution of angiosperms (Leitch et al., 1998; Soltis et al., 2003).

Species and genera previously reported

Data obtained for the species Polygala vazredae Costa is consistent with the 1C = 1.35 pg previously reported by Castro et al. (2007) also with flow cytometry. There is only another species from this genus (and from this family, according to the Plant DNA C-Values Database, Bennett & Leitch, 2010), P. calcarea F. W. Schultz, whose genome size has been assessed twice independently: 1C = 0.49 pg (Castro et al., 2007) and 1C = 0.43 pg (Hanson et al., 2005). Chromosome numbers for these species are 2n = 28 and 2n = ca. 30, respectively.

The ornamental shrub Stachyurus praecox Siebold & Zucc. was assessed for genome size and
chromosome number for the first time by Hanson et al. (2005), who obtained $1C = 0.35$ pg and $2n = 24$. Our result ($1C = 0.44$ pg) is approximately 25% larger than theirs. This difference exceeds the accepted range of variation within a species, as discussed in Garcia et al. (2006). Both genome size estimations

*Figure 1. Some of the species here studied: (A), Ochna serrulata; (B), Cercidiphyllum japonicum [picture by Jean-Pol Grandmont]; (C), Anredera cordifolia; (D), Corrigiola littoralis; (E), Stachyurus praecox [picture from home-and-garden.webshots.com]; (F), Corylopsis gotoana [picture by Krzysztof Zianerk]; (G), Hibbertia scandens; (H), Quillaja saponaria; (I), Paulownia tomentosa. All pictures except E taken from Wikimedia commons under the terms of GNU free documentation license (different versions) or under the Creative Commons Attribution-Share Alike license (different versions).*
have been performed with different techniques (Hanson’s with Feulgen microdensitometry and the present one with flow cytometry), and using different calibration standards (*Vigna radiata* (L.) R.Wilczek ‘Berken’, 2C = 1.06 pg, vs. *Petunia hybrida*, 2C = 2.85 pg), which could explain partly the difference between the measurements, although ideally they should be comparable.

The tamarisks had yet been represented, as for genome size data, by the species *Tamarix tetranandra* Szov. ex Bunge with a 1C value of 1.55 pg (also using flow cytometry with propidium iodide stain; Zonneveld *et al.*, 2005), a value which is also consistent with our present data. To our knowledge, this is also the only genus of family Tamaricaceae for which genome size has been previously assessed.

The evergreen shrub *Escallonia pulverulenta* Pers. is similar to the tamarisks in that it is a species tolerant to maritime exposure. There are two previous genome size estimations for other *Escallonia* species: *E. rubra* Pers. presents a value fairly similar to the present one (1C = 0.42 pg) albeit being obtained by Feulgen microdensitometry (Hanson *et al.*, 2005) whereas *E. langleyensis* Vilm. & Bois C-Value (1C = 6.15 pg, Zonneveld *et al.*, 2005) is much larger. It is likely that the latter is a polyploid but we cannot ascertain this in the absence of chromosome counts.

**Chromosome number and genome size**

The scarce available published data on chromosome numbers for the studied species gathered from the Index to Plant Chromosome Numbers (IPCN) from the Missouri Botanical Garden (Goldblatt & Johnson, 2010) and from Hanson *et al.* (2005) does not allow raising a pattern related to genome size. However, it is to note that the species with more chromosomes are among those with smaller genomes. Although the sample of plants here studied is very heterogeneous and the data incomplete, this coincides with previous findings reporting relatively small genome sizes in species with high chromosome numbers (Murray *et al.*, 2005; Leitch *et al.*, 2010). However, an analysis carried out on angiosperm 1C values and chromosome numbers (using the Plant DNA C-Values database) did not reveal any significant relationship between both datasets.

![Figure 2. Histogram showing the distribution of angiosperm 1C values](https://example.com/figure2.png)

**Genome sizes in trees**

Trees are a polyphyletic assemblage (including angiosperm and gymnosperm species) but they share key characters such as great size, height and longevity features that explain their ecological success (Petit & Hampe, 2006). Tree species are found in all eudicot orders except in Geraniales and Gunnerales (Groover, 2005). All the tree species of our sample (Table 1) present invariably very small C-Values (from 1C = 0.42 pg to 1C = 0.99 pg), and also those considered as shrub-trees rank very low. This is consistent with low genome sizes found in most angiosperm trees (see some examples of such low amounts in Table 2), despite widespread paleopolyploidy and high chromosome numbers with respect to herbaceous angiosperms (Mehra, 1976; Ehrendorfer, 1982; Morawetz, 1986); in this sense Levin & Wilson (1976) had estimated that tree genera have a mean basic chromosome number of 13.1 whereas it is only 9.3 in herbaceous plants.

A study about the consequences on genome size of climate and growth form in plants (Ohri, 2005) concluded that, in an angiosperm sample, woody growth was characterized by a significantly smaller genome size compared with the herbaceous growth form. It was also found that woody monocots had larger genome sizes than eudicot ones, and that woody gymnosperms presented even larger genome sizes. Although the author did not find a direct explanation for the association of small genome
size with woody habit, others claim that the woody growth implies constraints on maximum nuclear size of the small cambial cells forming wood fibres (Stebbins, 1950; Khoshoo, 1962). In the same line, size of the small cambial cells forming wood fibres growth implies constraints on maximum nuclear size with woody habit, others claim that the woody habit is also significant in subgenus Pinus (Grotkopp et al., 2004). Garcia et al. (2008) proved the same pattern for the species of genus Artemisia listed as weeds in the US invasive plants list (USDA Natural resources conservation service: invasive and noxious weeds, 2010) which always presented significantly lower C-Values as compared with the non-invasive Artemisia. Questions are still open regarding how (and why) selection operates against high nuclear DNA amounts in groups as different as weeds and trees.

The weed Proboscidea louisiana is also a carnivorous (murderous) plant, as the slime that it secretes on its leaves is the trap of the insects that approach it. Genome size of carnivorous plants has also been studied given the particularity of such plants. A search in the Plant DNA C-Values Database (Bennett & Leitch, 2010) demonstrated that the tempo of genome size evolution was strongly influenced by growth form, suggesting that life history alone could impose constraints in the evolution of genome size; however they did not find a consistent pattern of woody species having smaller genome sizes in genera consisting of both woody and herbaceous species. Nevertheless, angiosperm trees are reported to have large effective population sizes (Petit & Hampe, 2006), which would make selection more efficient at removing deleterious mutations or excess DNA (Lynch, 2007), and this might explain reduced genome sizes.

We have also compared genome sizes of deciduous vs. evergreen woody eudicots of our sample, even considering that our sample is not representative. Values differed approximately 4-fold both in deciduous and evergreen plants, but the analysis showed a non-significant difference between both categories ($P = 0.3466$). As the present one, a previous study did not find a significant difference between genome sizes of deciduous and evergreen woody dicots, using a sample of 773 woody species (Ohri, 2005).
Progress towards completing family representation

This study follows the Angiosperm Phylogeny Group, APG III (2009) which recognizes 415 families. However, the number of families can vary with time, as new ones are created while some are divided or merged, based on new molecular or morphological data. These changes complicate establishing how many families are there, what is the proportion represented in the Plant DNA C-Values Database and therefore which new percentages actually contribute papers like the present one. Since 2001, the Royal Botanic Gardens, Kew, has been targeting families for which no previous C-Value data had been published, with the purpose of achieving 75% familial coverage by 2009 (Hanson et al., 2005), as stated. At present, genome size data is listed in the Plant DNA C-Values Database for 55% of angiosperm families; the present research increases this representation in 1.45%, awaiting for the next update of this database, whose release (including new data for around 1600 angiosperm species) is expected for 2010 (Plant DNA C-values database, 2010).

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