

## Evolution of Genome Size in Brassicaceae

J. SPENCER JOHNSTON<sup>1,\*</sup>, ALAN E. PEPPER<sup>2</sup>, ANNE E. HALL<sup>4</sup>, Z. JEFFREY CHEN<sup>3</sup>,  
GEORGE HODNETT<sup>3</sup>, JANICE DRABEK<sup>2</sup>, REBECCA LOPEZ<sup>2</sup> and H. JAMES PRICE<sup>2</sup>

<sup>1</sup>Department of Entomology, <sup>2</sup>Department of Biology, <sup>3</sup>Department of Soil and Crop Sciences,  
Texas A&M University, College Station, TX 77843, USA and <sup>4</sup>Department of Molecular Genetics and Cell Biology,  
The University of Chicago, Chicago, IL 60637, USA

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• **Background and Aims** Brassicaceae, with nearly 340 genera and more than 3350 species, anchors the low range of angiosperm genome sizes. The relatively narrow range of DNA content (0.16 pg < 1C < 1.95 pg) was maintained in spite of extensive chromosomal change. The aim of this study was to erect a cytological and molecular phylogenetic framework for a selected subset of the Brassicaceae, and use this as a template to examine genome size evolution in Brassicaceae.

• **Methods** DNA contents were determined by flow cytometry and chromosomes were counted for 34 species of the family Brassicaceae and for ten *Arabidopsis thaliana* ecotypes. The amplified and sequenced ITS region for 23 taxa (plus six other taxa with known ITS sequences) were aligned and used to infer evolutionary relationship by parsimony analysis.

• **Key Results** DNA content in the species studied ranged over 8-fold (1C = 0.16–1.31 pg), and 4.4-fold (1C = 0.16–0.71 pg) excluding allotetraploid *Brassica* species. The 1C DNA contents of ten *Arabidopsis thaliana* ecotypes showed little variation, ranging from 0.16 pg to 0.17 pg.

• **Conclusions** The tree roots at an ancestral genome size of approximately 1x = 0.2 pg. *Arabidopsis thaliana* (1C = 0.16 pg; ~157 Mbp) has the smallest genome size in Brassicaceae studied here and apparently represents an evolutionary decrease in genome size. Two other branches that represent probable evolutionary decreases in genome size terminate in *Lepidium virginicum* and *Brassica rapa*. Branches in the phylogenetic tree that represent probable evolutionary increases in genome size terminate in *Arabidopsis halleri*, *A. lyrata*, *Arabis hirsuta*, *Capsella rubella*, *Caulanthus heterophyllus*, *Crucihimalaya*, *Lepidium sativum*, *Sisymbrium* and *Thlaspi arvense*. Branches within one clade containing *Brassica* were identified that represent two ancient ploidy events (2x to 4x and 4x to 6x) that were predicted from published comparative mapping studies. © 2005 Annals of Botany Company

**Key words:** *Arabidopsis*, Brassicaceae, ITS phylogeny, DNA content, genome size, chromosome number.

### INTRODUCTION

Genome size varies over 500-fold among angiosperms. The diploid species with the largest reported 1C DNA content is *Fritillaria davisii*,  $n = 12$  (90 pg DNA or ~88 000 Mbp; Bennett and Smith, 1976). One of the smallest 1C DNA contents is in *Arabidopsis thaliana*,  $n = 5$  (0.16 pg or ~157 Mbp DNA; Bennett *et al.*, 2003). The biological and evolutionary significance of the massive variation in DNA content is an unsolved puzzle of evolutionary biology. Research on the evolution of DNA content has been conducted in numerous laboratories over the last 40 years, but the lack of a clear phylogenetic framework limited the ability to resolve direction of evolutionary changes in DNA content. However, the recent use of DNA sequence data for assembling evolutionary trees has allowed variation in DNA content to be analyzed in a phylogenetic context (Bennetzen and Kellogg, 1997; Leitch, *et al.*, 1998, 2005; Wendel *et al.*, 2002; Soltis *et al.*, 2003; Price *et al.*, 2005).

Here we report a study of genome size in Brassicaceae. This family is large, consisting of about 340 genera and more than 3350 species (Al-Shehbaz, 1984). Brassicaceae is of particular interest from a genome evolution perspective

because it has nuclear DNA contents that anchor the low range of angiosperm values, and has a relatively narrow range of DNA content compared to families such as Poaceae (1C DNA content = 0.3 pg to 26.0 pg), Fabaceae (1C DNA content = 0.4 pg to 27.4 pg) and Liliaceae (1C DNA content = 12.4 pg to 127.4 pg; Plant DNA C-values Database <http://www.rbgekew.org.uk/cval/homepage.html>). No DNA contents above 1C = 1.95 pg have been reported for Brassicaceae (Plant DNA C-values Database). Furthermore, numerous molecular comparisons among species of Brassicaceae have been conducted, particularly among crop species in the genus *Brassica* (see Hall, *et al.*, 2002). In spite of the relatively conservative range of DNA content, extensive structural genome evolution has occurred involving chromosome duplication, rearrangements and fusions (Lagercrantz, 1998; Lan *et al.*, 2000).

The relatively small genomes also make Brassicaceae excellent choices for genome evolution studies. The *Arabidopsis* Genome Initiative (2000) produced over 73 % of the nuclear genomic sequence of *A. thaliana* (see Bennett *et al.*, 2003); and unprecedented genomic studies, which are now possible within a dicot species, are being extended to wild relatives of *Arabidopsis* through comparative studies (Acarkan *et al.*, 2000; Rossberg *et al.*, 2001) in which genome size evolution will be of additional interest.

\* For correspondence. E-mail spencerj@tamu.edu

## MATERIALS AND METHODS

### Plant material

The Brassicaceae species used and their sources are listed in Tables 1 and 2. Plants were grown in the University of Chicago greenhouse or in an environmental chamber at Texas A&M University on a light/dark regime of 14 h (22 °C)/10 h (17 °C).

### Chromosome counts

Chromosome counts were made by the protocol of Jewell and Islam-Faridi (1994) with slight modification as described by Price *et al.* (2005).

### Determination of DNA content

At least three plants for each species were analyzed to obtain the mean DNA content. Newly expanded leaves of the target species and of a standard species were co-chopped with a sharp razor blade and prepared using the procedures and flow cytometer as described by Price *et al.* (2005). *Arabidopsis thaliana* ecotype Columbia (1C DNA content = 0.16 pg; Bennett *et al.*, 2003) and/or *Sorghum bicolor* 'TX623' (1C DNA content = 0.835 pg; Price *et al.*, 2005) were used as internal calibration standards.

### DNA sequence analysis

DNA was extracted from leaves of single individuals, either by the method described in Pepper and Norwood (2001) or the CTAB procedure (Doyle and Doyle, 1987). DNA of ribosomal internal transcribed spacer (ITS) fragments was amplified using primers ITS4 and ITS5 (White *et al.*, 1990). Polymerase chain reaction (PCR) was performed using established conditions (Konieczny and Ausubel, 1993) in 50 mL reactions containing 10–20 ng of genomic DNA. The resulting PCR products were purified using the QIAquick PCR purification columns (Qiagen, Valencia, California, USA), then 20–30 ng of purified double-stranded PCR product was directly used as template for 35 cycles of sequencing using BigDye Version 3.0 terminator chemistry (Applied Biosystems, Foster City, California, USA). The primers used for direct sequencing were ITS4, ITS5, ITS11 (5'-ATCTCGGCTCTCGCATCG-ATG-3') and ITS12 (5'-CAAAGACTCGATGGTTCACG-3'). Cycle-sequencing products were purified by Bio-Gel P-30 size exclusion chromatography (Bio-Rad, Richmond, California, USA) and analyzed using an ABI3100 capillary sequencer (Applied Biosystems). Double-stranded DNA sequence contigs from each taxon were assembled and edited using Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA). Finished sequences from the various taxa were aligned using Clustal W (Thompson *et al.*, 1994). Alignments across insertion/deletion differences (indels) were then verified manually.

### Phylogenetic inference

Phylogeny reconstructions were performed using PAUP\* 4.0.1b3a (Swofford, 1999). For parsimony analysis, indels

of one nucleotide or longer were defined as a new (5th) character state. Ambiguous nucleotides (e.g. divergent paralogues) were defined as polymorphisms. Branch and bound searches using unweighted parsimony and accelerated transformation (ACCTRAN) of character state optimization were performed to identify the most-parsimonious trees. Branches of zero length were collapsed. For neighbour-joining analysis (Saitou and Nei, 1987), Kimura two-parameter distances (Kimura, 1980) and a minimum evolution objective function using the branch-and-bound method were employed, and ambiguous and 'missing' data (e.g. indels and divergent paralogues) were ignored. *Cleome hassleriana* (Brassicaceae) was used as an outgroup. Relative support for various clades was determined by bootstrap analysis (Felsenstein, 1985) employing 1000 replicates using a 'branch and bound' search strategy.

## RESULTS

### DNA content

DNA content and chromosome numbers of the 32 species of Brassicaceae are listed in Table 1. DNA content among the species of this study ranged over 8-fold from 1C = 0.16 pg (*A. thaliana*) to 1.31 pg (*B. carinata*). Excluding the allotetraploid *Brassica* species, 1C DNA content ranges 4.4-fold from 0.16 pg in *A. thaliana* to 0.71 pg in *B. oleracea*.

*Arabidopsis suecica* ( $2n = 4x = 26$ ) is a tetraploid with genomes contributed from *A. arenosa* ( $2n = 16$ ) and *A. thaliana* ( $2n = 10$ ) (O'Kane, *et al.*, 1996). The *A. arenosa* used in the current study is a tetraploid with  $2n = 4x = 32$  chromosomes and, therefore, the diploid 1C DNA content extrapolates to 0.21 pg. The 1C DNA content determined for *A. suecica* (0.355 pg) is proportionately less (96 %) than the sum of 0.21 pg and the 1C value of 0.16 for *A. thaliana*.

Three species, *B. napus*, *B. juncea* and *B. carinata*, are allotetraploids derived from *B. rapa* × *B. oleracea*, *B. rapa* × *B. nigra* and *B. nigra* × *B. oleracea*, respectively (U, 1935). The DNA content of these three amphidiploids is proportionately less (92 %, 92 % and 97 %, respectively) than the sum of the DNA content of their putative parental species.

The genome sizes of ten diverse ecotypes of *A. thaliana* are presented in Table 2. The 1C DNA content of all these biotypes is 0.16 pg, except for the ecotypes En-0 and Cvi-0, which were 0.17 pg. Although these differences are statistically significant, they are none-the-less small, and based upon three replicates may not be real.

### Phylogenetic reconstruction based on ribosomal DNA ITS sequences

ITS sequences for 22 taxa of Brassicaceae were determined (GenBank accession numbers AY662277–AY662298). Additional ITS sequences of *Caulanthus heterophyllus* var. *heterophyllus*, *C. heterophyllus* var. *pseudosimulans*, *C. amplexicaulis* var. *amplexicaulis* and *Guillenia lasiophylla* were determined previously (Pepper and Norwood, 2001), and ITS sequences of *A. thaliana* and *L. rapa* were obtained from Yang *et al.* (1999).

TABLE 1. Genomic characteristics of species of the Brassicaceae

Species	Source	Stock number	Chromosome number (2n)	1C Nuclear DNA Content (pg ± s.e)	Duncan Multiple Range Grouping <sup>7</sup>	Genome size (1x) (Mbp)
<i>Arabidopsis arenosa</i> (L.) Lawalrée	C. Pikaard		32 (4x)	0.417 ± 0.001	K	203
<i>Arabidopsis halleri</i> <sup>3</sup> (L.) O'Kane & Shehbaz ssp. gemmifera (Matsum.) O'Kane & Al-Shehbaz	SASSC <sup>1</sup>	J020	16 <sup>4</sup>	0.261 ± 0.004	Q	255
<i>Arabidopsis lyrata</i> (L.) O'Kane & Al-Shehbaz	S. O'Kane		32 <sup>4</sup> (4x)	0.468 ± 0.003	J	230
<i>Arabidopsis suecica</i> <sup>3</sup> (Fries) Norrlin	SASSC	JS6	26 (4x)	0.356 ± 0.001	M	174
<i>Arabidopsis thaliana</i> (L.) Heynh. ecotype Columbia	NASSC <sup>2</sup>		10 <sup>4</sup>	0.160 ± 0.001	T	157
<i>Arabis hirsuta</i> (L.) Scop.	SASSC	J023	32 <sup>6</sup> (4x)	0.686 ± 0.005	E	335
<i>Brassica carinata</i> L.	T. Osborn		34 (4x)	1.308 ± 0.012	A	642
<i>Brassica juncea</i> (L.) Czern.	T. Osborn		36 (4x)	1.092 ± 0.001	C	534
<i>Brassica napus</i> L.	T. Osborn		38 (4x)	1.154 ± 0.006	B	566
<i>Brassica nigra</i> (L.) Koch	T. Osborn		16	0.647 ± 0.009	F	632
<i>Brassica oleracea</i> L.	T. Osborn		18	0.710 ± 0.002	D	696
<i>Brassica rapa</i> L.	T. Osborn		20	0.539 ± 0.018	I	529
<i>Capsella bursa-pastoris</i> <sup>3</sup> (L.) Medik	SASSC	J022	32 <sup>4</sup> (4x)	0.414 ± 0.002	K	203
<i>Capsella rubella</i> <sup>3</sup> Reuter	Italy		16 <sup>4,6</sup>	0.257 ± 0.005	Q	250
<i>Cardamine amara</i>	L. Luczaj		16 <sup>6</sup>	0.225 ± 0.001	R S	221
<i>Cardamine hirsuta</i> <sup>3</sup> L.	SASSC	J027	16 <sup>4</sup>	0.229 ± 0.001	R S	225
<i>Cardamine impatiens</i> <sup>3</sup> L.	SASSC	J010	16 <sup>4</sup>	0.212 ± 0.009	S	206
<i>Caulanthus amplexicaulis</i> var. <i>barbarae</i>	A. Pepper		28 <sup>8</sup>	0.377 ± 0.001	L	372
<i>Caulanthus heterophyllus</i> (Nutt.) Payson var. <i>heterophyllus</i>	A. Pepper		28 <sup>8</sup>	0.701 ± 0.008	D E	686
<i>Caulanthus heterophyllus</i> (Nutt.) Payson var. <i>pseudosimulans</i> R. Buck	A. Pepper		28 <sup>8</sup>	0.687 ± 0.003	E	671
<i>Cleome hassleriana</i> Chodat	B&T Seeds		20 <sup>4</sup>	0.307 ± 0.003	P	299
<i>Crucihimalaya himalaica</i> (Edgeworth) Al-Shehbaz, O'Kane & R. A. Price	SASSC	J018	16 <sup>4</sup>	0.323 ± 0.004	N O P	319
<i>Crucihimalaya wallichii</i> <sup>3</sup> (J.D. Hooker & Thompson) Al-Shehbaz, O'Kane & R. A. Price	SASSC	J012	16 <sup>4</sup>	0.330 ± 0.011	N O	323
<i>Draba nemorosa</i> <sup>3</sup> L.	SASSC	J021	16 <sup>4</sup>	0.242 ± 0.002	Q R	235
<i>Guillenia lasiophyllum</i>	A. Pepper		28 <sup>8</sup>	0.384 ± 0.001	L	377
<i>Lepidium sativum</i> <sup>3</sup> L.	B&T Seeds	2309	24 <sup>4</sup> (3x)	0.582 ± 0.001	G	380
<i>Lepidium virginicum</i> <sup>3</sup> L.	SASSC	J26	32 <sup>4</sup> (4x)	0.333 ± 0.001	N	164
<i>Olimarabidopsis cabulica</i> <sup>3</sup> (J.D. Hooker and Thompson) Al-Shehbaz, O'Kane & R. A. Price	SASSC	JS4	48 <sup>5</sup> (4x?)	0.420 ± 0.004	K	206
<i>Olimarabidopsis pumila</i> <sup>3</sup> (Stephan) Al-Shehbaz, O'Kane & R. A. Price	SASSC	JS3	30 <sup>4</sup> (4x?)	0.416 ± 0.005	K	203
<i>Raphanus sativus</i> L.	T. Osborn		18 <sup>6</sup>	0.583 ± 0.006	G	573
<i>Sinapis alba</i> <sup>3</sup> L.	B&T Seeds	51709	24 <sup>6</sup>	0.566 ± 0.006	G H	553
<i>Sisymbrium irio</i> <sup>3</sup> L.	SASSC	J04	28 <sup>4</sup> (4x)	0.533 ± 0.002	I	262
<i>Sisymbrium orientale</i> <sup>3</sup> L.	SASSC	J19	14 <sup>4</sup>	0.312 ± 0.003	O P	304
<i>Thlaspi arvense</i> <sup>3</sup> L.	Chicago		14 <sup>4</sup>	0.548 ± 0.005	H I	539

<sup>1</sup> Arabidopsis Seed Stock Center, Sendai, Japan.<sup>2</sup> Arabidopsis Seed Stock Centre, Nottingham, United Kingdom.<sup>3</sup> Identification confirmed by Dr. Ihsan Al-Shehbaz at the Missouri Botanical Gardens.<sup>4</sup> Chromosome numbers determined in this study.<sup>5</sup> Koch *et al.*, 1999.<sup>6</sup> Hall *et al.*, 2002.<sup>7</sup> Alpha = 0.05.<sup>8</sup> Rollins, 1983.

Analysis using ClustalW yielded an alignment of ±599 nucleotides containing ITS1, the 5.8S ribosomal RNA gene, and ITS2. Nucleotide ambiguities consisted of a G/A dimorphism in ITS1 of *C. himalaica*, a G/A dimorphism in ITS2 of *Draba nemorosa*, and a C/T dimorphism in the 5.8S ribosomal RNA gene and a G/A dimorphism in ITS2 of *C. wallichii*. No other ambiguous nucleotides were observed.

A 20-nucleotide region (located in ITS1), in which alignments across several indel polymorphisms could not be confidently resolved, was excluded from further analyses. The modified alignment had 579 characters, of which 211

were parsimony-informative. Parsimony analysis yielded a single most-parsimonious tree of 910 steps. The most-parsimonious tree (Fig. 1) had a consistency index of 0.614 (0.543 excluding uninformative characters), retention index of 0.673, and homoplasy index of 0.393 (0.455 excluding uninformative characters). Neighbour-joining yielded a tree that differed from the parsimony tree only in the placement of *Guillenia lasiophylla* (this taxon was grouped with *Caulanthus heterophyllus* in the parsimony tree, while it was a sister group to the genus *Caulanthus* in the parsimony tree), and in the placement of a clade containing *Thlaspi* and *Lepidium* (sister to the clade containing

TABLE 2. Genome size of ten diverse ecotypes of *Arabidopsis thaliana*

Ecotype	Source/stock number*	Habitat	Origin	Elevation (m)	Latitude	1C DNA content (pg)	±s.e.	Duncan Grouping†
En-0	ABRC/CS1136	Sandy loam	Enkheim/Frankfurt, Germany	120	N50	0.172	0.003	A
Cvi-0	MartinKoornneef	Rocky wall with moss	Cape Verde Islands	1200	N16	0.168	0.001	A
Berkeley-0	Angus Murphy		Berkeley, CA, USA	50	N38	0.159	0.001	B
Buckhorn-0	Angus Murphy	Non-serpentine	Trinity Mountains, CA, USA		N30	0.162	0.002	B
Yo-0	ABRC/CS1622	Sandy valley floor	Yosemite National Park, CA, USA	1400	N37	0.160	0.001	B
Hod-O	ABRC/CS6178		Hodja-Obi-Garm, Tadjikistan	1800	~N38	0.161	0.001	B
La-er	MartinKoornneef		Landsberg, Germany	300	N48	0.159	0.001	B
Landsberg	Jeff Chen					0.162	0.001	B
Santa Clara-0	Angus Murphy	Serpentine outcrop	Santa Clara, CA, USA	100	N37	0.159	0.001	B
Tsu-1	ABRC/CS1640		Tsu, Japan	0 to 100	N34	0.158	0.001	B

\* ABRC = Arabidopsis Biological Research Center, Ohio State University, Columbus, Ohio, USA.

† Alpha = 0.05.

*Brassica* and *Cardamine* in neighbour-joining analysis, but sister to the clade containing *Arabidopsis* and *Capsella* in the parsimony tree). Neither pattern was strongly supported by bootstrap analysis.

Where there were overlapping taxa, the topological arrangements reported here were largely in agreement with those previously obtained through analysis of nucleotide sequences from the ITS (Koch *et al.*, 1999; Yang *et al.*, 1999; Pepper and Norwood, 2001; Hong *et al.*, 2003), coding and non-coding plastid DNA (Koch *et al.*, 2001; Pepper and Norwood, 2001; Yang *et al.*, 2002), chalcone synthase and alcohol dehydrogenase (Koch *et al.*, 2000; Koch *et al.*, 2001), and the S-locus related gene *SLR1* (Inaba and Nishio, 2002). Specifically, there were notable differences between our topological arrangements and that of Koch *et al.* (2001) in the placement of *Arabis hirsuta*, *Cardamine amara* and *Thlaspi arvense*. These differences most likely arise from overlapping but non-congruent sets of taxa sampled and different genes used (ITS vs. *CHS* and *matK*). For example, Koch *et al.* (2001) place *T. arvense* in a sister group to *Sisymbrium* and *Sinapis*, when using the combined *CHS* and *matK* dataset. These differences in taxonomic arrangement would not alter our interpretation of patterns of genome change in the taxa examined. Although not strongly supported throughout, our parsimony tree (which closely matched the neighbour-joining tree) was used as a framework for a preliminary phylogenetic analysis of genome size in Brassicaceae (Fig. 1).

The genome size and  $2n$  chromosome number are listed to the right of each species in the phylogeny (Fig. 1). The 1C value for known tetraploids, or species that are very likely tetraploids, was halved to give the 1x genome size. At each dichotomy, the presumed ancestral genome size was indicated.

Because the species sampled were a very small subset of the total Brassicaceae, and because the mode of genome size evolution is not known, a very conservative approach was used to calculate ancestral genome size. Assuming the genome size changes rarely and change is associated with speciation events, the assumed ancestral genome is the 1C value (rounded to one decimal place) common to members at each branch of the clade.

## DISCUSSION

Bennetzen and Kellogg (1997) proposed that plants may have a 'one-way ticket to genomic obesity' as a consequence of retroelement accumulation and polyploidy. Although this may be a general trend among the angiosperms as a whole (Leitch *et al.*, 1998; Soltis *et al.*, 2003), apparent decreases in genome size accompanying angiosperm phylogeny are also apparent (Soltis *et al.*, 2003). Evolutionary decreases in genome size have been detected in the evolution of cotton relatives (Wendel *et al.*, 2002) and in the evolution of species of the genus *Sorghum* (Price *et al.*, 2005). Although the evolution of DNA content in Brassicaceae is generally from low to high, the data support the concept of a dynamic nature of genome size evolution involving both increases and decreases.

The generally conservative nature of the evolution of genome size in Brassicaceae allows for the detection of changes in genome size, including polyploidization events, in a phylogenetic context. The ITS sequence phylogeny for Brassicaceae presents a tree (Fig. 1) that can be rooted with a genome size of about 0.2 pg (~200 Mbp). This is a tentative value. The number of species is small relative to the entire family, and the single outgroup has 1C = 0.3 pg. Further, the absence of a suitable model of the mechanism involved in genome change means no best method exists to estimate the base value. And yet, the value 0.2 pg, while tentative, is common to most branches in the phylogeny (Fig. 1) and, as such, serves as a good starting point to study genome size evolution in Brassicaceae. Assuming that the root 1C genome size is 0.2 pg, then the radiation of Brassicaceae has involved both increases and decreases in genome size. Since the family is rooted by small hypothetical genomes, we would expect that the general evolutionary trend should be to larger genomes. This was in fact observed; genome size decreased in one diploid species, *A. thaliana*, while genome size increased in eight other diploid species including *A. halleri*, *A. lyrata*, *C. rubella*, *C. himalaica*, *L. sativum*, *S. irio*, *S. orientale* and *T. arvense*. Two additional increases and one decrease that occurred concurrent with doubling and tripling of DNA content in diploidized species in the clade that includes *Brassica* and *Raphanus* are addressed below.

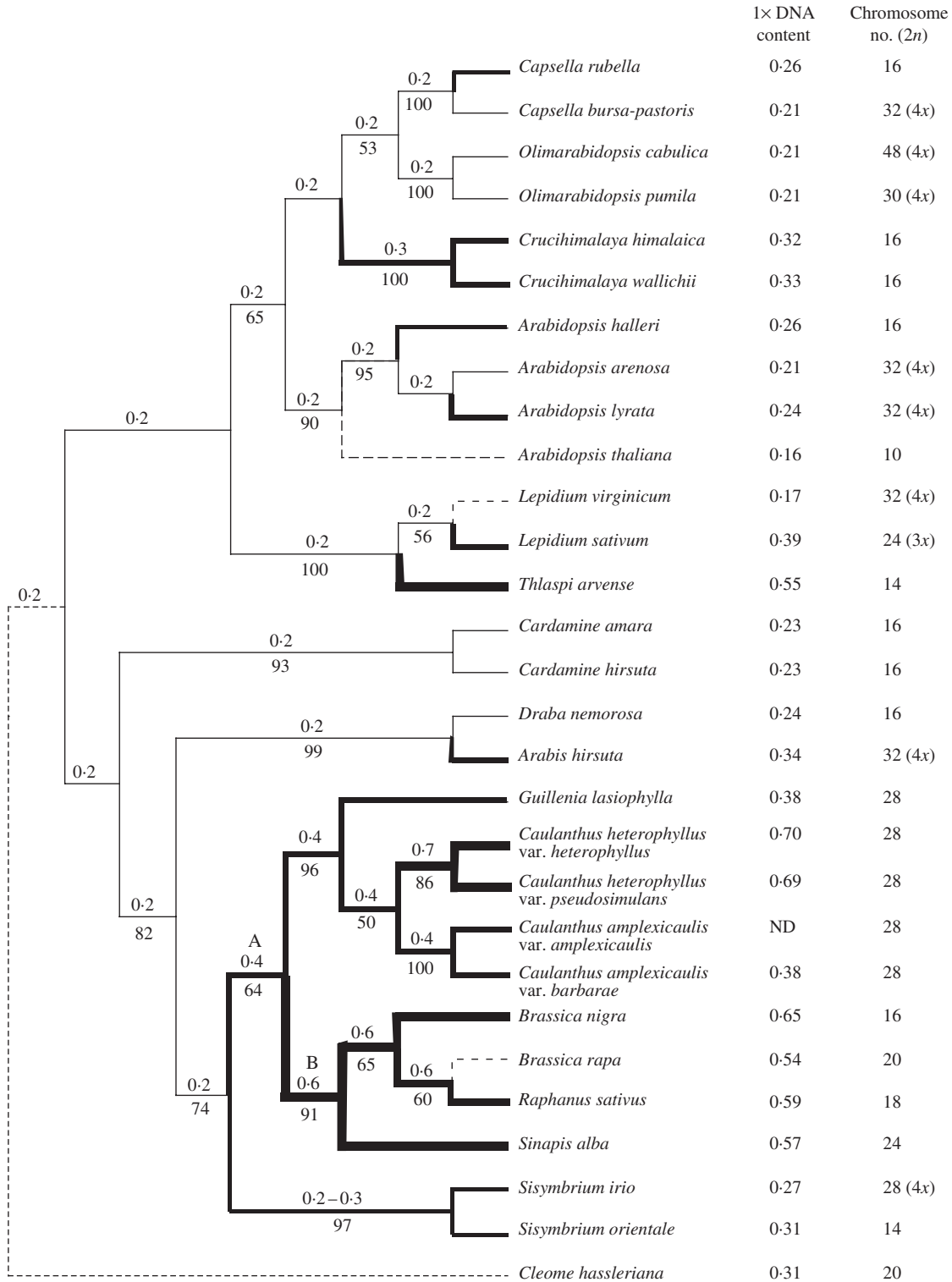


FIG. 1. Phylogenetic analysis of the evolution of genome size in representative members of Brassicaceae based on a single most-parsimonious ITS tree. Bootstrap support (%) for various nodes (1000 replicates) is indicated beneath the corresponding node. Nodes without an indicated bootstrap support had bootstrap values of less than 50 %. Genome size (1× DNA content) expressed in pg, and chromosome numbers for each taxon are indicated. Numbers above each node indicate a hypothetical ancestral genome size (pg). Thickness of the lines shown in the tree is used to illustrate hypothetical changes in genome size in the Brassicaceae family. A dashed line is used to indicate a decrease in genome size. *Cleome hassleriana* (Capperaceae) merely provides an outgroup for the phylogenetic analysis, and was not used to estimate the ancestral genome size of Brassicaceae. A and B indicate nodes discussed in the text.

Studies of comparative linkage maps (Lagercrantz and Lydiate, 1996; Lagercrantz, 1998; Lan *et al.*, 2000) indicate extensive duplication and triplication in the genomes of the 'diploid' *Brassica* species relative to *A. thaliana*, thus suggesting that modern *Brassica* species are descendants of a hexaploid ancestor. Evolution of extant diploid *Brassica* species involved extensive chromosomal rearrangement, including chromosome fusion events resulting in a reduction of chromosome number (Lagercrantz, 1998). In the phylogeny presented, branches where the putative polyploidization events occurred can be identified (Fig. 1, nodes A and B). Node A represents a hypothetical 2-fold increase (0.2 to 0.4 pg) in DNA content that would be expected in proceeding from the diploid to the tetraploid condition. Node B represents an increase from 0.4 to 0.6 pg, which would be expected going from tetraploid to hexaploid. When these putative polyploid events are assumed to be true, and the genome size of the putative polyploids is reduced to 1x to permit direct comparisons to the other 1x values in Fig. 1, the extent of genome size evolution in Brassicaceae is further apparent.

*Arabidopsis thaliana* has the smallest genome detected in this study and may represent the smallest genome in Brassicaceae. *Cardamine amara* has a reported genome size of 1C = 0.06 pg (Angiosperm DNA C-values Database) and until recently had been considered the smallest genome in Brassicaceae. However, we report a genome size of 1C = 0.23 pg (Table 1). Similar 1C values for *C. amara* was determined by L. Hanson and M. Bennett (see Bennett and Leitch, 2005) and J. Greilhuber (pers. com.).

The *Arabidopsis thaliana* genome of 1C = 0.16 pg (~157 Mbp) apparently represents an evolutionary decrease in genome size. Other branches representing probable evolutionary decreases in genome size terminate in *L. virginicum* and *B. rapa*. On the other hand, *Capsella rubella*, *Crucihimalaya*, *A. halleri*, *A. lyrata*, *L. sativum*, *Thlaspi arvense*, *Arabis hirsuta*, *Caulanthus heterophyllus* and *Sisymbrium* represent branches in the phylogenetic tree leading to increases in genome size.

Further decreases in DNA content have apparently occurred in the extant allopolyploids, *B. napus*, *B. juncea* and *B. carinata* formed from hybridization between *B. rapa* × *B. oleracea*, *B. rapa* × *B. nigra*, and *B. nigra* × *B. oleracea*. The DNA content of *B. napus*, *B. juncea* and *B. carinata*, respectively, is proportionally less (0.92, 0.92 and .97 pg, respectively) than the sum of the DNA content of their putative parental species. Similar reductions were seen for the tetraploid *A. suecica*, which has a genome size ~4 % less than the sum of its diploid ancestors. Narayan (1998) also observed that the genomes of these present-day allopolyploid *Brassica* species average >6 % less than the expected value. Genomic instability in artificially synthesized allopolyploids from crosses of *B. rapa* × *B. nigra* and of *B. rapa* × *B. oleracea* was detected as unexpected changes in restriction fragment profiles (Song *et al.*, 1995). Song *et al.* (1995) interpreted these observations as demonstrating rapid genomic changes following polyploid formation in Brassicaceae. If hybrid instability also influences DNA content, then the DNA content of polyploids is not necessarily expected to be strictly additive with respect to their progenitors.

Among angiosperms in general, DNA content variation greater than 2- to 3-fold is common among congeneric species. In well-studied genera such as *Microseris* (Price and Bachmann, 1975), *Lathyrus*, *Nicotiana*, *Clarkia* and *Allium* (Narayan, 1998) the DNA content variation is not continuous, but rather falls into discontinuous groupings. Although DNA content may vary greatly among congeneric species (Price, 1976) it is typically very constant within species, seldom exceeding the approximate 2–5 % resolution of microspectrophotometric and flow cytometric methods commonly used for determining nuclear DNA amount (see Bennett *et al.*, 2000). Relative constancy of C-value was observed among the ten geographically diverse ecotypes of *A. thaliana* included in the current study (Table 2). Only two of the biotypes were significantly different from the others and these differences were small.

Several observations suggest that genome size evolution is more than a slow equilibrium process resulting from accumulation of small insertions and deletions as hypothesized by Petrov (2002): (1) differences of 0.05 pg and 0.04 pg, respectively, were observed between the 1x genomes of sister species within the genera *Capsella* and *Sisymbrium*; while there are no differences between taxa separated for much greater periods of time such as *Capsella bursa-pastoris*, *Olimarabidopsis cabulica* and *O. pumila*. (2) DNA content variation among congeneric species is often discontinuous (Narayan, 1998), and (3) DNA content is normally controlled within narrow limits within species (Bennett *et al.*, 2000). Bennett *et al.* (2000) proposed that a 'counting' mechanism must exist that regulates genome size within tightly defined or preselected limits. It is likely that such canalization of genome size results from some ubiquitous property of DNA replication and/or repair that only occasionally allows jumps to higher or lower DNA amounts. Their primary role does not have to be regulation of genome size, as long as the consequences of intrinsic checkpoints generally ensure constancy of DNA amount within a species.

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