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

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Received: 20 November 2011; Returned for revision: 16 December 2011; Accepted: 17 February 2012; Published: 29 February 2012

Citation details: Bliss BJ, Suzuki JY. 2012. Genome size in *Anthurium* evaluated in the context of karyotypes and phenotypes. AoB PLANTS 2012: pls006; doi:10.1093/aobpla/pls006

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 collected from *Anthurium* species of different subgeneric groups and from commercial cultivars. The most current and well-supported topology of subgeneric, sectional relationships was applied to present 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.

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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 through 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 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 Kuehnl 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; Vicent 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 Spirdela (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 (Carlisen 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 Calomystrum 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; Prac¸a-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).

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; Carlsten and Croat 2007; Carlsten 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. Carlsten sampled 84 species that we did not include, and we sampled 16 species that Carlsten did not include (Carlsten 2011). These are placed according to their existing sectional assignment (Carlsten 2011). The relationship of the monotypic section Gymnopus to other sections has not been determined (Fig. 1).

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 meiosis (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
Table 1: Genome sizes of accessions sampled, listed alphabetically by Anthurium species, followed by cultivars

| Species name                      | Accession          | Genome sizing standard | Genome sizea (pg/2 C) ± S.D. (n=4) | DNA content (Mbp/1 C)b |
|----------------------------------|--------------------|------------------------|------------------------------------|------------------------|
| A. amnicola Dressler             | ABG 19911372       | W                      | 10.53 ± 0.11                       | 5147                   |
|                                  | MBG 84952          | W                      | 10.81 ± 0.57                       | 5287                   |
|                                  | MSBG 1976-0053-002A| W                      | 9.74 ± 0.21                        | 4765                   |
| A. andraeanum Linden             | ABG 19911368       | W                      | 9.59 ± 1.20                        | 4688                   |
| A. andraeanum sp. aff. (presumed Hort.) | USBG s.n.       | W                      | 8.92 ± 0.05                        | 4631                   |
| A. antioquiense Engler           | MBG 81407a/b       | W                      | 10.35 ± 0.07                       | 5059                   |
|                                  | MBG 1996-0276A     | W                      | 9.91 ± 0.06                        | 4845                   |
|                                  | NYBG 1383/78*A*C   | W                      | 9.23 ± 0.14                        | 4512                   |
| A. armeniense Croat              | MBG 63434e         | W                      | 11.38 ± 0.15                       | 5563                   |
|                                  | MSBG 1979-1055A    | W                      | 12.64 ± 0.35                       | 6180                   |
| A. bakeri Hooker f.              | MBG 78747c         | W                      | 9.89 ± 0.08                        | 4835                   |
|                                  | NYBG 897/63*A*B*C  | W                      | 8.71 ± 0.35                        | 4260                   |
|                                  | USBG 77-0090       | W                      | 9.24 ± 0.13                        | 4519                   |
| A. cerracampanense Croat         | MBG 76663/b        | W                      | 11.73 ± 0.18                       | 5734                   |
|                                  | MSBG 1980-0429A    | W                      | 11.16 ± 0.09                       | 5455                   |
| A. clavigerum Poepp.& Endl.      | MSBG 1991-0174A    | W                      | 13.27 ± 0.17                       | 6490                   |
|                                  | ABG 20011433       | T                      | 15.26 +/−0.28                      | 7462                   |
| A. clidemioides Standl.          | JBVL 850002        | W                      | 9.47 ± 0.17                        | 4632                   |
|                                  | MBG 79567          | W                      | 8.86 ± 0.65                        | 4333                   |
| A. coriaceum G. Don              | NYBG 574/65*A      | W                      | 14.32 ± 0.17                       | 7003                   |
|                                  | ABG 20072399       | W                      | 8.79 ± 0.17                        | 4297                   |
| A. flexile ssp muelleri (J.F. Macbr.) Croat & Baker | MBG 100348 | W                      | 9.46 ± 0.19                        | 4628                   |
| A. formosum Schott               | MSBG 1991-0158A    | W                      | 8.77 ± 0.28                        | 4286                   |
| A. fragrantissimum Croat         | MBG 76860          | W                      | 6.21 ± 0.39                        | 3036                   |
| A. gracile (Rudge) Lindl.        | ABG 19980680       | W                      | 9.66 ± 0.20                        | 4721                   |
|                                  | MBG 95664          | W                      | 13.51 ± 0.22                       | 6607                   |
|                                  | MSBG 2001-0232A    | W                      | 13.98 ± 0.06                       | 6835                   |
| A. gymnopus Griseb.              | CJBN 2009.3.213    | A. coriaceum NYBG 574/65*A | 11.21 ± 0.14                     | 5482                   |
| A. hoffmanni Schott              | MBG 66203          | W                      | 9.56 ± 0.38                        | 4674                   |
|                                  | MSBG 1993-0176A    | W                      | 9.89 ± 0.09                        | 4835                   |
| A. kamemotoanum Croat            | UH s.n.            | W                      | 9.47 ± 0.07                        | 4629                   |
| A. lentii Croat & Baker          | MBG 35902          | W                      | 13.96 ± 0.06                       | 6824                   |
| A. leuconeurm Lemaire            | MSBG 1980-1683B    | W                      | 14.35 ± 0.07                       | 7016                   |
| A. lucens Standl. ex Yuncker     | MBG 78702          | W                      | 13.24 ± 0.50                       | 6473                   |
|                                  | NYBG 103/81*A*B    | W                      | 12.71 ± 0.38                       | 6216                   |
|                                  | NYBG 103/81*A*B    | W                      | 13.16 ± 0.68                       | 6435                   |

Continued
### Table 1 Continued

| Species name                  | Accession | Genome sizing standard | Genome size \(a\) (pg/2 C) ± S.D. \((n=4)\) | DNA content (Mbp/1 C) \(b\) |
|-------------------------------|-----------|------------------------|---------------------------------------------|-----------------------------|
| A. microspadix Schott         | MBG 100186 | W                      | 12.25 ± 0.13                               | 5992                        |
| A. nympheaefolium 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       | W                      | 14.52 ± 0.11                               | 7103                        |
| A. ravenii Croat & Baker      | MBG 74778  | W                      | 13.32 ± 0.29                               | 6515                        |
| 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 | ABG 19911433 | W                      | 9.98 ± 0.57                                | 4882                        |
| Scheffer                      | ABG 19980667 | W                      | 9.67 ± 0.15                                | 4726                        |
|                              | MBG 47671  | W                      | 9.28 ± 0.16                                | 4537                        |
| A. schlechtendali ssp. schlechtendali 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                        |
| A. solitium Schott            | JBM 2083-2000 | W                      | 14.69 ± 0.23                               | 7181                        |
|                              | MBG 53699  | W                      | 16.03 ± 0.21                               | 7840                        |
| A. warocqueanum J. Moore      | ABG 19930478 | W                      | 8.65 ± 0.14                                | 4232                        |
|                              | MBG 101538 | W                      | 8.97 ± 0.09                                | 4386                        |
|                              | MSBG 2006-0001A | W                      | 8.95 ± 0.12                                | 4374                        |
| A. watermaliense Hort ex. L.H. Bailey & Nash | MBG 78766 | W                      | 9.47 ± 0.32                                | 4632                        |
|                              | MSBG 1977-2832A | T                      | 7.57 ± 0.02                                | 3701                        |
| A. wendlingeri Barroso        | ABG 20072507 | W                      | 7.87 ± 0.20                                | 3846                        |
|                              | MBG 95418  | W                      | 7.04 ± 0.15                                | 3441                        |
|                              | MSBG 1977-1989A | T                      | 6.59 ± 0.31                                | 3221                        |
|                              | USBG 01-1412 | W                      | 8.31 ± 0.22                                | 4066                        |
| cv. ‘Marian Seefurth’         | HAIA-MS1   | W                      | 9.22 ± 0.07                                | 4509                        |
| cv. ‘Midori’                  | HAIA-Md1   | W                      | 9.48 ± 0.25                                | 4636                        |
| cv. ‘Miss June Purple’        | HAIA-MJP   | W                      | 14.05 ± 0.32                               | 6870                        |
| cv. ‘New Pahoa Red’           | HAIA-NPR2  | W                      | 8.93 ± 0.08                                | 4367                        |
| cv. ‘Princess Aiko’           | NG-10-02-Aiko | W                      | 9.39 ± 0.27                                | 4592                        |
| cv. ‘Puanani’                 | NG-10-Pu   | W                      | 9.44 ± 0.10                                | 4616                        |
Table 1 Continued

| Species name         | Accession | Genome sizing standard | Genome size<sup>a</sup> (pg/2 C) ± S.D. (n=4) | DNA content (Mbp/1 C)<sup>b</sup> |
|----------------------|-----------|------------------------|------------------------------------------|----------------------------------|
| cv. ‘Purple Passion’ | NG-10-PP  | W                      | 10.82 ± 0.14                             | 5291                             |
| cv. ‘Regina’         | NG-10-1-Reg | W                      | 9.80 ± 0.22                              | 4792                             |
| cv. ‘Shibori’        | NG-10-Shi | W                      | 9.73 ± 0.20                              | 4758                             |

Mbp, million base pairs; W, wheat (Triticum aestivum cv. ‘Zok,’ 30.55 pg/2 C); T, tobacco (Nicotiana tabacum cv. ‘SR-1’ 9.32 pg/2 C); ABG, Atlanta Botanical Garden; CJB, 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; HAI, Hawaiian Anthurium Industry Association.

<sup>a</sup>Genome size and standard deviation have been rounded to two decimal places.

<sup>b</sup>Mbp/1 C DNA for plant species is based on 1 pg DNA (10<sup>−12</sup> Mbp, million base pairs).

Table 1 Continued

| Species name         | Accession | Genome sizing standard | Genom...
Calomystrium, Xialophyllium) with the exception of A. roseospadix (Fig. 1). The 2N = 35 chromosome count report for A. leuconeum 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).

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.
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 *Dactylophyllum*, 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 Kuehne 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) 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.

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) (Kame moto 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 Coot 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 *Pachyneuriun*, 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. nymphaeafolium* (*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 *Polyphyllum*, 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 Yogeewaran 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 *Polyphyllum* 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. cidemoides* is unknown. However, polyploids arising by interspecies hybridization (allopolyploidy) are subject to mismatch repair during recombination of homologous 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 investigation. 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 extra-chromosomal 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 31 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.

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.

Conflict of interest statement

None declared.

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