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DNA C-values in seven families fill phylogenetic gaps in the basal angiosperms

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The evolutionary significance of the *c*. 1000-fold range of DNA C-values in angiosperms (1C = *c*. 0.1–127.4 pg) has often attracted interest. A recent analysis, which superimposed available C-value data onto the angiosperm phylogeny, that placed Ceratophyllaceae as the most basal angiosperm family led to the conclusion that ancestral angiosperms were characterized by small genomes (defined as 1C ≤ 3.5 pg). However, with the recent increase in DNA sequence data and large-scale phylogenetic analyses, strong support is now provided for Amborellaceae and/or Nymphaeaceae as the most basal angiosperm families, followed by Austrobaileyales (comprising Schisandraceae, Trimeniaceae and Austrobaileyaceae). Together these five families comprise the ANITA grade. The remaining basal angiosperm families (Ceratophyllaceae, Chloranthaceae and magnoliids), together with monocotyledons and eudicotyledons, form a strongly supported clade. A survey showed that C-value data were scarce in the basal angiosperm families, especially the ANITA grade. The present paper addresses these phylogenetic gaps by providing C-value estimates for each family in ANITA, together with C-values for species in Chloranthaceae, Ceratophyllaceae and a previously unrepresented family in the magnoliids, the Winteraceae. © The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2002, **140**, 175–179.

ADDITIONAL KEYWORDS: Amborellaceae – ANITA – Austrobaileyaceae – Ceratophyllaceae – Chloranthaceae – DNA amount – evolution – genome size – magnoliids – Nymphaeaceae – phylogeny – Schisandraceae – Trimeniaceae – Winteraceae

INTRODUCTION

The amount of DNA in the unreplicated nuclear genome of an individual is referred to as its C-value (Swift, 1950) and in angiosperms this has been shown to vary over 1000-fold, ranging from *c*. 0.10 pg in *Aesculus hippocastanum* L. to 127.4 pg in *Fritillaria assyriaca* Baker (Bennett & Smith, 1976, 1991). Knowledge of C-values has continued to increase, with data now available for over 3500 different species corresponding to *c*. 1.4% of the angiosperm flora (Bennett & Leitch, 2001; Hanson *et al*., 2001a,b).

The origin and significance of this huge variation in C-values has often attracted interest (Stebbins, 1976; Cavalier-Smith, 1985a; Bennett, 1987, 1998) and there is general agreement that the differences can be attributed largely to changes in the proportion of non-coding, repetitive DNA sequences such as transposable elements (Flavell, 1988; Kubis, Schmidt & Heslop-Harrison, 1998) and the extent of genome duplication (Bennetzen, 2000; Sankoff, 2001). Indeed, recent studies are beginning to shed light on the mechanisms which can lead to both increases and decreases in DNA amount (Vicient *et al*., 1999; Kirik, Salomon & Puchta, 2000; Bensasson *et al*., 2001; Petrov, 2001).

In addition to this new understanding of how C-values may change, the evolutionary significance of the huge range of C-values has started to be evaluated. While earlier studies were sometimes flawed by the lack of a rigorous phylogenetic framework to underpin the analyses of the data (Stebbins, 1976; Price, 1988; Ohri, 1998), in more recent years evolutionary relationships between different families are increasingly being resolved through large-scale analyses. In 1998, Leitch, Chase & Bennett (1998) superimposed available C-value data (Bennett, Cox & Leitch, 1998) onto the angiosperm phylogenetic tree of Chase *et al*. (1993), which was based on DNA sequence

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information together with 252 non-molecular characters for 499 angiosperm species. Leitch *et al*. (1998) concluded that ancestral angiosperms were characterized by the possession of small genomes (defined as 1C ≤ 3.5 pg).

Since the analysis of Leitch *et al*. (1998), further DNA sequence data have increasingly resolved relationships between families and, in particular, amongst the earliest, most basal angiosperms (Qiu *et al*., 1999, 2000; Zanis *et al*., 2002). In contrast to the tree used by Leitch *et al*. (1998) that placed Ceratophyllaceae at the root of angiosperms, these recent studies have provided strong support for Amborellaceae and/or Nymphaeaceae as the most basal families that are sister to all other angiosperms (Zanis *et al*., 2002). The data have also revealed that the next diverging lineage is Austrobaileyales comprising Austrobaileyaceae, Trimeniaceae, and Schisandraceae (Angiosperm Phylogeny Group II, 2002). These five families have been described as the ANITA grade (Qiu *et al*., 1999). Initially, ANITA was defined as containing **A**morborellaceae, **N**ymphaeaceae and **I**lliciales-**T**rimeniaceae-**A**ustrobaileyaceae (Qiu *et al*., 1999). Subsequently, changes in taxonomy have led to a revision in the circumscription of some families (Angiosperm Phylogeny Group II, 2002). In the context of this work, Schisandraceae has been newly circumscribed to include Illiciaceae, therefore families comprising ANITA are now recognized as Amborellaceae, Nymphaeaceae, Trimeniaceae, Schisandraceae and Austrobaileyaceae. The present paper follows these new circumscriptions. All angiosperms outside ANITA form a strongly supported clade comprising the other basal angiosperms (Ceratophyllaceae, Chloranthaceae and magnoliids), together with the monocotyledons and eudicotyledons.

The analysis of Leitch *et al*. (1998) included only three C-values in the ANITA grade, no data for Ceratophyllaceae nor Chloranthaceae, and only 29 values in the magnoliids. The present paper aims to address these phylogenetic gaps by providing new C-value estimates for representatives of seven families, thereby completing familial coverage in ANITA, by providing C-value estimates for Chloranthaceae and Ceratophyllaceae, and adding an estimate for a previously unrepresented family in the magnoliid clade, the Winteraceae.

MATERIAL AND METHODS

Plant material

Taxonomic details of the seven species studied are given in Table 1, together with distribution data. Representative vouchers are lodged at the Royal Botanic Gardens, Kew Herbarium.

Estimation of DNA amounts

Either flow cytometry (Obermayer *et al*., 2002) or Feulgen microdensitometry (following methods described in Hanson *et al*., 2001a) was used to estimate the DNA C-values. Details of the calibration standard and method used for each species are given in Table 1.

Table 1. The seven angiosperm taxa studied in the present work, together with their distribution, cytology number, method used to estimate C-value and calibration standard used

Taxon	Distribution of genus	Cytology. no.	Method used to estimate C-value*	Calibration standard used†
Amborella trichopoda Baill.	New Caledonia, USA	01–174	FC: PI	*Oryza*
Austrobaileyales				
Austrobaileya scandens C.T. White	Queensland, Australia	02–76	FC: PI	*Pisum*
Piptocalyx moorei Oliver	Central Malaysia to Marquesas & Samoa	02–133	FC: PI	*Pisum*
Schisandra rubriflora‡	East Asia and East and North America	02–78	FC: PI	*Pisum*
Ceratophyllum demersum L.	Widespread	02–118	Fe	*Vigna*
Chloranthus spicatus Mak.	Indonesia to Malaysia & E. Asia	01–215	FC: PI	*Pisum*
MAGNOLIIDS				
Canellales				
Drimys vickeriana A.C. Smith	Trop. America, central Malaysia to Tahiti	02–70	FC: PI	*Oryza*

*Fe, Feulgen microdensitometry; FC: PI, flow cytometry using the fluorochrome propidium iodide.
†*Vigna* = *Vigna radiata* cv. Berken, 4C = 2.12 pg; *Oryza* = *Oryza sativa* cv. IR36, 4C = 2.02 pg; *Pisum* = *Pisum sativum* cv. Minerva Maple, 4C = 19.46 pg.
‡Authority unknown or unclear to the present authors.

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Chromosome counts

Where possible, chromosome counts were obtained from root tip squashes prepared using a standard Feulgen-stained squash technique as described previously in Hanson *et al*. (2001a). However, in many cases this was not possible, as only leaf material was available for analysis by flow cytometry.

RESULTS AND DISCUSSION

C-values in the most basal angiosperm lineage – the ANITA grade

Table 2 lists DNA C-value estimates for four species in the ANITA grade, including representatives of Amborellaceae, Austrobaileyaceae and Trimeniaceae in which C-values were previously unknown. Thus a C-value estimate is now available for at least one species in all five families comprising ANITA.

The smallest C-value estimated in ANITA was 1C = 0.89 pg for *Amborella trichopoda* in Amborellaceae, a monotypic family. Interestingly, it is similar to C-values reported previously for two species in Nymphaeaceae; *Nymphaea caerula*, 1C = 0.6 pg (Bharathan, Lambert & Galbraith, 1994) and *N. lotus*, 1C = 1.1 pg (Bennett & Leitch, 1995). Thus the two most basal angiosperm families (i.e. Amborellaceae and Nymphaeaceae) both have very small C-values (Leitch *et al*., 1998). Although no chromosome counts are available, the genome size for these species in the most basal angiosperm families cannot be greater than the 1C-values listed above because an estimated genome size is calculated from the 2C-value divided by ploidy level. If the material studied was polyploid rather than diploid, this would result in even smaller genome sizes in these species. Thus while further work will seek to obtain chromosome counts for the material studied, the C-value data given here are still informative.

Three new C-value estimates are reported for species in Austrobaileyales (comprising Austrobaileyaceae, Trimeniaceae and Schisandraceae). Those for *Austrobaileya scandens* (Austrobaileyaceae) of 1C = 9.52 pg and *Schisandra rubriflora* (Schisandraceae; 1C = 9.12 pg) are similar to estimates for three other Schisandraceae species reported recently by Morawetz and Samuel (pers. comm., listed in Bennett, Bhandol & Leitch (2000), i.e. *Kadsura longespicata*, 1C = 8.9 pg, *K. japonica*, 1C = 8.1 pg, and *K. coccinea*, 1C = 7.4 pg). In contrast, the 1C-value of 4.08 pg for *Piptocalyx moorei* (Trimeniaceae) is more similar to the estimate of 1C = 3.4 pg for *Illicium anisatum* (Schisandraceae) reported by Nagl, Habermann & Fusenig (1977). Overall, the C-value estimates in Austrobaileyales ranged nearly three-fold, from 1C = 3.4 to 9.52 pg.

C-values in Ceratophyllaceae and Chloranthaceae

The phylogenetic affinities of Ceratophyllaceae and Chloranthaceae still await determination and are currently controversial (reviewed in Qiu *et al*., 2000). Some analyses have suggested that Ceratophyllaceae might be sister to the monocotyledons. It is therefore of interest that the small value of 1C = 0.69 pg reported here for *Ceratophyllum demersum* is similar to those for the two species comprising Acoraceae

Table 2. DNA C-values for seven angiosperm species studied together with chromosome numbers (2*n*) obtained in the present work and counts taken from Goldblatt & Johnson (2002)

Taxon	Family*	2*n*	Published 2*n*	DNA amount 1C (Mbp)†	1C (pg)	2C (pg)	4C (pg) ± SD
Amborella trichopoda	Amborellaceae	–	26	870	0.89	1.78	3.55 ± 0.02
Austrobaileyales							
Austrobaileya scandens	Austrobaileyaceae	–	44	9327	9.52	19.04	38.07 ± 0.78
Piptocalyx moorei	Trimeniaceae	–	16	3998	4.08	8.17	16.33 ± 0.11
Schisandra rubriflora	Schisandraceae	–	28‡	8938	9.12	18.24	36.48 ± 0.38
Ceratophyllum demersum	Ceratophyllaceae	*c*. 70	24, 48	674	0.69	1.38	2.75 ± 0.*26*
Chloranthus spicatus	Chloranthaceae	30	30	3528	3.60	7.20	14.39 ± 0.29
MAGNOLIIDS							
Canellales							
Drimys vickeriana	Winteraceae	–	90–91‡	1104	1.13	2.26	4.51 ± 0.06

*Family nomenclature follows Angiosperm Phylogeny Group II (2002)

†1 pg = 980 megabase pairs, Mbp (Cavalier-Smith, 1985b)

‡Where no chromosome count is known for the species studied, counts for other species in the same genus are given and marked with an asterisk.

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(Bharathan *et al*., 1994), the most basal family in the monocotyledons (Chase *et al*., 2000). Further, the chromosome number obtained for the material studied ($2n = c.$ 70) suggests that it is hexaploid with an unreplicated genome size of 1C = 0.23 pg (the 2C-value divided by ploidy level), as previous counts have included $2n = 24$ and 48 for *C. demersum* and $2n = 72$ for the related species *C. submersum* (Les, 1988).

In Chloranthaceae, the C-value estimate of 3.6 pg for *Chloranthus spicatus* reported here is similar to previous reports for two other Chloranthaceae species: 1C = 2.9 pg for *Chloranthus officinalis* and 1C = 4.4 pg for *Sarcandra glabra* (Bennett *et al*., 2000). The chromosome number of $2n = 30$ obtained for *C. spicatus* agrees with previous reports for the species and is considered to be diploid (Song, 2000).

Together the 14 C-values now available for these basal angiosperms range 16-fold, from 1C = 0.6 pg in *Nymphaea caerula* (Nymphaeaceae) to 9.52 pg in *Austrobaileya scandens* (Austrobaileyaceae). This range falls towards the lowest 14% of the *c*. 1000-fold range encountered in angiosperms (see Introduction).

C-VALUES IN MAGNOLIIDS

Our estimate of 1C = 1.13 pg for *Drimys vickeriana* is the first value reported for the magnoliid family Winteraceae. It is only a fifth of the estimate of 1C = 5.8 pg for *Canella winterana* which is in the sister family Canellaceae. Together Canellaceae and Winteraceae comprise Canellales (Qiu *et al*., 2000), one of the four higher orders of the magnoliid clade, the other three being Laurales, Magnoliales and Piperales (Qiu *et al*., 2000). Since the analysis by Leitch *et al*. (1998), the number of C-values for magnoliids has doubled, with data now available for 60 species and ranging 14.5-fold, from 1C = 0.4 pg in *Friesodielsia obovata* to 5.8 pg in *Canella winterana* (Bennett & Leitch, 2001). The C-value reported for *Drimys vickeriana* therefore falls within this range.

THE FUTURE

New studies, particularly those targeting systematic gaps in the data, have contributed significantly to an increase in the phylogenetic coverage of C-value data. For example, two papers by Hanson *et al*. (2001a,b) have increased familial representation of data from 34% to 44.8%. With the new data presented in the current paper which have filled all familial gaps in basal angiosperm families, and with an increasingly robust angiosperm phylogeny now available, it is timely to repeat and enlarge the large-scale phylogenetic analysis of C-values by Leitch *et al*. (1998) to provide a deeper insight into the evolution of DNA amounts in angiosperms.

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