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Genome size variation in the Fagaceae and its implications for trees

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Abstract Polyploidization is a major source of diversification among plants, particularly during cladogenesis, but most evidence involves herbaceous temperate species. The prevalence of polyploidy among woody taxa is largely unknown, especially among tropical groups. In this study, we examined genome size variation globally and at several taxonomic levels within the Fagaceae. This family has diversified in the northern temperate zone (*Quercus*) and at least twice in the Asian tropics (*Lithocarpus* and *Castanopsis*), allowing us to examine genomic size evolution across a broad latitudinal range. We compared nuclear DNA contents from 78 species in six genera, including new measurements for 171 individuals from 47 Chinese species using standard flow cytometry methods. No evidence suggests that polyploidization or whole genome duplication has occurred in the family. Genome size varied among genera, but limited variation was present in each genus and species. In general, tropical species had larger genomes than temperate species, but the ancestral state cannot be determined given current evidence. Partial duplication does seem to occur among species as within genus variation was larger than within species variation. A review of the literature suggests that genome size and even chromosome structure is highly conserved among woody plants and trees. We propose that ploidy level and genome size are conserved among trees because they participate in diverse syngameons. This behavior would provide similar benefits to polyploidization but avoid exclusion from the syngameon. This conservatism in genome size and structure should enhance ongoing whole genome studies.

Keywords C-value · Polyploidization · Whole genome duplication · Flow cytometry · Syngameon

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Introduction

Genome size varies tremendously among angiosperms, spanning a remarkable ~2,400-fold range (Bennett and Leitch 2011; Pellicer et al. 2010), extending from 0.129 pg (*Genlisea margaretae*, Lentibulariaceae) to 304.46 pg (*Paris japonica*, Melanthiaceae). This variation can play an important role in speciation and diversification and strongly affects phenotypic variation (Gregory 2005; Bennett and Leitch 2011). The evolution of early plant diversity involved ancient polyploidization events, and almost 15 % of well-documented speciation events involved polyploidization (Soltis et al. 2009; Wood et al. 2009), particularly in temperate herbaceous plants (Bennett 1998; Soltis and Soltis 1999; Adams and Wendel 2005). Overall, with the advent of whole genome sequencing technologies and an increased awareness of the role of

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polyploidization and whole genome duplication (WGD) in plant diversification, interest in genome size evolution and variation in eukaryotes has increased substantially over the past decade (Oliver et al. 2007).

Surprisingly, the variation and evolution of genome size in woody angiosperms remain poorly understood, particularly among tropical species. Genome size has been measured in only a tiny fraction of woody tropical species, e.g., a few species of *Dalbergia* (Hiremath and Nagasampige 2004). While trees are usually predicted to have smaller genome sizes than herbs and to experience less frequent polyploidization (Ohri 2005; Petit and Hampe 2006; Beaulieu et al. 2010), little empirical data is available, particularly across taxonomic levels within a family. In the Rosaceae (Dickson et al. 1992) and Myrtaceae (Grattapaglia et al. 2012), genome sizes were relatively stable and small at both the family and genus levels. Despite the fact that plant species diversity is predominantly found in the tropics, the relationship in genome size between tropical and temperate plants is poorly understood, nor is it known how growth form (climbers, shrubs, and trees) or life history strategies (pioneer versus climax species) might affect genome size variation. Here, we focus on long-lived trees.

Genomes in tropical herbs are typically smaller than those in temperate herbs, as measured by chromosome length, although the relationship is not necessarily preserved at family level (Levin and Funderburg 1979). A comprehensive survey of the relationship between climate and growth form with nuclear DNA content found that tropical and temperate woody dicots generally had similar C-values, although within some families and for particular taxa, temperate species had larger C-values than tropical species (Ohri 2005). Neither of these studies controlled for phylogenetic relationships nor did they examine tropical and temperate groups in the same family. Given the great variability in genome size observed in other studies (Bennett 1987), more detailed and taxonomically controlled studies of families that have diversified in both temperate and tropical zones are necessary to understand whether polyploidization generally plays a global role in plant diversification or whether it is largely limited to temperate herbaceous plants.

In this study, we perform the first extensive survey of genome size variation in a large, diverse, and widely distributed woody plant family (Fagaceae), sampling among and within six genera for which the phylogenetic relationships are known (Oh and Manos 2008; Kremer et al. 2012). Because of their ecological and economic importance, this family is one of the most comprehensively studied groups of trees in the world (Manos and Stanford 2001). While most of this work has concentrated on the temperate oaks (*Quercus*) in the Northern Hemisphere, the family also includes two large tropical genera (*Lithocarpus* and *Castanopsis*) that represent significant diversifications in the Asian tropics (Manos et al. 2001). The number of species in each genus is highly variable (Govaerts and Frodin 1998; Manos et al. 2008), with *Quercus* (450), *Lithocarpus* (300), and *Castanopsis* (120) being the most diverse, whereas several other genera contain only a few species, including *Fagus* (12), *Castanea* (10), *Trigonobalanus* (3), *Chrysolepis* (2), and *Notholithocarpus* (1). The latter two genera, which are the only taxa missing from our survey, currently exist as small endemic populations in the Pacific Northwest of the USA.

The major groups in the Fagaceae are largely confined to either temperate or tropical regions, reflecting their fossil distributions (Manos and Stanford 2001; Crepet and Nixon 1989). Thus, each major group diversified within its current area of distribution, including the small tropical evergreen subgenus *Cyclobalanopsis* within the genus *Quercus*, which is also limited to tropical Asia (Nixon 1993; Manos et al. 1999). The smaller genera *Castanea* and *Fagus* are exclusively composed of deciduous temperate species, which are continuously distributed throughout the Northern Hemisphere. The two large evergreen tropical genera *Lithocarpus* and *Castanopsis* are completely confined to East Asia and share almost identical distributions and patterns of diversity (Soepadmo 1972). The distribution of the modern *Trigonobalanus* species spans tropical amphi-Pacific regions, this evergreen genus being a relict and basal in the phylogeny of the family (Manos and Stanford 2001), although the distribution of each species is highly restricted and localized in gregarious populations.

To investigate the stability and variation of genome size within and among genera and across biotic zones in the Fagaceae, we directly measured nuclear DNA content for 171 individuals from 47 tropical Chinese species, including three separate genera and the tropical subgenus *Cyclobalanopsis* within the genus *Quercus*. These new measurements are combined with published estimates for 31 species (Kremer et al. 2007, 2012; Bennett and Leitch 2010), within the genera *Quercus*, *Castanea*, and *Fagus*. Given the known phylogenetic and biogeographic framework of the family (Kremer et al. 2012; Manos et al. 2008), we address the following questions: (1) Is there any evidence of whole genome duplication or polyploidization? (2) What is the scale of genome size variation within and among genera and species? (3) Does genome size variation correlate with the historical region of diversification and current distribution? (4) Does this variation relate to the genus-level phylogeny of the family? (5) Are the patterns within the genus *Quercus*, between the temperate and tropical subgenera, similar to those seen at the family level among genera?

Materials and methods

Plant materials

Tissue samples were obtained from either the young roots of freshly germinated seeds or fresh young leaves taken from

transplanted seedlings. To obtain fresh roots, acorns were collected from natural forests of six sampling localities in Yunnan Province, Southwest China, during the autumn of 2010 and the spring of 2011 (Table 1). To shorten germination time, the seeds were removed from the acorns and were planted on 0.6 % agar plates at 25 °C with illumination from fluorescent lighting (100 μmol m^{-2} s^{-1}). Fresh leaves were collected from seedlings transplanted in the Horticulture Department of the Xishuangbanna Tropical Botanical Garden (XTBG). Voucher specimens for these acorns and seedlings were deposited at the herbarium of XTBG (HITBC). Tissue was harvested from young plants of *Pisum sativum* cv. Ctirad (9.09 pg/2C, 2*n*=14) to act as an internal standard for the measurements (Doležel et al. 2007).

Sample preparation

The tissue of tropical Fagaceae species contains high levels of cytosolic compounds (e.g., polyphenolics, tannic acid) that might interfere with flow cytometric analysis of nuclear DNA contents, due to the so-called "tannic acid effect" (Doležel et al. 2007; Loureiro et al. 2006). In order to abrogate this effect and optimize measurement conditions for the Fagaceae (Zoldos et al. 1998), we compared the use of different source materials, including adult leaves, young leaves, resting buds, and young roots, and explored supplementation of the chopping buffer with polymeric agents known to sequester polyphenolics. Changes in the nuclear fluorescence intensities for the plant standards were examined as a function of composition of the chopping buffer and the presence or absence of the Fagaceae species (Price et al. 2000; Jedrzejczyk and Sliwinska 2010). Under optimal conditions, only slight shifts in the peak positions were observed, and these were not statistically significant, illustrating abrogation of the tannic acid effect. In general, young roots were optimal source tissues for the measurements, although reliable results could also be obtained from young leaves. Therefore, we germinated seeds whenever possible, using young leaves only for species that did not fruit during the study period.

Equal amounts (100 mg) of tissue excised from the targeted species and from the internal standard (*P. sativum*) were immersed within a petri dish in 1 mL of modified Galbraith's chopping buffer and were co-chopped for approximately 30 s using a new razor blade (Galbraith et al. 1983). The buffer consisted of 45 mM $MgCl_2 \cdot 6H_2O$, 30 mM trisodium citrate, 20 mM 3-morpholine-1-propane-sulfonic acid (MOPS; #M3183, Sigma-Aldrich, St. Louis, MO, USA), supplemented with 1 % (*v*/*v*) Triton X-100 (#T8787, Sigma-Aldrich), and 1 % (*w*/*v*) Polyvinylpyrrolidone-3500 (PVP-3500; #27614, Acros, Morris Plains, NJ, USA), and adjusted to pH 7.2. These concentrations of Triton X-100 and PVP-3500 were appropriate for reduction of cellular debris and removal of polyphenolic compounds (Doležel et al. 2007). Coarse rather than fine chopping was found to improve the coefficients of variation (CV) of the histogram peaks (Zoldos et al. 1998). The homogenate was filtered through a 30-μm nylon mesh into a labeled tube containing 2.5 μL of 10 mg/mL DNase-free RNase A (#EN0531, Fermentas, St. Leon-Rot, Germany). The fluorochrome propidium iodide (PI; #P4170, Sigma-Aldrich) was then added to a final concentration of 50 μg/mL. The stained sample was incubated on ice in darkness for 1 h prior to analysis on the flow cytometer.

Flow cytometry measurements

Genome sizes were measured using an Accuri C6 flow cytometer (Accuri Cytometers, Inc., Ann Arbor, MI, USA), following a standard protocol (Galbraith 2009). Analysis was based on light-scatter and fluorescence signals produced from 20-mW laser illumination at 488 nm. Performance of the flow cytometer was validated using 8- and 6-peak fluorescent bead mixtures (Spherotech) as recommended by the manufacturer (CFlow User Guide, Accuri). Measurements were made to a total count of 10,000–15,000 nuclei, and noise derived from subcellular debris was eliminated by gating. Instrument

Table 1 Geographic location, climate, and vegetation type of the sampling locations

Location	Longitude	Latitude	Altitude (m)	Annual rainfall (mm)	Mean annual temperature (°C)	Vegetation type
Ailaoshan	101° 1′ E	24° 32′ N	2,450	1,931	11	a
Mengsong	100° 25′~100° 35′ E	21° 27′~21° 34′ N	1,500~1,900	1,700	18	b
Menglian	99° 22′ E	22°6'N	1,200~1,800	1,375	19.7	a
Jinghong	100° 35′ E	22° 14′ N	1,200	1,450	23.5	c
Mengla	101° 34′ 26"~47" E	21° 36′ 42"~58" N	710~867	1,400	21.5	d
XTBG	101° 15′ 10" E	21° 55′ 45" N	570	1,563	21.5	–
KIB	102° 44′ 42" E	25° 8′ 8" N	1,800	1,010	14.7	–

a subtropical evergreen broad-leaved forest, *b* tropical montane rainforest, *c* monsoon evergreen broad-leaved forest, *d* tropical monsoon rainforest, *XTBG* Xishuangbanna Tropical Botanical Garden arboreta, *KIB* Kunming Institute of Botany arboreta

linearity was routinely tested as described in Fig. S1. Using the optimized sample preparation procedure, the flow histograms showed sharp G0/G1 peaks, with CVs typically <5 %, for both the target plants and the internal standard (Fig. 1); replicate measurements were highly reproducible with low systematic errors. The population means for the FL2-A values of the G0/G1 peaks were recorded for further analysis.

Statistical analysis

Using the same procedure and the same instrument settings, each individual was made into three to five independent samples, and each sample was measured five to ten times by the flow cytometer. The nuclear DNA contents for the measurements were averaged to provide a value of the nuclear DNA content for that individual. The nuclear DNA content value for each species was then determined by calculating the mean value of all the measured individuals of that species. The nuclear DNA contents were enumerated from the equation by Doležel et al. (2007).

In addition, nuclear DNA contents for 24 species of *Quercus*, 5 species of *Castanea*, and 2 species of *Fagus* were obtained from the literature (Kremer et al. 2007; Bennett and Leitch 2010). For species having more than one published estimate, mean average values of nuclear DNA contents were calculated from all available estimates. We applied Student's *t* test to compare the mean 2C nuclear DNA contents between various groups, based upon distribution, taxonomy, and phenology. Statistical analysis was done using the basic R package (R Development Core Team 2010).

Results

For the 47 new estimates for the Fagaceae (Table 2), the largest genome (2.61 pg/2C, *Lithocarpus calolepis*) was roughly 50 % larger than the smallest (1.82 pg/2C, *Quercus rex*). Within a single genus or species, genome sizes fell within a narrow range, even among the 12 species for which five or more individuals were sampled from more than one location. Within the *Lithocarpus*, the two species in the section *Synaedrys* (*Lithocarpus corneus* and *Lithocarpus pachylepis*) have the smallest values (2.01 and 1.86 pg/2C, respectively), while several species of *Lithocarpus* possessed the largest genomes, although the ranges of genome size for *Lithocarpus* and *Castanopsis* were not significantly different. Among the *Castanopsis* species, the DNA values ranged from 1.95 pg/2C (*Castanopsis wattii*) to 2.35 pg/2C (*Castanopsis clarkei*), representing a 20 % difference. Except for the unusually large value from *Quercus austrocohinchinensis*, our

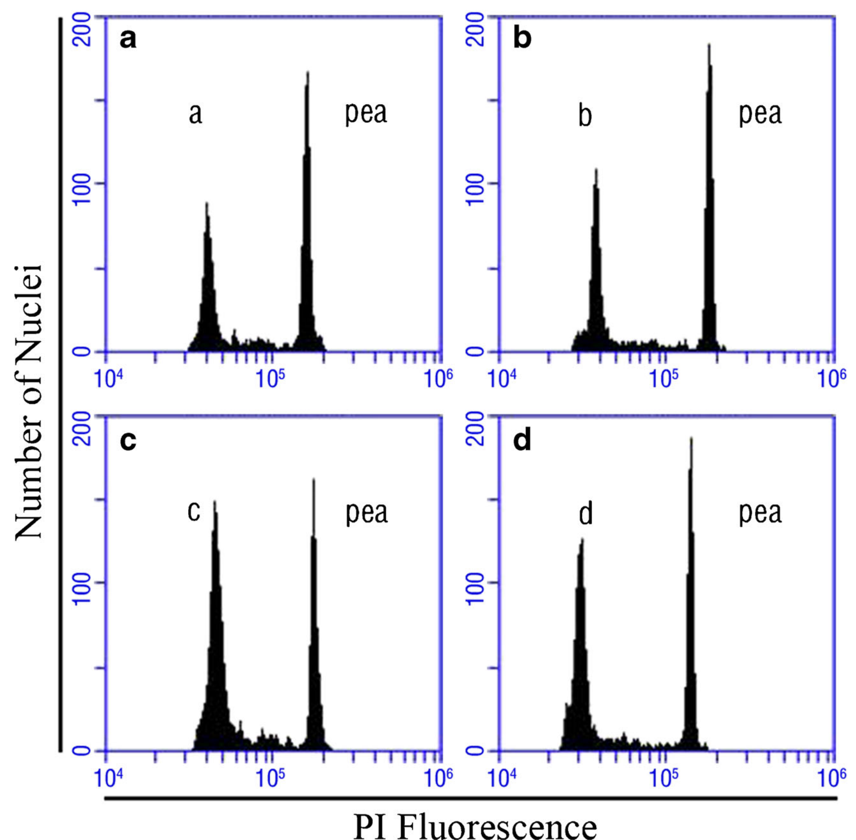

Fig. 1 Uniparametric histograms of fluorescence intensities of the nuclei of the representative Fagaceae plants and the *Pisum sativum* control, after staining with propidium iodide. The 2C nuclear DNA content of *P. sativum* (garden pea) is 9.09 pg. **a** *Lithocarpus elizabethiae* (*peak a*); **b** *T. doichangensis* (*peak b*), **c** *Castanopsis calathiformis* (*peak c*), **d** *Quercus griffithii* (*peak d*)

Table 2 2C nuclear DNA contents for the species in the genera of *Lithocarpus*, *Castanopsis*, *Quercus*, and *Trigonobalanus* measured in this study

Species	Nuclear DNA content (2C/pg) mean±sd (*N*)	Locality
Lithocarpus		
L. bacgiangensis (Hickel & A. Camus) A. Camus	2.34±0.04 (2)	Mengsong
L. balansae (Drake) A. Camus	2.21±0.01 (3)	XTBG
L. calolepis Y. C. Hsu & H. W. Jen	2.61±0.10 (2)	Menglian
L. corneus (Loureiro) Rehder in Bailey	2.01±0.05 (1)	Jinghong
L. crassifolius A. Camus	2.33±0.03 (1)	Ailaoshan
L. dealbatus (J. D. Hooker & Thomson ex Mi-quel) Rehder	2.36±0.06 (4)	Ailaoshan
L. echinophorus (Hu ex A. Camus) A. Camus	2.21±0.01 (2)	KIB
L. elizabethiae (Tutcher) Rehder	2.33±0.04 (4)	KIB
L. fenestratus (Roxb.) Rehder	2.15±0.09 (4)	Mengla
L. fohaiensis (Hu) A. Camus	2.28±0.06 (2)	XTBG
L. fordianus (Hemsl.) Chun	2.03±0.02 (2)	XTBG
L. grandifolius (D. Don) S. N. Biswas	2.39±0.06 (6)	Jinghong
L. hancei (Bentham) Rehder	2.37±0.04 (6)	Ailaoshan
L. litseifolius (Hance) Chun	2.46±0.04 (4)	Menglian
L. mairei (Schottky) Rehder	2.29±0.01 (3)	KIB
L. pachylepis A. Camus	1.86±0.06 (2)	XTBG
L. polystachyus (Wall.)Rehd.	2.35±0.08 (3)	XTBG and KIB
L. skanianus (Dunn) Rehder	2.25±0.04 (1)	Menglian
L. triqueter (Hickel & A. Camus) A. Camus	2.43±0.03 (5)	Menglian
L. truncatus (King ex Hook. f.) Rehder et E. H. Wilson	2.36±0.01 (3)	Mengsong
L. xylocarpus (Kurz) Markgraf	2.30±0.02 (3)	Ailaoshan
Castanopsis		
C. calathiformis (Skan) Rehder & E. H. Wilson	1.96±0.03 (8)	Mengsong & Ailaoshan
C. clarkei King ex Hook. f.	2.35±0.12 (4)	XTBG
C. delavayi Franch.	2.03±0.02 (2)	KIB
C. echinocarpa J. D. Hooker & Thomson ex Miquel	2.28±0.05 (6)	Menglian & XTBG
C. fissa (Champion ex Bentham) Rehder & E. H. Wilson	2.33±0.06 (4)	KIB
C. fleuryi Hickel & A. Camus	2.23±0.04 (5)	Ailaoshan
C. hystrix J. D. Hooker & Thomson ex A. de Candolle	2.13±0.06 (6)	Ailaoshan & XTBG
C. indica (Roxburgh ex Lindley) A. de Candolle in Hance	2.26±0.11 (4)	XTBG
C. mekongensis A. Camus	2.15±0.07 (5)	Mengsong & Mengla
C. orthacantha Franch.	2.03±0.02 (4)	KIB
C. rockii A. Camus	2.33±0.04 (3)	XTBG
C. wattii (King ex J. D. Hooker) A. Camus	1.95±0.02 (2)	Ailaoshan
Quercus		
Q. acutissima Carruthers	1.93±0.04 (5)	Mengsong & XTBG
Q. aliena Blume	2.09±0.06 (3)	KIB
Q. austrocochinchinensis Hickel et A. Camus	2.44±0.08 (2)	XTBG
Q. franchetii Skan	2.12±0.04 (4)	KIB
Q. glauca (Thunb.) Oerst.	2.05±0.02 (3)	KIB
Q. glaucoides Schottky	2.16±0.07 (7)	XTBG and KIB
Q. griffithii J. D. Hooker & Thomson ex Miquel	1.99±0.03 (6)	Ailaoshan
Q. hui (Chun) Chun ex Y. C. Hsu & H. W. Jen	2.09±0.02 (2)	Mengsong
Q. kerrii (Craib) Hu	1.93±0.01 (2)	XTBG
Q. rex (Hemsl.) Schottky	1.82±0.06 (4)	XTBG
Q. semiserrata (Roxb.) Oerst.	2.12±0.01 (2)	XTBG
Q. variabilis Blume	1.84±0.01 (2)	Ailaoshan

Table 2 (continued)

Species	Nuclear DNA content (2C/pg) mean±sd (*N*)	Locality
Q. yiwuensis C. C. Huang	2.01±0.01 (3)	XTBG
Trigonobalanus		
T. doichangensis (A. Camus) Nixon & Crepet	1.86±0.02 (10)	Menglian

Locality indicates the collection site (see detailed description in Table 1)

sd standard deviation, *N* number of individuals sampled per species, *XTBG* Xishuangbanna Tropical Botanical Garden arboreta, *KIB* Kunming Institute of Botany arboreta

measurements for *Quercus* species fell within the previously reported range for temperate oak species in the RBG Kew Angiosperm DNA *C*-values Database (maximum 2.18 pg/2C, *Quercus robur*), confirming the accuracy of our measurements and the compatibility of these datasets. Finally, the genome size of *Trigonobalanus doichangensis* was found to be 1.86 pg/2C, providing a baseline for genomic studies within this endangered genus.

Incorporating previously published genome size measurements for the family (Table 3), the genome sizes for the two major tropical genera were significantly larger than measurements for temperate groups (Table 4). Additionally, the genome sizes of tropical evergreen species in *Quercus* (mainly in subgenus *Cyclobalanopsis*) were significantly larger than those of the temperate *Quercus* (Table 4). Even within the temperate subgenus *Quercus*, evergreen species had significantly larger genome sizes than deciduous ones (Table 4). A couple of minor discrepancies were found between our measurements and previous reports. For the East Asian species *Quercus acutissima*, our estimate was substantially larger (1.93 vs 1.42 pg/2C) than those previously reported (Kremer et al. 2007). This difference might be due to technical issues (Doležel et al. 1998), since the two studies used different species as internal standards for flow cytometry analysis. The difference might also reflect real differences given sample provenance: we measured five indigenous individuals collected from natural forests, whereas the samples described by Arumuganathan et al. (Kremer et al. 2007) were obtained from three to four individuals transplanted in US arboreta (personal communication J. E. Carlson). Given the consistency of our results, we assume that our estimate is reliable for *Q. acutissima* and adopt it in further analysis.

Discussion

Our study is the first extensive survey of genome size variation within a large and species-rich family of trees, with genera representing significant and independent diversifications in both temperate and tropical regions. This research also considerably expands our general knowledge of genome size in the Fagaceae, previously known primarily from the temperate oaks (Bennett and Leitch 2010). While genome size among genera did vary as much as 50 %, a much narrower range of genome sizes was observed within each genus and species. Overall, genome size seems to be highly conserved in the family, across biomes and deep phylogenetic splits, particularly in comparison to many large herbaceous groups and in other families of woody plants (Dickson et al. 1992; Grattapaglia et al. 2012). The scale of genome size is consistent with previous observations that woody angiosperms have small and relatively stable genomes (Ohri 2005; Gmitter et al. 2012).

We found no evidence that whole genome duplication or polyploidization plays a major role in species diversification among the Fagaceae genera. Previous cytological studies confirm that chromosome and ploidy numbers in the Fagaceae have remained stable across most genera, being uniformly $2n=24$ (Armstron and Wylie 1965; Chokchaichamnankit et al. 2007), with occasional triploids (Dzialuk et al. 2007) and extra chromosomes ($2n=24+1$, 2, or 3) in some *Quercus* populations (Zoldos et al. 1998). One example of a different basal chromosome number and ploidy ($2n=14$ and $2n=42$) was reported in *Trigonobalanus verticillata* (Chen and Sun 2010; Chen et al. 2007), an endemic and relictual species. Given the consistent ploidy level in the family, genome size appears to evolve through relatively small segmental duplication instead of whole duplication or merger, with total variation being roughly 20 % across species in a single genus.

The role of polyploidization in cladogenesis is less clear. While tropical groups did have larger genomes than temperate groups (Fig. 2 and Table 3), the fraction of increase appears to be incremental, instead of involving whole genomes, but this does not rule out the possibility of ancient polyploidization with subsequent genome size reduction (Bowers et al. 2003). This scenario requires that each genus returned to the same basic ploidy level. Interestingly, tropical groups did always have larger genomes, both among genera and within a single genus. Within the genus *Quercus*, species in the tropical subgenus *Cyclobalanopsis* had significantly larger genomes than temperate oaks (Fig. 3). Additionally, the evergreen Mediterranean oaks in the subgenus *Quercus* section *Cerris* also have larger genome sizes than those in the rest of the

Table 3 Compiled literature data of mean average value for the nuclear DNA contents of 31 species in the genera *Quercus*, *Castanea*, and *Fagus*

Genus	Species	Mean nuclear DNA content (pg/2C)	Number of reports	Estimation method	Reference source
Quercus					
Section *Quercus*	*Q. alba*	1.59	1	FC:PI	b
	Q. bicolor	1.35	1	FC:PI	b
	Q. macrocarpa	1.62	1	FC:PI	b
	Q. montana	1.49	1	FC:PI	b
	Q. petraea	1.76	5	Fe/FC:EB	a
	Q. pubescens	1.88	2	FC:EB	a
	Q. pyrenaica	1.67	1	FC:EB	a
	Q. robur	1.82	7	FC:PI/FC:EB	a, b
	Q. stellata	1.55	1	FC:PI	b
Section *Cerris*	*Q. cerris*	1.90	1	FC:EB	a
	Q. coccifera	2.00	1	FC:EB	a
	Q. ilex	1.98	2	FC:EB	a
	Q. suber	1.86	3	FC:PI/FC:EB	a
Section *Lobatae*	*Q. coccinea*	1.64	1	FC:PI	b
	Q. falcata	1.72	1	FC:PI	b
	Q. imbricaria	1.81	1	FC:PI	b
	Q. nigra	1.52	1	FC:PI	b
	Q. nuttallii	1.39	1	FC:PI	b
	Q. pagoda	1.75	1	FC:PI	b
	Q. palustris	1.60	1	FC:PI	b
	Q. phellos	1.66	1	FC:PI	b
	Q. rubra	1.64	2	FC:PI/FC:EB	a, b
	Q. shumardii	1.47	1	FC:PI	b
	Q. velutina	1.17	1	FC:PI	b
Castanea	*C. crenata*	1.65	1	FC:PI	b
	C. dentata	1.67	1	FC:PI	b
	C. mollissima	1.65	1	FC:PI	b
	C. sativa	1.68	4	FC:PI/FC:EB	a, b
	C. seguinii	1.57	1	FC:PI	b
Fagus	*F. grandifolia*	1.27	1	FC:PI	b
	F. sylvatica	1.20	6	FC:PI/FC:EB	a

FC:EB flow cytometric measurement with ethidium bromide as the fluorochrome, *FC:PI* flow cytometric measurement with propidium iodide as the fluorochrome, *Fe* Feulgen microdensitometry, *a* according to the RBG Kew Angiosperm DNA *C*-values Database (Bennett and Leitch 2010), *b* according to Chapter of Fagaceae trees in the book *Forest Trees* (Kremer et al. 2007)

subgenus *Quercus* (Table 3). The historical separation among genera and subgenera is quite ancient, easily on the scale of tens of millions of years. Beeches (*Fagus*) possess the smallest genomes. This genus is basal in the family, relatively poor in species, and has a similar current distribution and biogeographic history as the temperate chestnuts (*Castanea*) (Manos and Stanford 2001), suggesting that a small genome size is ancestral in the family.

Genome size in trees

Our results clearly support the hypothesis that, among woody angiosperms, genome size is generally small and conserved, with polyploidization being rare. The genomes of several different species of *Eucalyptus* in the largely woody family of Myrtaceae exhibit a great deal of synteny and colinearity, even among species in different taxonomic sections (Grattapaglia et al. 2012; Hudson et al. 2011). The entire family has a fixed base ploidy number of $x=11$, including the extremely diverse genus *Syzyium*, although the genomic properties of very few species have been investigated. Stebbins (1938) first proposed several possible reasons for the observed cytological stability in woody taxa: (1) whole genome reorganization generally leads to major phenotypic and developmental shifts, and woody plants cannot afford the same morphological plasticity as herbs, particularly forest trees which compete directly for sunlight and space; (2) the increase in genome size disrupts cell growth in woody plants, particularly in the woody cambium; and (3) relatively high degree of compatibility

Table 4 Comparisons of mean 2C nuclear DNA content between various groups of the Fagaceae, based upon distribution, taxonomy, and phenology

Comparison	*N*	Mean 2*C*-value (pg)	*t*	df	*P*	Significance
Tropical genera	34	2.23	9.35	74.00	3.57e-14	***
Temperate genera	44	1.75				
Tropical species[a]	41	2.21	10.62	68.05	4.31e-16	***
Temperate species	37	1.69				
Evergreen species	46	2.18	11.43	60.81	2.20e-16	***
Deciduous species	32	1.64				
Subgenus *Cyclobalanopsis*	7	2.09	4.25	10.24	1.60e-3	**
Subgenus *Quercus*	30	1.73				
Evergreen *Quercus*	12	2.05	5.92	27.69	2.36e-06	***
Deciduous *Quercus*	25	1.67				
Evergreen in subgenus *Quercus*	5	1.99	5.39	14.25	8.99e-05	***
Deciduous in subgenus *Quercus*	25	1.67				

[a] Subgenus *Cyclobalanopsis* species included

*Statistically different ($P<0.05$); **statistically significant ($P<0.01$); ***statistically highly significant ($P<0.001$). *df* degree of freedom; *t* *t*-value.

among interspecific hybrids, suggesting that interspecific gene flow is important.

Our results do not support the hypothesis that large genomes evolve under low temperature because of limits to growth (Grime and Mowforth 1982). This hypothesis emerged from a study on grasses and herbaceous plants and their rapid growth response to seasonal changes. This type of growth behavior may not be important to trees, particularly

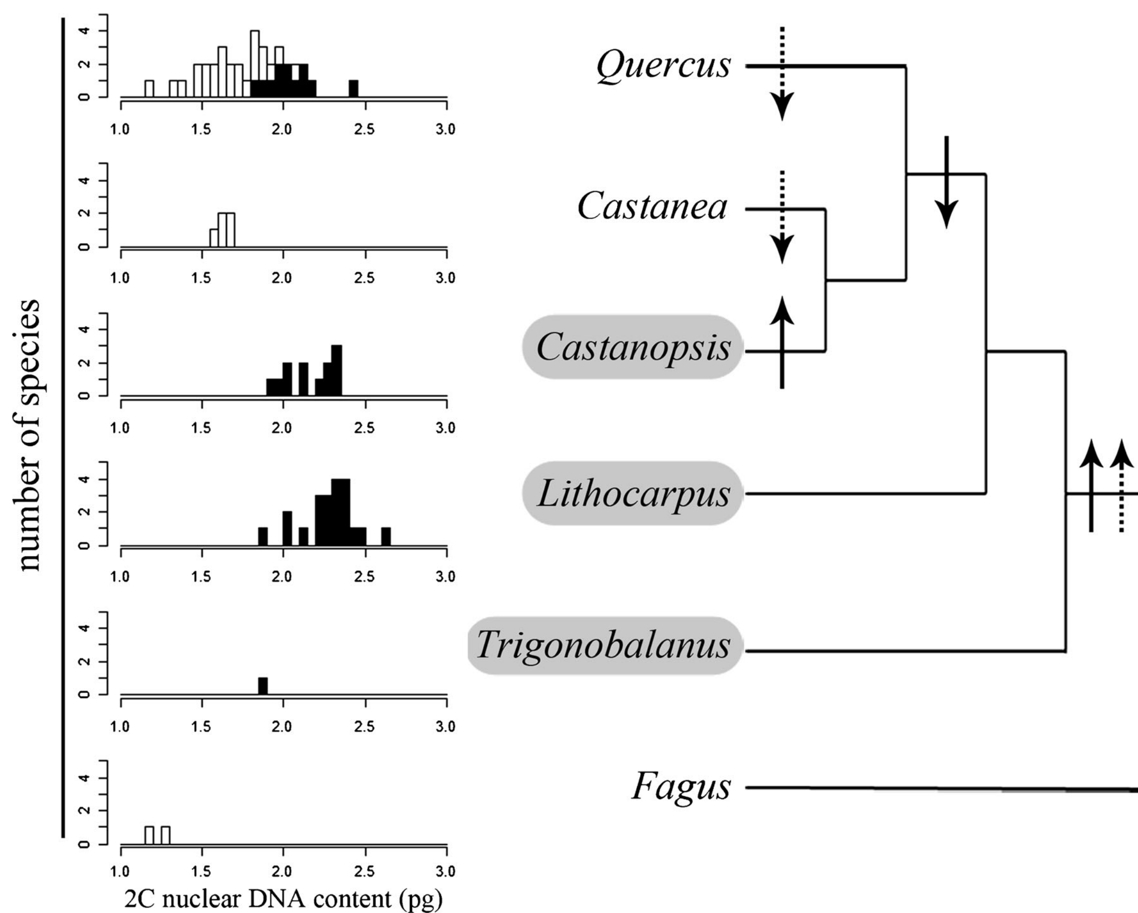

Fig. 2 Distributions of 2C nuclear DNA contents for 78 species of genera *Castanea*, *Castanopsis*, *Quercus*, *Lithocarpus*, *Trigonobalanus*, and *Fagus*, given the phylogenetic relationship of the genera. The phylogeny is adapted from Oh and Manos (2008) and Manos et al. (2008). *Black bars* represent evergreen species and *white bars* represent deciduous species. The *arrows* indicate two equally likely scenarios of genome size evolution, with the direction of the arrow indicating either genome size expansion or reduction. *Shaded boxes* denote tropical genera, the remainder being temperate genera

tropical groups where individuals often take decades to reach maturity. The larger genome sizes of tropical species suggest that these plants may have longer cell cycling time, lower growth rates, longer life spans, and higher mutation rates (Bennett 1972). By contrast, a small genome size in the temperate region permits greater competitive success because of greater evolutionary flexibility, for example, rapid seedling establishment, short minimum generation times, reduced cost of reproduction, and an increased reproductive rate (Leitch et al. 1998). Our results are in agreement with this proposition, hence, the reduction in genome size may have occurred during the historical diversification of temperate elements of this family.

Unfortunately, we cannot conclusively determine the sequence and direction of genome size change because the ancestral condition of genome size at the point of diversification in both the temperate and tropical regions cannot be objectively determined. Given the small genome size of the basal *Fagus* and the subsequent expansion of genome size in the tropics, two scenarios can be proposed: (1) genome size was subsequently reduced as the two temperate groups (*Quercus* and *Castanea*) expanded and diversified in the Northern Hemisphere, or (2) genome size was reduced during a single northward expansion with a subsequent expansion in the tropical chestnuts (*Castanopsis*) (Fig. 2). No matter which scenario is correct, an overall trend exists toward genome size decreases in derived branches, such as *Quercus* and *Castanea*.

Although the hypothesis of a "one-way ticket to genomic obesity" (Bennetzen and Kellogg 1997) and the general trend of genome expansion within the angiosperms (Soltis et al. 2003) are widely accepted, several studies indicate that genomic decreases repeatedly occur within specific herbaceous groups. For example, Wendel et al. (2002) observed increases as well as decreases in genome sizes in the diploid members of the cotton genus (*Gossypium*), terming this ambiguous evolutionary change as "bidirectional variation." Enke et al.

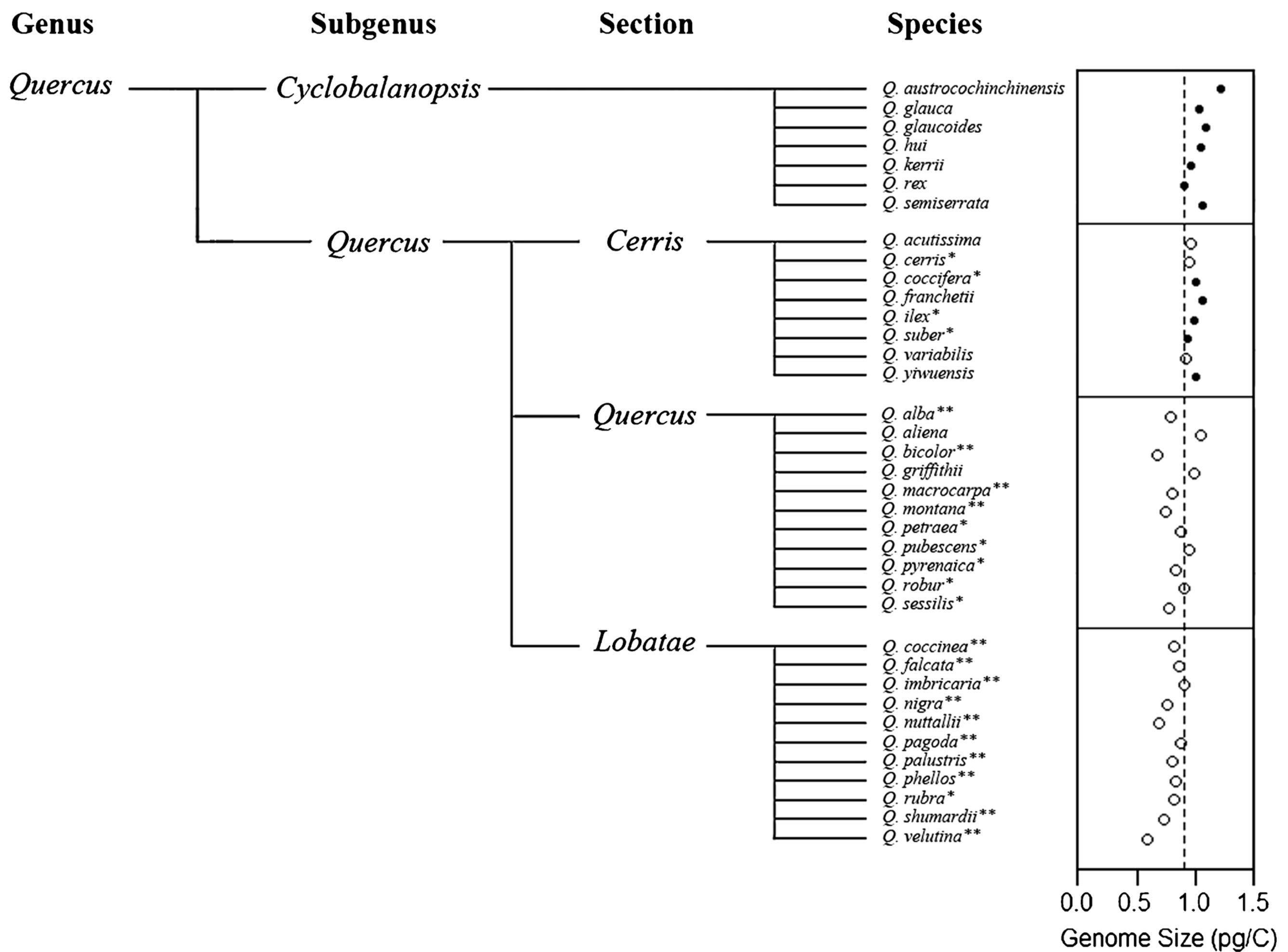

Fig. 3 Genome size variation in the genus *Quercus* aligned with taxonomic classification based on Camus (1934–1954). *Filled circles* represent evergreen species and *open circles* represent deciduous species. The mean genome size for all *Quercus* (0.87 pg/C) is indicated by a *dashed vertical line*. Genome sizes with an *asterisk* (*) are taken from the RBG Angiosperm *C*-value Database (Bennett and Leitch 2010), and those with two *asterisks* (**) are taken from Arumuganathan et al. (Kremer et al. 2007)

(2011) also described obvious genome shrinking within the genus *Crepis* within a phylogenetic context and provided a summary of ambiguous variations of genome size within certain taxonomic groups found by other studies. These studies mostly focused on species within a particular genus or subgenus. Although we cannot specify the overall evolutionary direction of genome size changes in the Fagaceae, the reduction of genome sizes observed among the genera and also within the genus *Quercus* corresponds with previous findings and extends them to a woody family (Fig. 3).

The dynamics of genome size may be due to rapid speciation at the primary center of diversity for the Fagaceae. According to a study of the New World *Pinus* genus, species across old provenances have a slight tendency toward larger genome sizes (Hall et al. 2000). The center of origin of the Fagaceae is generally assumed to be tropical, with a history of ancient displacement events (Manos and Stanford 2001). Thus, our observation that tropical genera of the Fagaceae in old provenances possess larger genome sizes is congruent with the suggestion that larger genomes are found in ancestral areas, with subsequent reduction accompanying range expansion. Previous studies suggest that the overall tropical-temperate difference primarily is a consequence of the geographical replacement of the plant groups with different genome sizes rather than the product of parallel evolution within these taxa in response to similar environmental changes (Levin and Funderburg 1979). Adaptations among tropical species are governed by biotic interactions, while those among temperate taxa are largely caused by strong selection pressures in hard environmental and climatic conditions (Mittelbach et al. 2007), so genomic mechanisms of adaptation should differ between tropical and temperate groups.

Participation in syngameons

We propose that genome size and structure is strongly conserved primarily because the species participate in a syngameon (Lotsy 1925, 1931), where partial interfertility among species can be advantageous (Arnold et al. 2003). The occasional and selected gene flow among the members of the syngameon could provide many of the same benefits as polyploidization, without committing to the genomic merger entirely. The genomic interaction among syngameon members would be limited by geographic distribution and probably be episodic as selection pressures change and advantageous alleles arise in various members of the syngameon. Given a particular environment or community, a particular combination of traits may provide a selective advantage to a species. Given the life history of trees and their very long lives, environmental and community change will always be rapid in comparison to other organisms. Participation in the syngameon should be a particularly effective mechanism for increasing heterozygosity and capturing advantageous alleles, which partially addresses the paradox recognized in trees, with their slow microevolution but rapid macroevolution (Petit and Hampe 2006). Polyploidization immediately places an individual outside of this syngameon, excluding it for the occasional benefits of interspecific gene flow and genomic introgression.

Overall, our survey of genome size in the Fagaceae, across taxonomic levels and geographic scales, strongly supports the hypothesis that woody taxa rarely experience polyploidization, which is consistent with the reported observation that the genome sizes of woody angiosperms are small and fall within a narrow range (Ohri 2005). This conservatism in genome size should enhance whole genome approaches (Neale and Kremer 2011). Two large tropical genera (*Lithocarpus* and *Castanopsis*), representing major and independent diversifications in the Asian tropics, both possessed significantly larger genome sizes than their sister temperate genera, particularly between the closely related genera of *Castanopsis* and *Castanea*. This pattern was also observed within the genus *Quercus*, where species in the tropical subgenus *Cyclobalanopsis* possessed significantly larger genomes than those in close temperate relatives in the subgenus *Quercus*. We feel that a more complete picture on genome size variation of the Fagaceae will lead to a better understanding of genome size evolution in these important components of global forests and its relationship to biogeographic distribution. Ongoing work is aimed to distinguish between genome size expansion within tropical species and genome size reduction among temperate groups and uncover possible mechanisms. These basic observations concerning nuclear genome size will improve the efficiency of genomics applications, provide targets for the bioinformatic analysis of genomic diversification, and assist in ongoing whole genome sequencing efforts for the Fagaceae.

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Data Archiving Statement The data in this analysis consists of flow cytometry measurements of nuclear DNA content for 171 individuals from 47 tropical Chinese species, including three separate genera and the tropical subgenus *Cyclobalanopsis* within the genus *Quercus*. This data will be deposited at the Plant DNA C-values Database (http://data.kew.org/cvalues/) hosted by the Royal Botanic Gardens, Kew, UK.

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