AoB PLANTS
About
Author Guidelines
Editorial Board

AoB Plants. 2012; 2012: pls006.
Published online 2012 Apr 16. doi: 10.1093/aobpla/pls006
PMCID: PMC3326669
PMID: 22509462

Genome size in *Anthurium* evaluated in the context of karyotypes and phenotypes

Barbara J. Bliss and Jon Y. Suzuki*

Little is known about the genome of *Anthurium* other than chromosome observations, which frequently indicate supernumerary ("B") chromosomes. New genome size estimates for 34 species and nine cultivars presented here provide insights into genome organization and evolution in this very large genus.

Abstract

Background and aims

Anthurium is an important horticultural crop from the family Araceae, order Alismatales, a lineage considered to have diverged from other monocots prior to the cereals. Genome size and its distribution in *Anthurium* were investigated to gain a basic understanding of genome organization in this large genus and to forge a firm foundation for advancement of molecular approaches for the study of *Anthurium*. Currently, genome size estimates have been reported for only two *Anthurium* samples

Methodology

Bulk nuclear DNA content estimates were obtained by flow cell cytometry using leaf tissue co
from *Anthurium* species of different subgeneric groups and from commercial cultivars. The m
current and well-supported topology of subgeneric, sectional relationships was applied to pr

genome size estimates in the context of reported chromosome counts, karyotypes, putative phylogenetic relationships, observed phenotypes and pedigree.

Principal results

Genome size estimates based on bulk nuclear DNA content for 77 accessions representing 34 species and 9 cultivars were obtained, including initial estimates for 33 *Anthurium* species, and both the smallest (*Anthurium obtusum*; *Tetraspermium*) and largest (*Anthurium roseospadix*; *Calomystrium*) *Anthurium* genome sizes reported to date. Genome size did not distinguish any subgeneric section, but ranged 5-fold (4.42–20.83 pg/2 C) despite consistent 2N= 30 chromosome counts. Intraspecies genome size variation >20 % is reported for *Anthurium ravenii*, *A. watermaliense* and *A. gracile*.

Conclusions

Genome size estimates for *Anthurium* species spanning 13 recognized subgeneric sections indicate that genome size does not generally correlate with chromosome count or phylogenetic relationships. Mechanisms of genome expansion and contraction, including amplification and reduction of repetitive elements, polyploidy, chromosome reorganization/loss, may be involved in genome evolution in *Anthurium* as in other species. The new information on *Anthurium* genome sizes provides a platform for molecular studies supporting further research on genome evolution as well as cultivar development.

Introduction

Anthurium is the most speciose genus in the Araceae, a monocot family defined by its unique inflorescence composed of a spadix and spathe. The spadix holds hundreds of minute flowers compacted on a spike, which is subtended by a more or less showy sterile leaf-like organ, the spathe. *Anthurium* is comprised of ~900 published and 1500 estimated species endemic to the neotropical zones of northern Mexico and south through Central America to southern Brazil, and on the Caribbean Islands (Croat 1988, 1989; Mayo *et al.* 1997; Govaerts *et al.* 2011; Boyce and Croat 2012). *Anthurium andraeanum*, native to Colombia, was first introduced to the island of Oahu in 1889, where it flourished and became widely cultivated by amateur breeders and hobbyists who developed many attractive new varieties throughout the 1940s. Beginning in 1950, an intensive breeding programme at the University of Hawai`i at Manoa yielded many unique and improved cultivars using selective breeding, hybridization and *in vitro* propagation of *Anthurium* species. The novel colours and forms, as well as desirable horticultural attributes, generated in these cultivars contributed to the dominance of the anthurium industry by Hawaiian growers for much of the second half of the

20th century (Kamemoto and Kuehnle 1996). We anticipate that future improvements to anthurium cultivars will utilize molecular resources developed to contribute to the basic understanding of this large genus while supporting applied science.

Genome size has implications for molecular biology work, genomics and overall successful implementation as a study organism. Genome size is also correlated with seed mass, cell size, stomatal size, stomatal density, length of cell cycle and a host of derivative phenotypes important for plant success (Beaulieu *et al.* 2007, 2008; Leitch *et al.* 2007; Hodgson *et al.* 2010). Genera with larger genome sizes have limited photosynthetic rates, tend to have limited distribution and tend to be less speciose (Knight *et al.* 2005). Genome size in angiosperms varies ~1000-fold (Leitch *et al.* 2005) with the largest genomes found among the monocots (Leitch *et al.* 2010; Zonneveld 2010). Variations in angiosperm DNA content (Bennett and Leitch 2005) have been interpreted in a robust phylogenetic context to reconstruct genome size evolution, revealing the ancestral genome size to be relatively small (1.4 pg/1 C) (Soltis *et al.* 2003). Significant increases in genome size have been attributed to polyploidy and to amplification of repetitive DNA content (SanMiguel *et al.* 1996; Vicient *et al.* 1999; Hawkins *et al.* 2006). Secondary downsizing in lineages embedded within clades having larger genome sizes counters the overall trend towards genome size growth (Leitch *et al.* 1998; Bennetzen *et al.* 2005).

In the family Araceae, genome sizes tend to be moderate, including *Orontium aquaticum* (30 pg/2 C), a species derived from an early-diverging lineage in Araceae (Cabrera *et al.* 2008; Cusimano *et al.* 2011). Genome size estimates have been reported for only two *Anthurium* accessions: *A. schlechtendalii* (15.27 pg/2 C) and *A. grande* Hort. (27.00 pg/2 C) (26 July 2011; http://data.kew.org/cvalues). These are also of moderate size with the genome size estimate for *A. schlechtendalii* 2.8 times that of *Zea mays* (5.45 pg/2 C) (Bennett and Smith 1976), and the estimate for the accession *A. grande* Hort. nearly twice as large as that of *A. schlechtendalii* (Ghosh *et al.* 2001). However, in Lemnoideae, the sister group to the true Araceae, the group to which *Anthurium* belongs (Cabrera *et al.* 2008; Cusimano *et al.* 2011), the genera *Lemna* (1.20 pg/2 C) and *Spirodela* (0.60 pg/2 C, 0.74 pg/2 C) have quite small genome sizes (Geber 1989). The evolutionary relationship of these lineages suggests that the common ancestor of both Lemnoideae and the true Araceae may have had in place the genomic machinery to secondarily generate species with small genomes and that there may also be *Anthurium* species with small genome sizes.

Understanding the organization and composition of the *Anthurium* genome is a prerequisite to the development of molecular resources to support improvement of the *Anthurium* Hort. complex. We set out to document a wider range of *Anthurium* genome sizes and interpret them in the context of the most recent phylogenetic analysis of *Anthurium* species (Carlsen 2011), referencing cytological observations and known mechanisms of genome size evolution to identify trends in genome evolution in *Anthurium*. We sampled most deeply the natural, easily recognized sections *Calomystrium* and *Cardiolonchium* from which the *Anthurium* Hort. complex mainly derives in order to better explore the extent of evolutionary change and gain insight into the events influencing genome evolution in these clades.

Materials and methods

Nuclear genome size estimations were obtained by flow cell cytometry following the protocol described by Arumuganathan and Earle (1991). Genome sizes were obtained for 81 accessions obtained from botanical gardens, anthurium industry growers and cultivar developers. Tissue samples of 50 mg fully expanded, non-senescing leaf tissue were collected and shipped to arrive for analysis within 24 h of collection. Flow cytometry involves chopping of fresh plant material together with an internal standard (Galbraith *et al.* 1983). Ideal internal standards display minimal variability (Baranyi and Greilhuber 1996), match as closely as possible the configuration of DNA (e.g. chromosome structure) in the nucleus of the sample, and have a genome size larger than that of the sample, but not >4 times larger (Suda and Leitch 2010; Praça-Fontes *et al.* 2011). Monocots display a greater variability in chromosome organization and amount of DNA in the genome (Leitch *et al.* 2010), so we provided the monocots wheat (*Triticum aestivum* cv. 'Zak' 30.55 pg/2 C) and barley (*Hordeum vulgare* line NE86954 9.69 pg/2 C) as internal standards based on the existing 2-fold range of genome sizes for *Anthurium* found in the plant DNA C values database (26 July 2011; http://data.kew.org/cvalues). We also provided tobacco (*Nicotiana tabacum* cv. 'SR-1' 9.32 pg/2 C). Each nuclear preparation was sampled four times, under the direction of K. Arumuganathan, at the Flow Cell Core Lab, Benaroya Research Institute at Virginia Mason, 1202 Ninth Avenue, Seattle, WA 98101, USA. The mean bulk nuclear DNA content (2 C) of each sample (expressed as picograms) was based on 1000 scanned G0 + G1 nuclei from sample tissue, compared with nuclei of the internal standard.

Results

Genome sizing

The terms 'C value' and 'genome size' have specific meaning independent of the number of chromosomes or base pairs in the cell. The term 'C value' originally referred to a constant value observed across all different tissue types in animals, whereas the term 'genome size' is used to describe the bulk nuclear DNA content of cells, both the more easily obtained somatic (holoploid) cells and also gametic (monoploid) cells (Bennett and Leitch 2005; Greilhuber *et al.* 2005). The terms 2 C and 1 C have been proposed to distinguish between the DNA content of holoploid somatic cells and monoploid gametes, respectively, and we follow this convention (Greilhuber *et al.* 2005). Measurements were derived from somatic (i.e. leaf) tissue, therefore values for 1 C are obtained by dividing the measured 2 C value in half and are useful for estimating and comparing the DNA content of the monoploid genome. After excluding four accessions having uncertain provenance, we report the arithmetic mean of four instrument readings of nuclei, ±standard deviation, for 77 accessions, increasing reported genome size estimates for *Anthurium* spp. by 33 species and 9 cultivars (Table 1).

Table 1

Genome sizes of accessions sampled, listed alphabetically by *Anthurium* species, followed by cultivars

	103/81*A*B			
A. microspadix Schott	MBG 100186	W	12.25 ± 0.13	5992
A. nymphaefolium C. Koch & Bouché	MBG 55262	W	9.45 ± 0.27	4623
A. obtusum (Engl.) Grayum	ABG 19970478	T	5.80 ± 0.08	2835
	MBG 82905	T	4.42 ± 0.09	2160
A. ochranthum K. Koch	MBG 69861a	W	10.87 ± 0.12	5317
	MBG 75190a	W	10.64 ± 0.10	5201
A. pittieri Engl.	JBM 84-2010	T	5.61 ± 0.03	2745
A. radicans K. Koch & A. Haage	ABG 19911495	W	15.10 ± 0.32	7103
	MSBG 1975-0053-0003A	W	14.52 ± 0.11	7103
	USBG 98-2591	W	13.46 ± 0.33	6581
A. ravenii Croat & Baker	MBG 74778	W	13.32 ± 0.29	6515
	MSBG 1980-0425A	W	7.54 ± 0.15	3689
A. roseospadix Croat	MBG 74076	W	20.83 ± 0.36	10187
A. scandens (Aubl.) ssp. *pusillum* Engler	USBG 98-1900	W	5.12 ± 0.14	2506
A. scandens (Aubl.) ssp. *scandens* Scheffer	ABG 19911433	W	9.98 ± 0.57	4882
	ABG 19980667	W	9.67 ± 0.15	4726
	MBG 47671	W	9.28 ± 0.16	4537
A. schlechtendalii ssp. *schlechtendalii* Kunth	MBG 78640	W	11.54 ± 0.13	5643
	MSBG 1977-3108A	W	14.33 ± 0.15	7008
	NYBG 933/79*A	W	12.25 ± 0.18	5991
	NYBG 993/93*A-C	W	12.07 ± 0.10	5903

Mbp, million base pairs; W, wheat (*Triticum aestivum* cv. 'Zak,' 30.55 pg/2 C); T, tobacco (*Nicotiana tabacum* cv. 'SR-1' 9.32 pg/2 C); ABG, Atlanta Botanical Garden; CJBN, Conservatoire et Jardins Botaniques de Nancy; JBM, Jardin Botanique de Montréal; JBVL, Jardin Botanique de la Ville de Lyon; MBG, Missouri Botanical Garden; MSBG, Marie Selby Botanical Gardens; NYBG, New York Botanical Garden; UH, University of Hawai`i College of Tropical Agriculture and Human Resources; USBG, United States Botanic Garden; NG, Novelty Greens; HAIA, Hawaiian Anthurium Industry Association.

[a]Genome size and standard deviation have been rounded to two decimal places.

[b]Mbp/1 C DNA for plant species is based on 1 pg=978 Mbp according to Doležel and Greilhuber (2010) and was calculated prior to rounding genome size (pg/2 C) to two decimal places.

Wheat and barley, both monocots, were chosen as internal standards to better reflect the DNA configuration in our *Anthurium* samples. However, when barley was used, sample peaks frequently overlapped those of the internal standard, so most genome sizes are reported using wheat as the internal standard. When overlapping peaks prevented interpretation of results with wheat, or if the genome size was closer to that of the eudicot tobacco, results are reported using that species as an internal standard. In one case (*A. gymnopus*) the sample was processed using a previously evaluated *Anthurium* species as the internal standard (Table 1) because standards were not available at the time of sampling. Genome sizes for 26 accessions obtained using both tobacco and wheat as internal standards were generally within 10 % of the mean, confirming that the use of either standard produces essentially the same results [see Additional Information].

The mean pg/2 C genome size of all accessions sampled for each species is presented (Fig. 1) along with previously published chromosome counts [see Additional Information], organized according to accepted sectional assignments based on traditional characters of morphology, habit, flower/inflorescence, secondary metabolites, karyotype and, most recently, molecular data (Croat 1983, 1991; Croat and Sheffer 1983; Carlsen and Croat 2007; Carlsen 2011). The most recent phylogenetic analysis of 102 samples broadly retains the composition and identity of natural sections, those found least controversial by traditional systematics, and proposes relative relationships among them, which can be extended to other species assigned to those sections. Carlsen sampled 84 species that we did not include, and we sampled 16 species that Carlsen did not include (Carlsen 2011). These are placed according to their existing sectional assignment (Carlsen 2011). The relationship of the monotypic section *Gymnopus* to other sections has not been determined (Fig. 1).

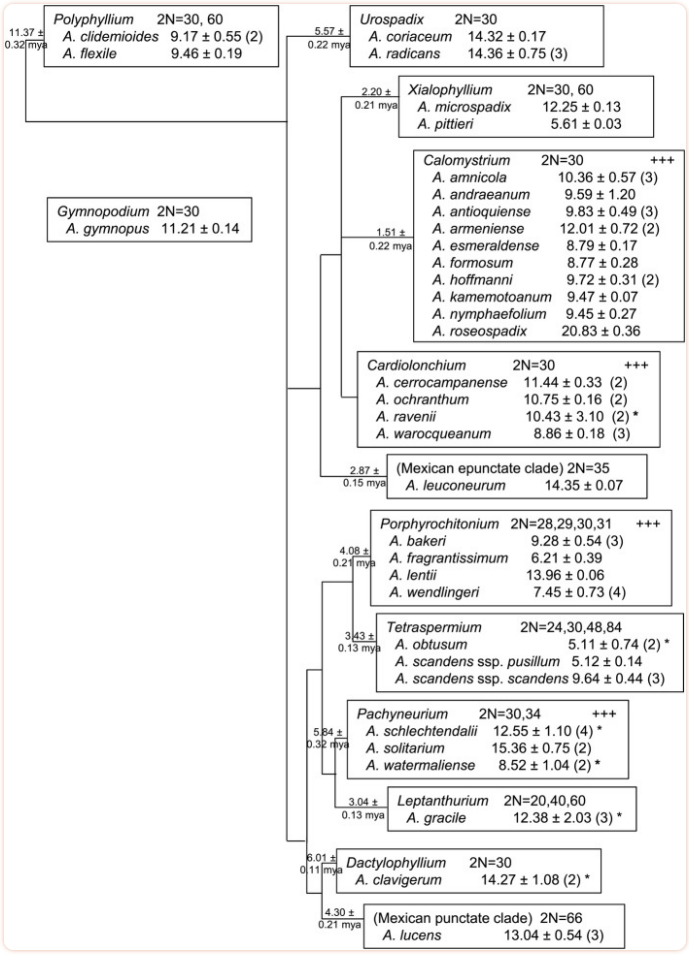

Fig. 1

Genome size and chromosome counts of *Anthurium* species presented by subgeneric sections. Clades of punctate and epunctate Mexican species are proposed new sections (Carlsen 2011). Relative relationships of sections and estimated dates of crown group divergence are indicated at nodes, as per Carlsen (2011). Dates expressed in millions of years ago (mya). Chromosome counts for each section represent values reported for species in that section. '+++' indicates supernumerary chromosomes have been observed in that section. Genome size is reported as the mean of all accessions sampled, in pg/2 C ± S.D., followed by number of accessions sampled in parentheses. Superscript asterisk indicates between-sample (accession) variance >10 % of the species mean.

Published chromosome counts report 2N= 30 (N= monoploid chromosome number) for most *Anthurium* species (Fig. 1), with frequent reports of supernumerary chromosomes ('B', chromosomes, satellites or fragments) (Petersen 1989), distinguished mainly by size, dispensability and behaviour at meioisis (Jones and Rees 1982) [see Additional Information]. Of the species we sampled, recent cytogenetic analyses report supernumerary chromosomes exclusively in species assigned to

sections *Calomystrium, Cardiolonchium, Porphyrochitonium* and *Pachyneurium* (Fig. 1) (Sharma and Bhattacharyya 1961; Sheffer and Kamemoto 1976; Sheffer and Croat 1983; Marutani *et al.* 1993; Cotias-de-Oliveira *et al.* 1999).

Genome sizes in a phylogenetic framework

The organization of *Anthurium* species chromosome counts and genome sizes according to the accepted subgeneric grouping of species into sections suggests all clades derived from a progenitor lineage having 2N= 30 chromosomes, with occasional polyploidy and cytological variation in most sections. The genome sizes associated with 2N= 30 vary >4-fold (Table 1, Fig. 1). The genome sizes of the two species sampled in section *Polyphyllium* (9.17–9.46 pg/2 C) are within 10 % of each other, a variance not greater than within-sample variance (K. Arumuganathan, Flow Cell Core Lab, Benaroya Research Institute, Seattle, WA, USA, pers. commun.). There is evidence for polyploidization in section *Polyphyllium*, with twice as many chromosomes reported for *A. flexile* (2N= 60) as for *A. clidemioides* (2N= 30) (Table 1, Fig. 1). The near equivalence of genome size despite the doubled number of chromosomes may reflect an early polyploidization in the *Polyphyllium* crown group which arose over 11 million years ago (mya) (Fig. 1). The genome sizes of the two species sampled in section *Urospadix* (14.32–14.36 pg/2 C) are also within 10 % of each other, but are reported to share the same chromosome count (Table 1, Fig. 1) [see Additional Information].

The phylogenetic relationships among the traditionally described sections *Xialophyllium*, *Calomystrium* and *Cardiolonchium* are unclear, although it is certain that section *Cardiolonchium* as traditionally defined is not monophyletic (Carlsen 2011). The genome sizes of two species sampled in section *Xialophyllium* are 5.61–12.25 pg/2 C, displaying an approximately proportional relationship between genome size and chromosome count between the two species, suggesting a polyploid event occurring no more than 2.20 mya, the estimated crown group divergence date (Fig. 1).

In section *Calomystrium*, the most recently established clade (1.5 mya), genome sizes of the 10 species sampled range from 8.77 to 20.83 pg/2 C, a 2.4-fold difference (Figs 1 and 2), despite a consistent number of chromosomes (Fig. 1). Among the *Calomystrium* species, the genome sizes of *A. formosum* and *A. esmeraldense* are very similar (8.77–8.79 pg/2 C), as are the genome sizes of *A. nymphaefolium*, *A. kamemotoanum*, *A. andraeanum*, *A. hoffmanni* and *A. antioquiense* (9.45–9.83 pg/2 C) (Table 1, Fig. 1). The genome sizes of *A. amnicola* and *A. armeniense* are larger, but the genome size of *A. roseospadix* (20.83 pg/2 C), the largest *Anthurium* genome size to date, is two or more times that of most other species sampled in section *Calomystrium* (Table 1, Fig. 1). Although B chromosomes have been occasionally reported in section *Calomystrium*, there have been no reports of polyploidy as in other *Anthurium* sections having such large inter- and intraspecies differences in genome size (Fig. 1).

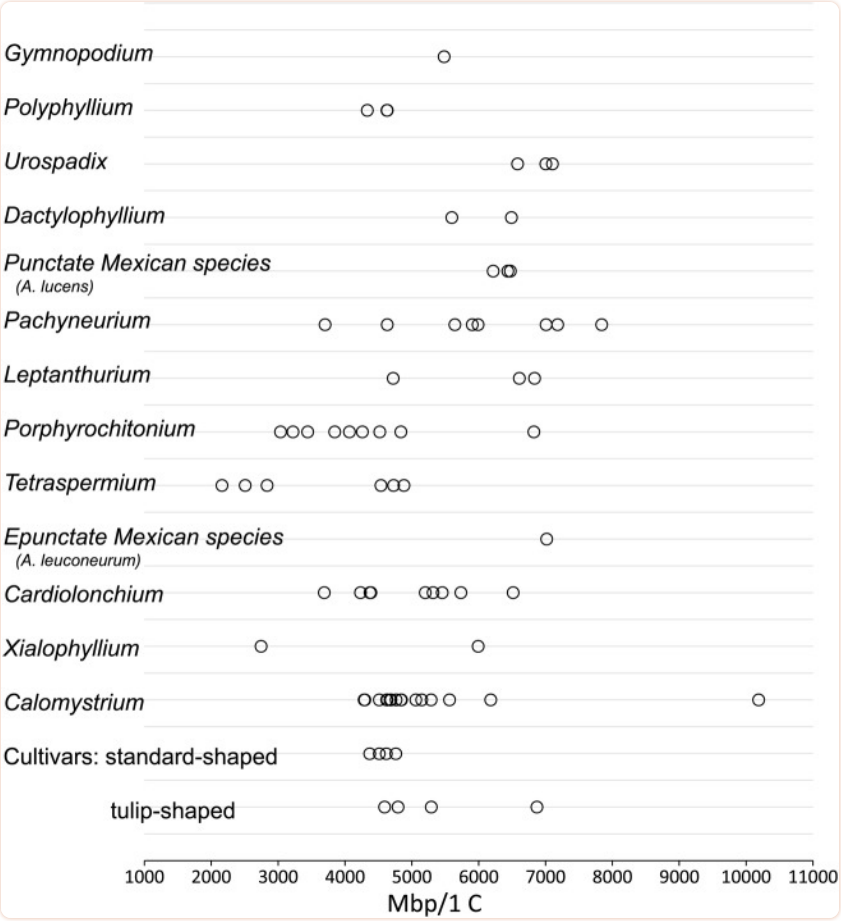

Fig. 2

Genome sizes for *Anthurium* accessions sampled. Estimated genome sizes are shown as million base pairs (Mbp) per 1 C.

The mean genome size estimates for the four species represented in section *Cardiolonchium* range from 8.86 to 11.44 pg/2 C, with consistent 2N= 30 and reported occurrence of B chromosomes (Figs 1 and 2). The range of the mean genome sizes for these species is less than the range of genome size estimates reported for the two accessions sampled for the species *A. ravenii* (Table 1). The intraspecies variation reported for *A. ravenii* (7.54–13.32 pg/2 C) reflects ~43 % difference in genome sizes between the two accessions sampled, each of which had reliable provenance information and resembled no species other than *A. ravenii*. The larger genome size reported for this species is 1.76 times that of the smaller one, suggesting some extent of autopolyploidy in *A. ravenii* and that we may have sampled an accession with a different cytotype. Other species sampled in this section display unremarkable intraspecies variance.

The genome size of *A. leuconeurum*, a member of a newly described (Carlsen 2011) and yet unnamed clade of epunctate Mexican species, is much larger than all those in its sister clade (consisting of *Cardiolonchium*, *Calomystrium*, *Xialophyllium*) with the exception of *A. roseospadix* (Fig. 1). The 2N= 35 chromosome count report for *A. leuconeurum* has not been confirmed in over 50 years (Mookerjea 1955), although it is speculated to have possibly included observation of 1–5 B chromosomes (Sheffer and Kamemoto 1976).

The four species sampled in section *Porphyrochitonium*, a section arising ~ 4.08 mya, display a more than two-fold range in genome sizes (6.21–13.96 pg/2 C). Supernumerary chromosomes have been reported in *A. bakeri*, the only species sampled from section *Porphyrochitonium* varying from 2N = 30. In section *Tetraspermium*, sister clade to *Porphyrochitonium*, *A. obtusum* and *A. scandens* ssp. *pusillum* have similar genome sizes, with one accession of *A. obtusum* having the smallest genome size (4.42 pg/2 C) reported to date in *Anthurium* (Fig. 1, Table 1). *Anthurium obtusum* has been reported as having 2N= 30, and also as 2N= 24, suggesting a ready loss of six chromosomes, or a 20 % decrease. The genome sizes of the two accessions for this species differ by ~25 % (Table 1, Fig. 1), suggesting that our accessions may have had the different numbers of chromosomes reported for this species. *Anthurium scandens* ssp. *pusillum* has been reported as having 2N= 24. A polyploid event in *A. scandens* may be responsible for the 2N= 48 chromosomes found in *A. scandens* ssp. *scandens*. A different loss of six chromosomes appears evident in the 2N= 84 variant of *A. scandens* ssp. *scandens*, which could arise by the *A. scandens* ssp. *scandens* 2N= 48 cytotype losing six chromosomes to 2N= 42, followed by a polyploidization event to yield the observed 2N= 84.

The mean genome sizes of the three species in section *Pachyneurium* (estimated divergence 5.8 mya) range from 8.52 to 15.36 pg/2 C (Fig. 1). Relatively wide intraspecies variance is observed between accessions in *A. watermaliense*, which varied ~20 % from the mean, approaching the intraspecies genome size changes that correlate with chromosome count changes in *A. scandens* of section *Tetraspermium*. However, supernumerary chromosome are the only cytotypic changes reported to occur in section *Pachyneurium*, suggesting that the genome size differences between different accessions observed here might be related to the presence of extra-chromosomal DNA, or that somatic changes in the accessions sampled may have been extensive.

The species sampled of section *Leptanthurium*, section *Dactylophyllium*, and *A. lucens*, representing a newly described (Carlsen 2011) and yet unnamed clade of punctate Mexican species, had relatively larger genome sizes (Table 1, Figs 1 and 2). In section *Leptanthurium*, one of the three different accessions sampled of *A. gracile* has a genome size nearly 30 % smaller than the other two, which are very similar, suggesting that we sampled two accessions having the same cytotype and a third accession having a different one.

Genome size and phenotype

Species contributing to the pedigree of cultivars were expected to be reflected in the genome sizes of those cultivars. *Anthurium andraeanum* hybridizes most easily with *A. amnicola*, *A. antioquiense*, *A. armeniense*, *A. formosum*, *A. hoffmanni*, *A. kamemotoanum*, *A. lindenianum*, *A. nymphaefolium* and *A. roseospadix*, all members of section *Calomystrium* (Kamemoto and Kuehnle 1996). Of these, *A. lindenianum* was not available for sampling. The cultivars 'Marian Seefurth', 'Midori', 'New Pahoa Red', 'Puanani' and 'Shibori' all share the 'standard' blistered, heart-shaped spathe of *A. andraeanum* (Fig. 3A–F) and lack any documented contribution by species other than *A. andraeanum*. Genome sizes for those five cultivars (Table 1, Fig. 2) are similar to those of most members of the *Calomystrium* series (Figs 2 and 4), but only the species *A. andraeanum* (Fig. 3A) has a heart-shaped spathe.

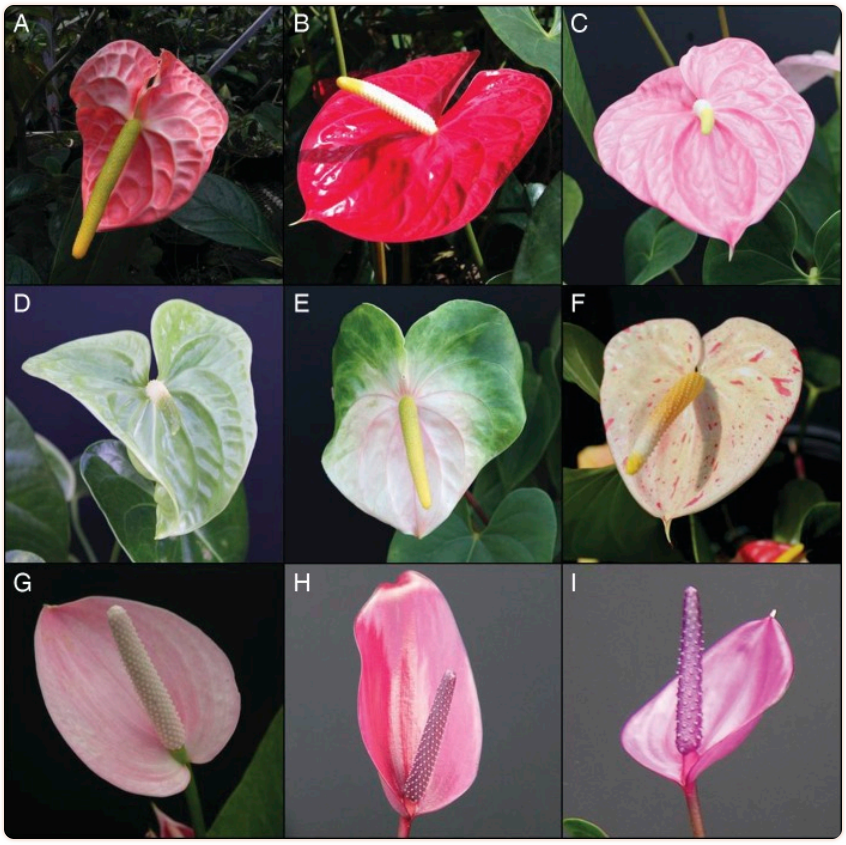

Fig. 3

Images of *A. andraeanum* and related cultivars. (A) *A. andraeanum* Linden (used with permission of Michael Wenzel), (B) *A. andraeanum* Hort. cv. 'New Pahoa Red', (C) *A. andraeanum* Hort. cv. 'Marian Seefurth', (D) *A. andraeanum* Hort. cv. 'Midori', (E) *A. andraeanum* Hort. cv. 'Puanani', (F) *A. andraeanum* Hort. cv. 'Shibori', (G) *Anthurium* Hort. 'Princess Aiko', (H) *Anthurium* Hort. 'Regina' and (I) *Anthurium* Hort. 'Miss June Purple.'

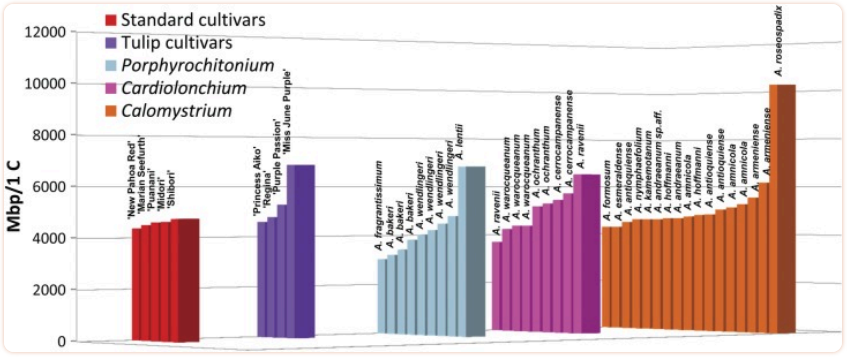

Fig. 4

Genome size estimates of accessions of cultivars and species from *Calomystrium*, *Cardiolonchium* and *Porphyrochitonium*, subgeneric sections with known contributors to commercial anthurium hybrids. Individual accessions within each series are ordered by increasing genome size.

The cultivars with tulip-shaped spathes (Fig. 3G–I), ‘Princess Aiko’, 'Regina’, ‘Purple Passion’ (photograph not available) and ‘Miss June Purple’, may have been derived from tulip-shaped species in section *Calomystrium*, or from species in other sections (*Cardiolonchium*, *Porphyrochitonium*) known to contribute to hybrids derived from *A. andraeanum* (e.g. *A. antrophyoides*, *A. ochranthum*, *A. cerrocampanense* and *A. lentii*) (Kamemoto and Kuehnle 1996). Alternatively, cultivars with tulip-shaped spathes may be derived entirely from species reproductively incompatible with *A. andraeanum* (e.g. *Anthurium wendlingeri*, *A. bakeri*, *A. scherzerianum*, *A. lancifolium*, *A. caperatum* and *A. garagaranum*). The pedigrees of the cultivars ‘Aiko’ and ‘Regina’ are in fact known, as they were developed in the University of Hawai`i, Manoa, anthurium breeding programme. The genome size of the ‘Princess Aiko’ cultivar (Table 1, Fig. 3G) is similar to that of the cultivars with standard-shaped spathes (Figs 2 and 4) and other accessions sampled in *Calomystrium*, reflecting its derivation from the standard cultivar ‘Tatsuta Pink Obake’ and the tulip-shaped section *Calomystrium* species, *A. antioquiense* (Kuehnle *et al.* 2004). The cultivar ‘Regina’ (Table 1, Fig. 3H) is derived from earlier cultivars composed of contributions from the smaller-genome-sized *Calomystrium* species (i.e. *A. amnicola*, *A. formosum*, *A. andraeanum* and *A. kamemotoanum)* (Kamemoto and Kuehnle 1996; Kuehnle *et al.* 2004), and the genome size of ‘Regina’ is consistent with that pedigree (Figs 2 and 4). The cultivar ‘Purple Passion’ resembles (phenotypically) no species other than *A. amnicola*, and the genome size estimate is consistent with that (Table 1, Figs 2 and 4). The cultivar ‘Miss June Purple’ phenotypically resembles ‘Regina’ in many aspects (Fig. 3H and I), but the genome size is ~43 % larger (Table 1, Fig. 4), suggesting a species with a larger genome size in its pedigree (perhaps *A. roseospadix* or *A. lentii*), or possibly an endogenous genome size change occurred in this cultivar.

Discussion

Genome sequencing and comparative mapping have revealed the ancient polyploid nature of angiosperms and provided an insight into the effects of polyploidy on genome evolution in plants (The Arabidopsis Genome Initiative 2000; Vision *et al.* 2000; Blanc and Wolfe 2004; Adams and Wendel 2005; Cui *et al.* 2006; Fawcett *et al.* 2009), while studies in *Oryza* (Wang *et al.* 2005), *Arabidopsis* (Lagercrantz 1998; Yogeeswaran *et al.* 2005) and others (Wendel 2000; Leitch and Bennett 2004; Leitch *et al.* 2008) reveal downsizing in genomes occurring after polyploidization events. We have used genome size to gain insight into some aspects of genome evolution in *Anthurium*, a genus for which the angiosperm genome size database previously contained data only for one species and one cultivar. In this study, apparent correlation between chromosome count and genome size is only clearly evident in species found in sections *Xialophyllium* and *Tetraspermium*. Although the correlations may be incidental, their importance as clues for the various ongoing processes involved in genome evolution in *Anthurium* should not be excluded.

Genome sizes and reported chromosome counts of two species are suggestive of polyploidy events in section *Xialophyllium*. In section *Tetraspermium*, with the smallest genome sizes reported to date, genome sizes reflect reported intraspecies variations in chromosome counts in *A. scandens* (Sheffer and Kamemoto 1976; Sheffer and Croat 1983), including polyploidy, and suggest additional, subsequent genome changes occurred. The age of genome changes such as polyploidy influences the confidence with which they can be identified since subsequent mutations tend to obscure the original event (Doyle and Egan 2009). Although the ages of polyploidy events discussed here are not known, they are maximally the ages of the relatively young crown groups, *Xialophyllium* and *Tetraspermium*, to which they belong, which are estimated to have arisen 2.2 and 3.43 mya, respectively. In sections other than *Xialophyllium* and *Tetraspermium*, our data display incongruity between interspecific and intraspecific genome size and chromosome counts reported by others for *Anthurium* and other genera.

In sections *Calomystrium, Cardiolonchium, Porphyrochitonium*and *Pachyneurium*, we report interspecies genome size variation without any apparent relationship between genome size and base chromosome number, suggesting that the size difference may be unrelated to a polyploidization event. Therefore, interspecies genome size variation in these sections, particularly in the youngest crown group, *Calomystrium*, suggests a mechanism of genome size change capable of producing large differences in a short span of time. Transposable elements are capable of producing such changes. Up to 80 % of the current *Z. mays* genome is composed of retroelements, most inserted in the last 1–3 million years (Rabinowicz and Bennetzen 2006). Transposable elements may be deleted after initial amplification (Shirasu *et al.* 2000), or may persist and play a part in local adaptation, as exemplified by the intraspecific expansion of BARE-1 retroelements in barley in response to elevation and aridity (Kalendar *et al.* 2000). In *Arabidopsis* and *Oryza*, genome size variations are associated with changes in repetitive DNA content occurring in the last 3 million years (Bennetzen *et al.* 2005). Considering *Calomystrium* is estimated to have arisen ~1.5 mya, genome changes due to

rapid invasion and evolution of repetitive elements may play a role in genome size differences. However, the timeframe for comparable changes to occur in *Anthurium*, a long-lived tropical perennial, may be different than that of the annuals *Zea* spp., *Arabidopsis* spp. and *Oryza* spp.

Some genome size changes in these sections may be attributable to DNA changes associated with chromosomal reorganization (Kalendar *et al.* 2000). Chromosome reorganization in *Anthurium* was reported by Marutani *et al.* (1993), who detected differences in the karyotypes of *A. nymphaefolium* (*Calomystrium*) and *A. ochranthum* (*Cardiolonchium*), which she proposed to have resulted from chromosomal rearrangement. She also observed very similar karyotypes among closely related species in section *Calomystrium*, noting that the *A. roseospadix* karyotype resembled those of *A. kamemotoanum* and *A. formosum* (Marutani *et al.* 1993). This is particularly interesting given that the genome size estimate for *A. roseospadix* is more than twice that of each of the other two, suggesting that this may be an example where similar karyotypes among related species may be composed of chromosomes of different structure or DNA mass. However, the cytotype of the *A. roseospadix* accession we sampled would have to be determined before further inferences can be made.

In *Polyphyllium*, the oldest crown group in *Anthurium*, we have a case of extreme differences between reported chromosome count and expected potential genome size, with reported ploidy difference between two species with measured genome sizes that were essentially the same. As ploidy differences within other *Anthurium* species do exist, it is possible that we may have sampled a previously unreported cytotype, as is suspected for *A. ravenii* in section *Cardiolonchium*. However, if verified, this apparent incongruity between chromosome count and measured genome size allows us to consider a loss or gain of bulk nuclear DNA and permits inferences based on evidence from mechanisms of genome evolution elucidated by studies in other species. For example, in rice, Wang *et al.* (2005) estimate that 35–60 % of duplicated genes were lost shortly after genome size expansion as recently as 5 mya. In *Arabidopsis*, Lagercrantz (1998) estimated ~90 chromosomal rearrangements since *Arabidopsis* and *Brassica* diverged ~14–24 mya, and Yogeeswaran *et al.* (2005) estimated ~10 chromosomal rearrangements occurred in the divergence of *Arabidopsis thaliana* and *Arabidopsis lyrata* ~5 mya. Carlsen (2011) estimated the crown group *Polyphyllium* arose ~11 mya, well within the time required to accomplish the scope of chromosomal changes as observed in the genus *Arabidopsis*.

The polyploid origin of *A. clidemioides* is unknown. However, polyploids arising by interspecies hybridization (allopolyploidy) are subject to mismatch repair during recombination of homeologous chromosomes which may generate large-scale deletions contributing to chromosome loss and reorganization (Leitch and Bennett 2004). The more broadly applicable mechanisms of ongoing unequal recombination and illegitimate recombination of homologous chromosomes also contribute to genome size reduction (Shirasu *et al.* 2000; Devos *et al.* 2002), in part by double-strand break repair, an essential but error-prone housekeeping function causing increases, decreases and chromosomal reorganizations, which can lead to chromosome loss (Gorbunova and Levy 1999; Kirik *et al.* 2000). Although the suppositions are intriguing, the disparity between measured genome size and reported chromosome count of *A. flexile* compared with *A. clidemioides* warrants further investiga-

tion. It was not possible to evaluate cytotypes for the accessions included in this study. Most samples were contributed by botanical gardens, and thus we did not have the plants available locally for fresh root tip sampling and cytotype determination.

The genus *Anthurium* displays considerable flexibility of nuclear DNA quantity and organization, even within species. We report here different genome sizes for different accessions of *A. ravenii*, *A. watermaliense* and *A. gracile* varying >20 % from the mean for the species. Conceptually, intraspecies variation can be viewed as incipient interspecies genome size variation (Greilhuber 1998). Once, genome size seemed to offer promise for delineating species (Ohri 1998; Ghosh *et al.* 2001), so reports of intraspecies genome size variations have been scrutinized to identify and eliminate systematic sources of variation, leading to standardization of methods, attention to detail in sample handling and careful selection of internal standards (Greilhuber 1998, 2005). Still, intraspecies variations persist: approximately 10 % of *Curcuma* species sampled displayed intraspecies variation in genome size estimates (2007), while both genome sizes and ploidy levels varied widely in a survey of 244 *Dianthus broteri* individuals collected from 25 populations (Balao *et al.* 2009), similar to results reported here for *A. scandens*. While variant cytotypes may explain the largest differences observed, a lesser amount of intraspecific variation in bulk nuclear DNA content may be attributed to the presence of extrachromosomal material, which can only be convincingly excluded from genome size estimates by determining the cytotype of each accession sampled (Teoh and Rees 1976). In particular, the origin and evolution of B chromosomes seems to be associated with amplification of tandem repeats on A chromosomes, and can be generated spontaneously following allopolyploidization (Jones and Houben 2003). It may be that activity of extra-chromosomal DNA in sections *Calomystrium*, *Cardiolonchium, Porphyrochitonium* and *Pachyneurium* has contributed to the range of genome sizes among accessions of the same species in those sections.

Furthermore, *Anthurium* cultural practices, including *in vitro* cultivation, clonal propagation and selection for sports, impose extreme selective pressures, capable of activating transposable elements causing intraspecies genome size variations without imposing a reproductive barrier (Peschke and Phillips 1991; Hirochika *et al.* 1996). Indeed, individual cultivars may be selected for phenotypes associated with transposable element activity which has affected genome size, but has more noticeably affected phenotype. For example, variegated cultivars of maize (McClintock 1965–1966), *Antirrhinum* (snapdragon) (Coen and Carpenter 1986), *Convolvulus* (morning glory) (Hoshino *et al.* 1995), *Dahlia* (Ohno *et al.* 2011) and *Sorghum* carry transposable elements associated with variegation (Chopra *et al.* 1999), and it may be that the mottled 'Shibori' cultivar (Figs 3I and 4), with a slightly larger genome than that of the other standard cultivars, is accomplishing its variegation by similar means.

Conclusions and forward look

Genome sizes in *Anthurium* display variation suggestive of repeated polyploidy, with evidence for possible re-diploidization in the oldest crown group *Polyphyllium*, and ongoing expansion in the youngest crown group, *Calomystrium. Anthurium* genome size distribution was not distinctly demarcated by ploidy level, as Leong-Škorničková *et al.* (2007) similarly reported in an analysis of nearly half the *Curcuma* (Zingiberales) species found on the Indian continent. Also, as in *Curcuma*, we found genome size to be useful, together with phenotypic similarities, for insight into the pedigree of cultivars (Leong-Škorničková *et al.* 2007). The new information on genome sizes in *Anthurium* will serve as a useful framework from which to launch molecular investigations including map- and sequence-based studies, which may provide further insight into the processes resulting in genome size variation observed in *Anthurium*, which may be similar to those described in other monocots.

Additional information

The following additional information is available in the online version of this article –

File 1. Genome size estimates for 26 *Anthurium* accessions evaluated with two internal standards.

File 2. References for cytological observations summarized for *Anthurium* species sampled.

Sources of funding

This work was funded by the United States Department of Agriculture, Agriculture Research Service.

Contributions by the authors

B.J.B. and J.Y.S. designed the study. B.J.B. coordinated sampling and genome sizing, analysed data, organized figures and authored the manuscript. J.Y.S. supported the research and contributed to the development and revision of the manuscript.

Conflict of interest statement

None declared.

Acknowledgements

Colleagues at public and private botanical gardens provided plant tissues: Michael Wenzel of Atlanta Botanical Garden (ABG), Jon Peter of New York Botanical Garden (NYBG), Bruce Holst of the Marie Selby Botanical Gardens (MSBG), Kyle Wallick of United States Botanic Garden, David Scherberich of

Jardin Botanique de la Ville de Lyon (JBVL), Renée Gaudette of Jardin Botanique de Montréal (JBM), Geneviève Ferry of Conservatoire et Jardins Botaniques de Nancy (CJBN), Thomas Croat of Missouri Botanical Garden (MBG). Monica Carlsen, University of Missouri St Louis (UMSL), contributed immensely to our understanding of *Anthurium* systematics and current phylogenetic relationships. Claudia Henriquez, Washington University in St Louis, selected, collected, packaged and shipped plant material from Missouri Botanical Garden. The Hawaiian Anthurium Industry Association (HAIA) donated cultivars. Teresita Amore and Joanne Lichty from University of Hawai`i, Manoa (UH) provided plant tissues, propagules and historical perspective. Special thanks to Thomas Croat, who verified determinations of some accessions, as well as Monica Carlsen, University of Missouri St Louis (UMSL), who shared pre-publication findings to provide us with the most current hypothesis of phylogenetic relationships in *Anthurium*.

References

Adams KL, Wendel JF. Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology.* 2005;8:135–141. [PubMed] [Google Scholar]

The Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature.* 2000;408:796–815. [PubMed] [Google Scholar]

Arumuganathan K, Earle E. Estimation of nuclear DNA content of plants by cell-flow cytometry. *Plant Molecular Biology Reporter.* 1991;9:229–233. [Google Scholar]

Balao F, Casimiro-Soriguer R, Talavera M, Herrera J, Talavera S. Distribution and diversity of cytotypes in *Dianthus broteri* as evidenced by genome size variations. *Annals of Botany.* 2009;104:965–973. [PMC free article] [PubMed] [Google Scholar]

Baranyi M, Greilhuber J. Flow cytometric and Feulgen densitometric analysis of genome size variation in *Pisum*. *TAG Theoretical and Applied Genetics.* 1996;92:297–307. [PubMed] [Google Scholar]

Beaulieu JM, Moles AT, Leitch IJ, Bennett MD, Dickie JB, Knight CA. Correlated evolution of genome size and seed mass. *New Phytologist.* 2007;173:422–437. [PubMed] [Google Scholar]

Beaulieu JM, Leitch IJ, Patel S, Pendharkar A, Knight CA. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist.* 2008;179:975–986. [PubMed] [Google Scholar]

Bennett MD, Leitch IJ. Nuclear DNA amounts in angiosperms: progress, problems and prospects. *Annals of Botany.* 2005;95:45–90. [PMC free article] [PubMed] [Google Scholar]

Bennett MD, Smith JB. Nuclear-DNA amounts in angiosperms. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences.* 1976;274:227–274. [PubMed] [Google Scholar]

Bennetzen JL, Ma J, Devos KM. Mechanisms of recent genome size variation in flowering plants. *Annals of Botany.* 2005;95:127–132. [PMC free article] [PubMed] [Google Scholar]

Blanc G, Wolfe KH. Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. *The Plant Cell Online.* 2004;16:1667–1678. [PMC free article] [PubMed] [Google Scholar]

Boyce PC, Croat TB. 2012. *The Überlist of Araceae: Totals for published and estimated number of species in aroid genera*http://www.aroid.org/genera/120110uberlist.pdf. 12 January 2012.

Cabrera LI, Salazar GA, Chase MW, Mayo SJ, Bogner J, Dávila P. Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. *American Journal of Botany.* 2008;95:1153–1165. [PubMed] [Google Scholar]

Carlsen M. St Louis, MO, USA: University of Missouri; 2011. *Understanding the origin and rapid diversification of the genus Anthurium Schott (Araceae), integrating molecular phylogenetics, morphology and fossils*. PhD Dissertation. [Google Scholar]

Carlsen MM, Croat TB. Taxonomic revision of *Anthurium* section Semaeophyllium Schott (Araceae) *Harvard Papers in Botany.* 2007;12:173–234. [Google Scholar]

Chopra S, Brendel V, Zhang J, Axtell JD, Peterson T. Molecular characterization of a mutable pigmentation phenotype and isolation of the first active transposable element from *Sorghum bicolor*. *Proceedings of the National Academy of Sciences of the USA.* 1999;96:15330–15335. [PMC free article] [PubMed] [Google Scholar]

Coen ES, Carpenter R. Transposable elements in *Antirrhinum majus*: generators of genetic diversity. *Trends in Genetics.* 1986;2:292–296. [Google Scholar]

Cotias-de-Oliveira ALP, Guedes MLS, Barreto EC. Chromosome numbers for *Anthurium* and *Philodendron* spp. (Araceae) occurring in Bahia, Brazil. *Genetics and Molecular Biology.* 1999;22:237–242. [Google Scholar]

Croat TB. A revision of the genus *Anthurium* (Araceae) of Mexico and Central America. Part I. Mexico and Middle America. *Annals of the Missouri Botanical Garden.* 1983;70:211–420. [Google Scholar]

Croat TB. Ecology and life forms of Araceae. *Aroideana.* 1988;11:4–56. [Google Scholar]

Croat TB. Ecology and life forms of Araceae: a follow-up. *Aroideana.* 1989;12:6–8. [Google Scholar]

Croat TB. A revision of *Anthurium* section Pachyneurium (Araceae) *Annals of the Missouri Botanical Garden.* 1991;78:539–855. [Google Scholar]

Croat TB, Sheffer RD. The sectional groupings of *Anthurium* (Araceae) *Aroideana.* 1983;6:85–123. [Google Scholar]

Cui L, Wall PK, Leebens-Mack JH, Lindsay BG, Soltis DE, Doyle JJ, Soltis PS, Carlson JE, Arumuganathan K, Barakat A, Albert VA, Ma H, dePamphilis CW. Widespread genome duplications throughout the history of flowering plants. *Genome Research.* 2006;16:738–749. [PMC free article] [PubMed] [Google Scholar]

Cusimano N, Bogner J, Mayo SJ, Boyce PC, Wong SY, Hesse M, Hetterscheid WLA, Keating RC, French JC. Relationships within the Araceae: comparison of morphological patterns with molecular phylogenies. *American Journal of Botany.* 2011;98:654–668. [PubMed] [Google Scholar]

Devos KM, Brown JKM, Bennetzen JL. Genome size reduction through illegitimate recombination counteracts genome expansion in *Arabidopsis*. *Genome Research.* 2002;12:1075–1079. [PMC free article] [PubMed] [Google Scholar]

Doležel J, Greilhuber J. Nuclear genome size: are we getting closer? *Cytometry Part A.* 2010;77A:635–642. [PubMed] [Google Scholar]

Doyle JJ, Egan AN. Dating the origins of polyploidy events. *New Phytologist.* 2009;186:73–85. [PubMed] [Google Scholar]

Fawcett JA, Maere S, Van de Peer Y. Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction event. *Proceedings of the National Academy of Sciences of the USA.* 2009;106:5737–5742. [PMC free article] [PubMed] [Google Scholar]

Galbraith D, Harkins K, Maddox J, Ayres N, Sharma D, Firoozabady E. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science.* 1983;220:1049–1051. [PubMed] [Google Scholar]

Geber G. Vienna: University of Vienna; 1989. Zur Karyosystematik der Lemnaceae. PhD Dissertation. [Google Scholar]

Ghosh P, Mukherjee S, Sharma A. Cytophotometric estimation of *in situ* DNA content in several species of Araceae. *Cytobios.* 2001;105:177–183. [PubMed] [Google Scholar]

Gorbunova V, Levy AA. How plants make ends meet: DNA double-strand break repair. *Trends in Plant Science.* 1999;4:263–269. [PubMed] [Google Scholar]

Govaerts R, Bogner J, Boos J, Boyce P, Cosgriff B, Croat T, Gonçalves E, Grayum M, Hay A, Hetterscheid W, Ittenbach S, Landolt E, Mayo S, Murata J, Nguyen VD, Sakuragui CM, Singh Y, Thompson S, Zhu G. 2011. *World checklist of Araceae*http://www.kew.org/wcsp/ 16 September 2011.

Greilhuber J. Intraspecific variation in genome size: a critical reassessment. *Annals of Botany.* 1998;82:27–35. [Google Scholar]

Greilhuber J. Intraspecific variation in genome size in angiosperms: identifying its existence. *Annals of Botany.* 2005;95:91–98. [PMC free article] [PubMed] [Google Scholar]

Greilhuber J, Doležel J, Lysák MA, Bennett MD. The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. *Annals of Botany.* 2005;95:255–260. [PMC free article] [PubMed] [Google Scholar]

Hawkins JS, Kim H, Nason JD, Wing RA, Wendel JF. Differential lineage-specific amplification of transposable elements is responsible for genome size variation in *Gossypium*. *Genome Research.* 2006;16:1252–1261. [PMC free article] [PubMed] [Google Scholar]

Hirochika H, Sugimoto K, Otsuki Y, Tsugawa H, Kanda M. Retrotransposons of rice involved in mutations induced by tissue culture. *Proceedings of the National Academy of Sciences of the USA.* 1996;93:7783–7788. [PMC free article] [PubMed] [Google Scholar]

Hodgson JG, Sharafi M, Jalili A, Díaz S, Montserrat-Martí G, Palmer C, Cerabolini B, Pierce S, Hamzehee B, Asri Y, Jamzad Z, Wilson P, Raven JA, Band SR, Basconcelo S, Bogard A, Carter G, Charles M, Castro-Díez P, Cornelissen JHC, Funes G, Jones G, Khoshnevis M, Pérez-Harguindeguy N, Pérez-Rontomé MC, Shirvany FA, Vendramini F, Yazdani S, Abbas-Azimi R, Boustani S, Dehghan M, Guerrero-Campo J, Hynd A, Kowsary E, Kazemi-Saeed F, Siavash B, Villar-Salvador P, Craigie R, Naqinezhad A, Romo-Díez A, de Torres Espuny L, Simmons E. Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? *Annals of Botany.* 2010;105:573–584. [PMC free article] [PubMed] [Google Scholar]

Hoshino A, Inagaki Y, Iida S. Structural analysis of *Tpn1*, a transposable element isolated from Japanese morning glory bearing variegated flowers. *Molecular and General Genetics.* 1995;247:114–117. [PubMed] [Google Scholar]

Jones N, Houben A. B chromosomes in plants: escapees from the A chromosome genome? *Trends in Plant Science.* 2003;8:417–423. [PubMed] [Google Scholar]

Jones RN, Rees H. *B chromosomes.* London: Academic Press; 1982. [Google Scholar]

Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH. Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. *Proceedings of the National Academy of Sciences of the USA.* 2000;97:6603–6607. [PMC free article] [PubMed] [Google Scholar]

Kamemoto H, Kuehnle AR. *Breeding Anthuriums in Hawai`i.* Honolulu: University of Hawai`i Press; 1996. [Google Scholar]

Kirik A, Salomon S, Puchta H. Species-specific double-strand break repair and genome evolution in plants. *EMBO J.* 2000;19:5562–5566. [PMC free article] [PubMed] [Google Scholar]

Knight CA, Molinari NA, Petrov DA. The large genome constraint hypothesis: evolution, ecology and phenotype. *Annals of Botany.* 2005;95:177–190. [PMC free article] [PubMed] [Google Scholar]

Kuehnle AR, Amore TD, Kamemoto H, Kunisaki JT, Lichty JS, Uchida JY. 2004. New plants for Hawai'i anthurium cultivar release: 'Princess Aiko' ('Imperial') and 'Regina', two novelty anthuriums. Research Extension Series 152, Publication NPH-A-7. 2 pp.

Lagercrantz U. Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that *Brassica* genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. *Genetics.* 1998;150:1217–1228. [PMC free article] [PubMed] [Google Scholar]

Leitch IJ, Bennett MD. Genome downsizing in polyploid plants. *Biological Journal of the Linnean Society.* 2004;82:651–663. [Google Scholar]

Leitch IJ, Chase MW, Bennett MD. Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. *Annals of Botany.* 1998;82:85–94. [Google Scholar]

Leitch IJ, Soltis DE, Soltis PS, Bennett MD. Evolution of DNA amounts across land plants (Embryophyta) *Annals of Botany.* 2005;95:207–217. [PMC free article] [PubMed] [Google Scholar]

Leitch IJ, Beaulieu JM, Cheung K, Hanson L, Lysak MA, Fay MF. Punctuated genome size evolution in Liliaceae. *Journal of Evolutionary Biology.* 2007;20:2296–2308. [PubMed] [Google Scholar]

Leitch IJ, Hanson L, Lim KY, Kovarik A, Chase MW, Clarkson JJ, Leitch AR. The ups and downs of genome size evolution in polyploid species of *Nicotiana* (Solanaceae) *Annals of Botany.* 2008;101:805–814. [PMC free article] [PubMed] [Google Scholar]

Leitch IJ, Beaulieu JM, Chase MW, Leitch AR, Fay MF. Genome size dynamics and evolution in monocots. *Journal of Botany.* 2010;2010 Available at **http://dx.doi.org/10.1155/2010/862516**. [Google Scholar]

Leong-Škorničková J, Šída O, Jarolímová V, Sabu M, Fér T, Trávníček P, Suda J. Chromosome numbers and genome size variation in Indian species of *Curcuma* (Zingiberaceae) *Annals of Botany.* 2007;100:505–526. [PMC free article] [PubMed] [Google Scholar]

Marutani M, Sheffer RD, Kamemoto H. Cytological analysis of *Anthurium andraeanum* (Araceae), its related taxa and their hybrids. *American Journal of Botany.* 1993;80:93–103. [Google Scholar]

Mayo SJ, Bogner J, Boyce P, Catherine E Royal Botanic Gardens Kew. *The genera of Araceae.* London: Royal Botanic Gardens, Kew; 1997. [Google Scholar]

McClintock B. *Regulation of pattern of gene expression by controlling elements in maize.* Washington, DC: Yearbook of The Carnegie Institution of Washington; 1965–1966. [Google Scholar]

Mookerjea A. Cytology of different species of aroids with a view to trace the basis of their evolution. *Caryologia.* 1955;7:221–291. [Google Scholar]

Ohno S, Hosokawa M, Hoshino A, Kitamura Y, Morita Y, Park K-I, Nakashima A, Deguchi A, Tatsuzawa F, Doi M, Iida S, Yazawa S. A bHLH transcription factor, DvIVS, is involved in regulation of anthocyanin synthesis in dahlia (*Dahlia variabilis*) *Journal of Experimental Botany.* 2011;62:5105–5116. [PMC free article] [PubMed] [Google Scholar]

Ohri D. Genome size variation and plant systematics. *Annals of Botany.* 1998;82:75–83. [Google Scholar]

Peschke VM, Phillips RL. Activation of the maize transposable element *Suppressor-mutator* (*Spm*) in tissue culture. *Theoretical and Applied Genetics.* 1991;81:90–97. [PubMed] [Google Scholar]

Petersen G. Cytology and systematics of Araceae. *Nordic Journal of Botany.* 1989;9:119–166. [Google Scholar]

Praça-Fontes M, Carvalho C, Clarindo W, Cruz C. Revisiting the DNA C-values of the genome size-standards used in plant flow cytometry to choose the 'best primary standards. *Plant Cell Reports.* 2011;30:1183–1191. [PubMed] [Google Scholar]

Rabinowicz PD, Bennetzen JL. The maize genome as a model for efficient sequence analysis of large plant genomes. *Current Opinion in Plant Biology.* 2006;9:149–56. [PubMed] [Google Scholar]

SanMiguel P, Tikhonov A, Jin Y-K, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL. Nested retrotransposons in the intergenic regions of the maize genome. *Science.* 1996;274:765–768. [PubMed] [Google Scholar]

Sharma AK, Bhattacharyya UC. Structure and behavior of chromosomes in species of *Anthurium* with special reference to the accessory chromosomes. *Proceedings of the National Institute of India, Part B, Biological Science.* 1961;27:317–328. [Google Scholar]

Sheffer RD, Croat TB. Chromosome numbers in the genus *Anthurium* (Araceae) II. *American Journal of Botany.* 1983;70:858–871. [Google Scholar]

Sheffer RD, Kamemoto H. Chromosome numbers in genus *Anthurium*. *American Journal of Botany.* 1976;63:74–81. [Google Scholar]

Shirasu K, Schulman AH, Lahaye T, Schulze-Lefert P. A contiguous 66-kb barley DNA sequence provides evidence for reversible genome expansion. *Genome Research.* 2000;10:908–915. [PMC free article] [PubMed] [Google Scholar]

Soltis DE, Soltis PS, Bennett MD, Leitch IJ. Evolution of genome size in the angiosperms. *American Journal of Botany.* 2003;90:1596–1603. [PubMed] [Google Scholar]

Suda J, Leitch IJ. The quest for suitable reference standards in genome size research. *Cytometry Part A.* 2010;77A:717–720. [PubMed] [Google Scholar]

Teoh SB, Rees H. Nuclear DNA amounts in populations of *Picea* and *Pinus* species. *Heredity.* 1976;36:123–137. [Google Scholar]

Vicient CM, Kalendar R, Anamthawat-Jonsson K, Schulman AH. Structure, functionality, and evolution of the BARE-1 retrotransposon of barley. *Genetica.* 1999;107:53–63. [PubMed] [Google Scholar]

Vision TJ, Brown DG, Tanksley SD. The origins of genomic duplications in *Arabidopsis*. *Science.* 2000;290:2114–7. [PubMed] [Google Scholar]

Wang X, Shi X, Hao B, Ge S, Luo J. Duplication and DNA segmental loss in the rice genome: implications for diploidization. *New Phytologist.* 2005;165:937–946. [PubMed] [Google Scholar]

Wendel JF. Genome evolution in polyploids. *Plant Molecular Biology.* 2000;42:225–249. [PubMed] [Google Scholar]

Yogeeswaran K, Frary A, York TL, Amenta A, Lesser AH, Nasrallah JB, Tanksley SD, Nasrallah ME. Comparative genome analyses of *Arabidopsis* spp.: inferring chromosomal rearrangement events in the evolutionary history of *A. thaliana*. *Genome Research.* 2005;15:505–515. [PMC free article] [PubMed] [Google Scholar]

Zonneveld BJM. New record holders for maximum genome size in eudicots and monocots. *Journal of Botany.* 2010;2010 Available at **http://dx.doi.org/10.1155/2010/527357**. [Google Scholar]