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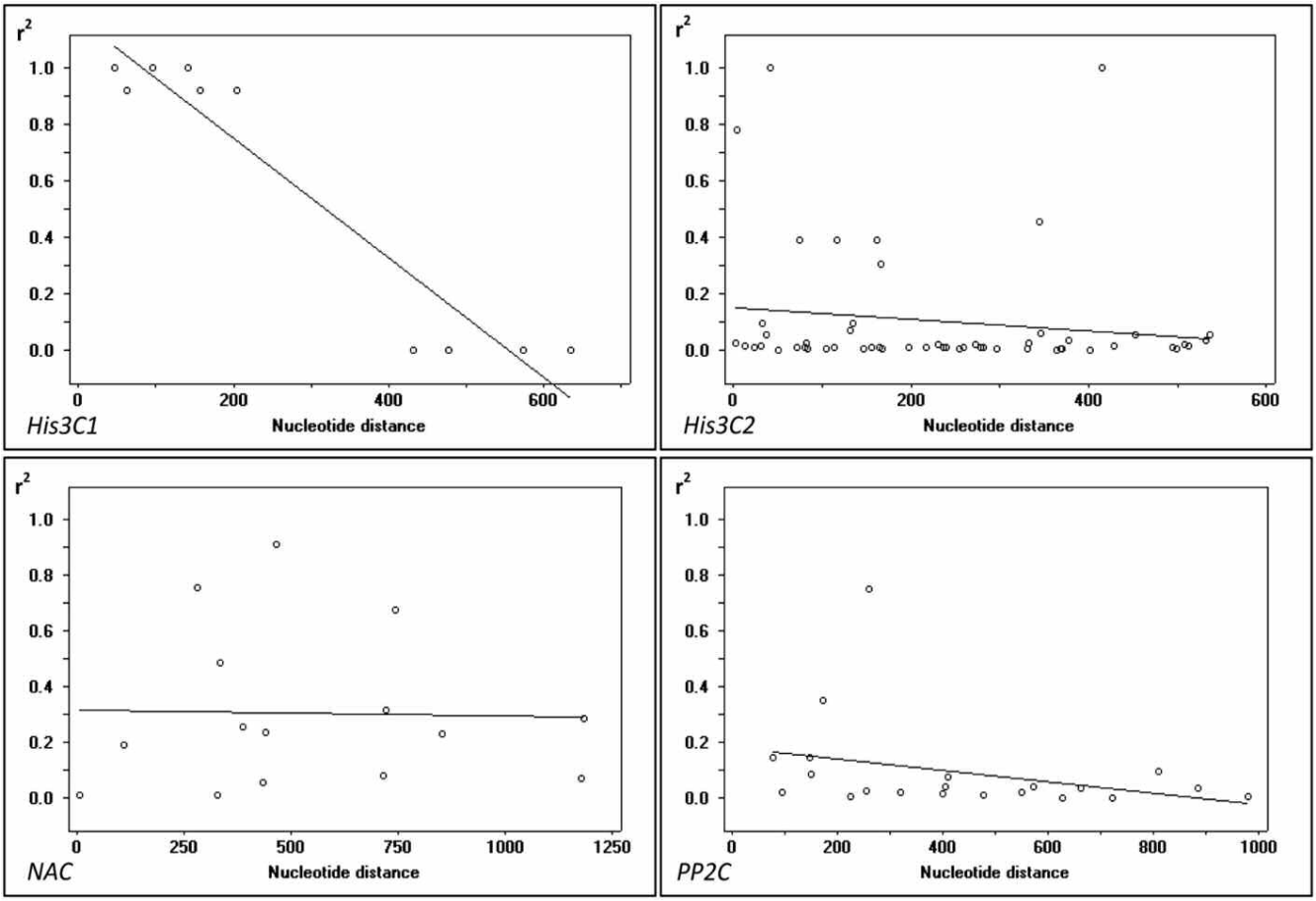

Figure S1. – Continued.

How small and constrained is the genome size of angiosperm woody species

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Abstract

Angiosperm hardwood species are generally considered to show an average smaller genome size with a narrow range of variation than their herbaceous counterparts. Various explanations pertaining to limitations of cell size exerted by wood fibers, the requirement of smaller stomata, longer generation time, large population size, etc., have been put forward to account for their small and constrained genome size. Yet studies done in the past several years show that genomically as well as evolutionarily, hardwoods are as diverse and active as their herba-

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ceous counterparts. This is entirely supported by the presence of well developed inter and intraspecific polyploid series and natural triploidy in many genera. Polyploidy, in some instances has been shown to confer adaptability to arid and salt stress conditions and in colonization of new areas. Moreover, hardwoods also show reasonable amenability to the induced polyploidy which abruptly changes the balance between nuclear and cell size. Polyploidy has been induced in many hardwoods to restore fertility in interspecific hybrids and for the production of triploids.

Furthermore, some cases studied show that genome size variation in hardwoods can be as variable as that of herbaceous species. Genome size has been shown to vary remarkably both at homoploid level as well as by polyploidy in certain genera. In the same way, the genome size is not correlated with the habit in certain groups having both herbaceous and woody taxa. This point is further proved by the presence of secondary and insular woody habit in certain cases where either the transition to woodiness is not followed by any diminution in the genome size, or the genome size of insular woody species may be even more than that of the congeneric herbaceous species. This shows that woody habit does not by itself put any constraints on the genome size either at homoploid or at polyploidy levels. The genome size in fact, not only varies significantly in many congeneric woody species but also may not show any correlation with the habit when woody and herbaceous species are compared in some narrow taxonomic groups studied.

Key words: hardwoods, constrained and small genome size, genome size variation, natural and induced polyploidy, insular and secondary woody habit.

Introduction

Genome size in angiosperms varies 2300-fold from 1C value of just 0.063 pg in *Genlisea margaretae* (Lentibulariaceae) (Greilhuber *et al.,* 2006) to 152.23 pg in *Paris japonica* (Melanthiaceae) (Pellicer *et al.,* 2010). The mechanisms underlying this large variation are now fairly known but its non-random distribution in organisms of varying complexity is still a big puzzle which is known as C-value enigma (Gregory, 2001). Although it is known that most of the DNA in a eukaryote genome is comprised of repetitive DNA, there are no empirical data which identify the forces responsible for causing variation of this component of DNA. Nevertheless, the availability of genome size data for about 4500 angiosperm species (Bennett and Leitch, 2010), allows us to seek various explanations in terms of phenotype, function, or ecological adaptations for this wide range of variation. Earlier work provided a functional view to this non-informative DNA because of strong positive correlation of genome size with nuclear and cell size, cell cycle time and minimum generation time of herbaceous plants leading to the concept of nucleotype (Bennett, 1971, 1972, 1987). Later studies have further reinforced the straight positive relationship of genome size with cell size (Knight and Beaulieu, 2008; Beaulieu *et al.,* 2008; Lomax *et al.,* 2009), cell cycle duration regardless of ploidy level (Francis *et al.,* 2008) particularly the S-phase (Simova and Herben, 2011). Genome size has been seen to have negative effect on root meristem growth rate in 8 herbaceous species as those with larger genome size show slower root growth velocity (Gruner, 2010). The direct positive relation of genome size with cell cycle time manifests itself in various growth and life history conditions. A comparison of 156 species of weeds and 2685 other species shows that both mean 4C DNA amount (11.74 pg) and genome size (3.79 pg) of weeds was significantly smaller than mean 4C (28.13 pg) and genome size (12.14 pg) of other species (Bennett *et al.,* 1998). Similarly, invasiveness has been observed to be negatively associated with genome size in *Pinus* species belonging to subg. *Pinus* (Grotkopp *et al.,* 2004), *Artemesia* species (Garcia *et al.,* 2008), and various genotypes of *Phalaris arundinacea* (Lavergne *et al.,* 2010). Likewise, species naturalized in Czech Republic have been shown to possess smaller genome size than their congeners which are otherwise not naturalized or invasive in other parts of the world (Kubesova, 2010). Moreover, a broad comparison of 3676 angiosperm species has shown a significantly negative effect of 1C and basic genome size on invasiveness among herbs, dicots, monocots, perennials, non-perennials, diploids, polyploids, Compositae and Poaceae and non-significant correlation among trees and Fabaceae (Chen *et al.,* 2010). Furthermore, a differential sensitivity of cell division and cell expansion, to low temperatures, operates through differences in DNA amounts. It has been demonstrated in grassland community in North England that the early spring sprout-

ing plants are those which undergo mitotic divisions in preceding warmer conditions followed with rapid growth by water uptake in spring (GRIME *et al.,* 1985). OHRI and PISTRICK (2001) however, failed to observe a tight correlation between genome size and early spring growth in 75 Allium species studies. In a broad comparison based on 3874 angiosperm species OHRI (2005) found that both mean 4C (5.63 pg) and 1Cx DNA (1Cx = 2C/ploidy level) values (1.21 pg) of woody angiosperms were significantly lower than those of herbaceous angiosperms (28.85 and 5.63 pg respectively). This was also true of all the subsamples including temperate and tropical angiosperms and dicots, tropical monocots and the family Fabaceae, when 4C and 1Cx values of woody and herbaceous species included in these samples are compared. Therefore, the woody species consistently showed smaller holoploid and 1Cx values with a narrow variance than their herbaceous counterparts (OHRI, 2005). Woody monocots which do not produce true wood, however, have 4C and 1Cx values 3.75 and 4.0-fold respectively, greater than those of woody dicots (OHRI, 2005). The present discussion would therefore be restricted to woody dicots only. The 4C DNA amounts in tropical woody dicots differ 47.8-fold ranging from 0.74 pg in *Dissotis canescens* (2n = c.28-32) to 35.4 pg in *Kadsura longespicata* (2n = 28) and is restricted to 50.5% of the range known for tropical herbaceous dicots. The 1Cx values of tropical woody dicots, however, show 55.6-fold variation from 0.08 pg in octoploid *Coriaria myrtifolia* (2n = c.72) to 4.45 pg in diploid *Bougainvillea spectabilis* (2n = 34). It is important to note that both temperate and tropical woody dicots had significantly lower mean 4C (4.45 pg and 4.49 pg respectively) and 1Cx values (0.89 pg and 0.94 pg respectively) than their herbaceous counterparts (OHRI, 2005) which falls in the range of very small <1.4 pg according to LEITCH *et al.* (1998). Moreover, the range of variation shown by woody species is also much narrow than that shown by the herbaceous species (OHRI, 2005). Similar results were obtained by BEAULIEU *et al.* (2010) who found a strong influence of growth form on genome size, with woody lineages being slow in accumulating changes in genome size as related to herbaceous species. This pattern was consistent in both Monocotyledons and Fabaceae irrespective of 1C or 1Cx DNA content (BEAULIEU *et al.,* 2010). This is despite the presence of widespread palaeoploidy and high basic numbers in woody species (MEHRA, 1976; MORAWETZ, 1986; EHRENDORFER, 1987). However, gymnosperms despite their long generation time possess large genomes (OHRI and KHOSHOO, 1986; MURRAY, 1998).

The question here arises is, which common constraints influence the genome sizes of this polyphyletic assemblage of woody species, belonging to all eudicot orders except Geraniales and Gunnerales (GROOVER, 2005). Nevertheless, tree habit though being polyphyletic has some common features such as size, longevity, reproductive output (prodigious seed production), predominantly outcrossing behavior, high levels of intra and interspecific gene flow, very large effective population sizes, slower mutation rates per unit time, nucleotide substitution and speciation (PETIT and HEMPE, 2006).

Some explanations have been provided to account for the retention and perpetuation of small genome size of woody dicot species. These pertain to both structural constraints related to growth form and life history traits. The robust relationship between cell size and genome size would mean that small cambial cells which form wood fibers put constraints on nuclear and chromosome size (STEBBINS, 1950; KHOSHOO, 1962). Another such constraint has been shown in case of guard cell length and stomatal density by BEAULIEU *et al.* (2008). This constraint manifests in small and dense stomata of angiosperm trees which confer greater stomatal conductance and transpiration rate required to move water and nutrients through longer xylem pathways (WOODWARD, 1998). At the same time, in dry environment, smaller stomata respond quickly to water stress (due to their greater membrane surface area to volume ratio) while high stomatal density maximizes CO_2 diffusion during optimal photosynthetic conditions (AASAMAA *et al.,* 2001; HETHERINGTON and WOODWARD, 2003). Furthermore, longer generation time and large population sizes (PETIT and HAMPE, 2006) of woody species allow for fewer opportunities for the accumulation of genomic changes and the removal of deleterious mutations and excess DNA, therefore maintaining small genome size (BEAULIEU *et al.,* 2010). Gymnosperms also have longer generation time however; it is worth mentioning here that they have lower photosynthetic rates and on average marginally less dense wood than the angiosperms (KNIGHT and BEAULIEU, 2008).

Nevertheless, the data accumulated over the past years on the chromosome and genome size evolution of woody dicots shows that their genome size though small, is not as constrained as it has been commonly considered. The genome size does not show very tight relationship with structural and life history traits as mentioned above, on the contrary it can show dramatic increases by polyploidy (both induced and natural) and by retrotransposons. Some of the mechanisms responsible for producing the sharp variation in genome size of woody species are discussed here.

Inter and Intraspecific polyploidy

A large number of hardwood families and genera possess the same haploid number and have evolved at diploid level (Mehra, 1972; Mehra *et al.,* 1972; Kremer *et al.,* 2007; Ohri and Khoshoo, 1987; Ohri and Ahuja, 1990, 1991; D'Emerico *et al.,* 1995; Zoldos *et al.,* 1999; Kumar and Rao, 2002; Wang *et al.,* 2005; Oudjehih and Bentouati, 2006; Chokchaichamnankit *et al.,* 2008; Ribeiro *et al.,* 2011; Hynniewta *et al.,* 2011; Coulleri *et al.,* 2012). However, a large heterogeneity of somatic chromosome numbers is exemplified by Meliaceae with 2n=16, 20, 22, 24, 26, 28, 30, 32, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 72, 78, 80, 84, 92, 100, c150, c280, c360 (Style and Vosa, 1971; Khosla and Styles, 1975). Furthermore, besides the presence of palaeoploidy and high basic numbers certain families and genera are characterized by the evolution of well developed polyploid series. Notable among these are *Alangium* (2x,3x), *Randia* (2x,4x), *Rauwolfia* (4x,6x) *Callicarpa* (2x,8x,10x), *Litsea* (2x,4x), *Trema* (2x,8x) (Mehra and Bawa, 1969), *Antidesma* (2x,6x,8x,9x) (Hans, 1970), *Terminalia* (2x,4x,6x) (Gill *et al.,* 1982; Ohri, 1996), *Fraxinus* 2x,4x,6x) (Nesom, 2010), *Rhododendron* (2x,6x) (De *et al.,* 2010), *Tetrameranthus* (2x,4x), *Rollinia* (2x,4x,6x,8x) (Morawetz, 1986), *Magnolia* (2x,4x,6x) (Biswas and Sharma, 1984; Parris *et al.,* 2010), *Annona* (2x,4x), *Cochlospermum* (2x,3x,4x,6x), *Barringtonia* (2x,4x) (Morawetz, 1986), and *Betula* (2x,4x,6x,10x) (Furlow, 1990), *Leucaena* (2x,4x) (Palomino *et al.,* 1995), *Trigonobalanus* (2x,6x) (Chen *et al.,* 2007), *Desoxylum* (2x,8x) (Khosla and Styles, 1975), *Aglaia* (2x,4x) (Mehra *et al.,* 1972), *Urvillea* (2x,4x,8x) (Ferrucci, 2000, Urdampilleta *et al.,* 2006).

Some of the examples of intraspecific polyploid series are *Swietenia mahagoni* (2x,3x,4x,6x,8x,9x,10x), *Aphanomixis polystachya* (2x,4x,8x) (Khosla and Styles, 1975), *Annona squamosa*, *Jatropha curcas*, *Leea indica*, *Ricinus communis*, *Salix tetrasperma*, *Trema orientalis*, *Wrightia tinctoria*, *Xeromphis dumetorum*, *Zizyphus oenoplia*, *Terminalia bellirica* (2x,4x), *Grewia hainesiana*, *Lawsonia alba* (3x,4x), *Emblica officinalis* (4x,8x), *Terminalia chebula*, *Toona ciliata* (2x,4x,6x), *Lantana camara* (2x,3x,4x,6x), *Syzygium cumini* (2x,3x,4x,5x,6x), *Zizyphus mauritania* (2x,4x,5x, 6x,8x) (Gill *et al.,* 1990; Mathur *et al.,* 1995), *Hydnocarpus laurifolia* (2x,4x), *Malpighia glabra* (2x,4x), *Geijera parviflora* (12x,16x,18x), *Eugenia jambolana* (2x,3x,4x, 5x,6x), *Eugenia uniflora* (2x,3x), *Duabanga sonneratioides* (3x,4x) (Singhal *et al.,* 1985), *Ixora rosea* (2x,3x), *Xeromphis spinosa* (2x,4x) (Bedi *et al.,* 1981), *Ulmus americana* (2x,4x) (Whittemore and Olsen, 2011) *Betula schmidtii* and *B. utilis* (2x,4x), *B. chinensis* (6x,8x), *B. glandulosa* (2x,3x), *B. devurica* (4x,6x,8x) (Furlow, 1990), *Leucaena esculenta* subsp. *esculanta* (2x,4x) (Palomino *et al.,* 1995), *Zizyphus jujube* (4x,5x,8x), *Z. rotundifolia* (4x,6x) (Khoshoo and Singh, 1963), *Antidesma acuminatum* (2x,6x) (Hans, 1970), *Cipadessa baccifera* (2x,4x) (Khosla and Styles, 1975), *Turnera ulmifolia* complex (2x,4x,6x,8x) (Lopez *et al.,* 2011), *Acacia tortilis* (2x,3x,4x,8x) (Oballa and Olngotie, 1993; Bukhari, 1997a, b; El Ferchichi *et al.,* 2009), *Acacia dealbata* (2x,3x,4x) (Blakesley *et al.,* 2002), *Eugenia hyemalis*, *E. punicifolia* (2x,4x), *E. uniflora* (2x,3x) (Costa and Fornis-Martins, 2006a), *Psidium cattleianum* (4x,3x, 7x,8x) (Costa and Fornis-Martins, 2006b), *Acacia senegal* (2x,3x,4x,6x) (Odee *et al.,* 2015), *Callicarpa macrophylla* (2x,4x) (Contreras, 2011), *Urvillea ulmacea* (2x,8x) (Urdampilleta *et al.,* 2006), *Psidium cattleianum* (4x,6x,7x,8x) (Costa and Forni-Martins, 2006b), *Olea europaea* complex (2x,4x,6x) (Besnard *et al.,* 2008).

Some studies on woody species have shown that polyploids exhibit larger stomata in low density, a lower osmotic potential at full turgor, and a thick epidermis, all of which tend to reduce water loss or maintain turgor in the case of lower water potential. This is depicted by the studies on different cytotypes. In *Atriplex canescens* of the three cytotypes 2x, 4x, 6x, those of higher ploidy levels show greater

resistance to embolism through a trade off between water conducting efficiency (decreased due to large vessel diameter) and safety against hydraulic failure therefore possessing better adaptation to grow in areas of water stress as compared with 2x cytotype which is more mesic (HAO *et al.,* 2013). The tetraploid (2n = 36, 2C = 5.11 pg) populations of *Atriplex halimus* also show greater adaptability to arid conditions of North Africa and eastern Mediterranean as compared to the diploid (2n = 18, 2C = 2.40 pg) populations which occur in western Mediterranean (WALKER *et al.,* 2005). Adaptability to more arid conditions is also shown by the tetraploid (2n = 4x = 52, 2C = 2.95–3.03 pg) as compared with the diploid populations (2n=2x=26, 2C=1.39-1.40 pg) of *Acacia tortilis* ssp. *raddiana* (EL FERCHICHI OUARDA *et al.,* 2009). Similarly *Larrea tridentata*, a North American desert shrub shows habitat differentiation with respect to its 2x,4x,6x cytotypes where higher ploidy levels show distribution in more arid regions. This ability of 4x and 6x cytotypes to grow well in regions of water stress could be due to decrease in stomatal density by increasing the cell size resulting in reduced water loss (HUNTER *et al.,* 2001). Such a difference of geographic distribution has also been found in 2x and 4x cytotypes of *Ulmus americana* where 4x shows a much wider and northern distribution than 2x (WHITTEMORE and OLSEN, 2011). Similar observations have been made in *Coffea canephora* and *C. arabica* which show decrease in stomatal and epidermal cell frequency and increase in guard cell length with increase in ploidy level (MISHRA, 1997). A study on 2x and 4x cytotypes of *Robinia pseudoacacia* showed that 4x has better salt tolerance capacity than 2x after treatment. This is manifested by decrease in photosynthesis and defense related enzyme activities and increase in reactive oxygen species, malondialdehyde content and relative increase in electrolyte leakage in 2x after salt treatment (WANG *et al.,* 2013).

Induced polyploidy

Polyploidy has been successfully induced in a number of woody species for producing triploids, increase in fiber length, enhancing fruit and ornamental traits, and restoring fertility in interspecific hybrids. Commonly observed effects manifested by induced tetraploidy are compact restricted growth (reduction in internodal distance), thicker leaves (low leaf index ratio), increase in leaf area, increase in guard cell length and number of chloroplasts per guard cell and decrease in number of stomata per unit area as observed in *Morus alba* (DWIVEDI *et al.,* 1989), *Buddleia globosa* (ROSE *et al.,* 2000), *Rhododendron hybrids* (VAINOLA, 2000), *Pyrus pyrifolia* (KADOTA and NIIMI, 2002), *Punica granatum* (SHAO *et al.,* 2003), *Zizyphus jujube* (GU *et al.,* 2005), *Platanus acerifolius* (LIU *et al.,* 2007), *Lespedeza formosa* (WEI *et al.,* 2007), *Populus* (WANG *et al.,* 2013), *Acacia mangium* (HARBARD *et al.,* 2012), *Betula platyphylla* (MU *et al.,* 2012), *Acacia mearnsii* (BECK *et al.,* 2003), *Paulownia tomentosa* (TANG *et al.,* 2010), *Bougainvillea* (ZADOO *et al.,* 1975), *Morus* (VERMA *et al.,* 1986), *Betula* spp. (SARKILAHTI and VALANNE, 1990), *Lagerstroemia indica* (ZHANG *et al.,* 2010), *Eucalyptus globulus* (LIN *et al.,* 2010), *Betula platyphylla* (MU, 2012), *Acacia crassicarpa* (LAM *et al.,* 2014).

A detailed anatomical study of diploid and induced tetraploid types of *Manihot esculenta* has shown a clear difference with respect to secondary vascular tissues which are more developed in 2x than in 4x types. In the secondary xylem of 4x plants the radial parenchyma cells are wider with thinner walls and little starch and more vessel groupings with little tylose inside elements leading to retention of larger quantity of water contrary to 2x plants with narrower parenchyma cells with much starch, thicker walls, and more solitary vessel elements with tylose inside (NASSAR *et al.,* 2008).

Occurrence of natural triploids

Natural triploids have been found among populations of otherwise diploid species. They generally originate by the production of unreduced gametes (OHRI and ZADOO, 1986; BUTORINA, 1993). Rare triploid individuals have been observed in different species of *Quercus*, and identified by various methods. They generally show increase in stomatal and overall size as compared to the diploids (NAUJKOS *et al.,* 1995; DZIALUK *et al.,* 2007; LEFORT *et al.,* 1998, 2000; BURDA and SHCHEPOTIEV, 1973; BUTORINA, 1993). Similar observations have been made in triploid *Populus tremuloides* (EINSPAHR *et al.,* 1963; BENSEN and EINSPAHR, 1967). These triploids are known to have comparatively larger wood cells, a positive character for paper industry. A high rate (69%) of triploidy has been recorded

in *Populus tremuloides* in western USA. However, the highest frequency of triploidy occurs in unglaciated drought prone regions of North America where largest clone sizes have been recorded (Mock *et al.,* 2012).

Genome size variation at homoploid level and by polyploidy

The hardwoods generally possess a small genome size with a narrow range of variation (Ohri and Kumar, 1986; Ohri *et al.,* 1986; Ohri and Khoshoo, 1987; Ohri *et al.,* 2004; Ohri, 1996, 2002, 2005). However, two issues need to be considered here: the presence of remarkable interspecific genome size variation in some hardwood genera and the extent of genome size variation in woody and herbaceous elements within some narrow taxonomic groups.

Studies done in past several years have shown interesting patterns of variation at both holoploid and 1Cx levels in many hardwood genera. The 2C DNA contents of 12 *Eucalyptus* species studied conform to their sectional classification. They range from 0.77 pg in *E. citriodora* to 1.47 pg in *E. saligna* therefore showing a 1.9-fold difference. The species of subgenus *Symphomyrtus* show 2C DNA values from 1.09 pg (*E. globulus*) to 1.47 pg (*E. saligna*), while those of subgenus *Corymbia* have much smaller 2C DNA values varying from 0.77 pg in *E. citriodora* to 0.80 pg in *E. torelliana* (Grattapagalia and Bradshaw, 1994). The fleshy fruited species of Myrtaceae however, possess smaller genome size than capsular species of Eucalyptae and Melaleuceae. Twenty eight diploid (2n = 2x = 22) species show a variation in 2C values from 0.486 pg in *Gomidesia schaueriana* to 0.636 pg in *Eugenia multicostata* while two tetraploids *Psidium acutangulum* and *P. cattleianum* show 1.053 pg and 1.167 pg respectively (Costa *et al.,* 2008).

Cros et al. (1995) observed a 2-fold variation in 12 diploid (2n = 22) *Coffea* species belonging to subgenus *Coffea* with 2C values ranging from 0.95 pg in *C. racemosa* to 1.78 pg in *C. humilis*. *C. arabica* which is an allotetraploid (2n = 44) has 2C value of 2.61 pg. A more detailed study on 15 species showed a 1.6-fold difference in 2C values from 1.03 pg in *Coffea racemosa* to 1.76 pg in *C. humilis* (Noirot *et al.,* 2003). An ecological trend relating small genome size with water deficit and large genome size with more humid conditions has been shown in both the studies. Similarly, a 1.47-fold variation ranging from *C. humboldtiana* and *C. mauritiana* (0.96 pg) to *C. millottii* (1.41 pg) has been shown in 44 *Coffea* species (2n = 2x = 22) (Mascarocoffea) from Indian Ocean islands (Razafinarivo *et al.,* 2012). Here again small genome size is associated with drier habitats. Moreover, a significant correlation was found between leaf length and 2C DNA content and a weak but significant correlation between guard cell length (GCL) and 2C DNA content (Razafinarivo *et al.,* 2012). Ten diploid (2n = 20) species of *Dalbergia* show a 1.3-fold variation in 4C DNA contents from 5.85 pg *D. lanceolaria* to 7.88 pg in *D. horrida*. While the tree species show a range from 5.85 pg in *D. lanceolaria* to 7.22 pg in *D. sissoides* the shrubby and climber species included in the sample show a much narrow but higher range from 7.36 pg in *D. rubiginosa* to 7.88 pg in *D. horrida* (Hiremath and Nagasampige, 2004). In *Lonchocarpus* 51 diploid (2n = 22) taxa including 42 species show 4C DNA values ranging from 1.92 pg in *L. santarosanus* to 2.86 in *L. xuul* depicting a 1.49-fold difference, which does not show any correlation with altitude (Palomino and Sousa, 2000).

Detailed studies have been made on 39 species of Sapindaceae belonging mainly to the tribe Paullinieae in which 1C values show a 9-fold variation varying from 0.305 pg in *Lophostigma plumosum* to 2.710 pg in *Cardiospermum heringeri* (Coulleri *et al.,* 2014). The genus *Cardiospermum* which has four basic numbers (x = 7, 9, 10, 11) depicts a 5.23-fold difference varying from *C. bahianum* (2n = 4x = 36, 1C = 0.519 pg) to *C. heringeri* (2n = 14, 1C = 2.715 pg). Eight species of *Paullinia* which are all diploid (2n = 2x = 24) show 1C values from 1.0 pg in *P. thallictrifolia* to 2.061 pg in *P. elegans* showing a 2.0-fold variation. In *Serjania* (2n = 2x = 24) 1C values differ from 0.974 pg (*S. marginata*) to 2.68 pg (*S. caracasana*) showing a 2.75-fold difference (Coulleri *et al.,* 2014). Similarly, new estimates for 47 species have been made in Fagaceae (Chen *et al.,* 2014) combining this with 31 estimates made earlier (Bennett and Leitch, 2010, Kremer et al., 2007) the overall difference in the family is 2.2-fold between 1.2 pg/2C in *Fagus sylvestris* (2n = 24) and 2.61/2C in *Lithocarpus calolepis* (2n = 24). In the genus *Quercus* (2n = 24) 24 species studied show 1.8-fold difference between *Q. bicolor* (1.35 pg/2C) and *Q. austrocochinchinensis* (2.44 pg/2C). The overall results show that two major tropical genera *Lithocarpus*

(2n=24) and *Castanopsis* (2n=24) have significantly larger 2C values than the temperate genera and genome sizes of tropical evergreen species of *Quercus* (subgenus *Cyclobalanopsis*) are significantly larger than those of temperate subgenus *Quercus*. Furthermore, within temperate subgenus *Quercus* the evergreen species depict significantly larger genome sizes than the deciduous ones (CHEN *et al.,* 2014).

Some examples show interesting patterns of variation taking place both at diploid and at polyploidy levels. This is exemplified by the genus *Terminalia* which shows a 3.5-fold difference between *T. oliveri* (2n=2x=24, 3.60 pg) and *T. bellirica* (2n=4x=48, 12.80 pg). Out of the six species studied *T. oliveri*, *T. myriocarpa* and *T. arjuna* are diploid (2n=24), *T. chebula* and *T. bellirica* are tetraploid (2n=48) and *T. muelleri* is triploid (2n=36). Difference of 1Cx DNA values is greatest (1.97-fold) between *T. oliveri* and *T. arjuna*. Two species groups are therefore well demarcated on the basis of their 1Cx values: *T. oliveri* (2x) and *T. chebula* (4x) (mean 1Cx=1.81 pg) and *T. myriocarpa*, *T. arjuna*, *T. muelleri*, and *T. bellirica* (mean 1Cx=3.34 pg). Within diploids and tetraploids there is 1.97-fold and 1.76-fold variation respectively in 1Cx values (OHRI, 1996). A study on 62 species of *Magnolia* having 2x, 4x and 6x ploidy levels shows a range of 2C values from 3.43 pg in *M. virginiana* var. *australis* (2n=2x=38) to 13.47 pg in *M. denudata* (2n=6x=114) a 3.9-fold variation. However, 1Cx values vary from 1.86 pg in *M. virginiana* (2x) to 2.76 pg in *M. thailandica* (2x). This shows that increase or decrease in genome size takes place both at diploid and polyploid levels (PARRIS *et al.,* 2010). Eight taxa of *Leucaena* have been studied of which five diploid (2n=56) taxa show 2C values from 1.35 pg in *L. esculenta* subsp. *esculenta* to 1.81 pg in *L. diversifolia* subsp. *diversifolia* showing 1.34-fold difference and three tetraploid taxa vary from 2.66 pg in *L. esculenta* subsp. *paniculata* to 3.31 pg in *L. confertifolia* subsp. *adenotheloidea* showing a 1.24-fold difference (PALOMINO *et al.,* 1995). Similarly the *Ternera ulmifolia* complex (x=5) comprising of 2x, 4x and 8x species show a range of 2C values from 1.38 pg in the diploid *T. subulata* (2n=10) to 5.94 pg in octoploid *T. fernandezii* (2n=40). The 1Cx values reflect genome downsizing ranging from 0.44 pg in *T. cuneiformis* (8x) to 0.98 pg in *T. grandidentata* (4x) (LOPEZ *et al.,* 2011). Eleven species of *Berberis* studied show a range of 2C values from 1.463 pg in *B. bidentata* (2x) to 6.7 pg in *B. buxifolia* (4x) showing a 4.6-fold difference. Three groups could be clearly indentified on the basis of 2C values and ploidy levels: first group comprises of diploid species (2n=28) with 2C values ranging from 1.46 pg to 1.85 pg which grow in high elevation sites having greater rainfall with lower water availability, the second includes diploid species with 2C values from 2.9 pg to 3.8 pg and these are found at lower elevation with higher water availability and temperature and the third group consists of tetraploid (2n=56) species with 2C values ranging from 5.8 pg to 6.8 pg and these show considerably wider distribution than the diploid species (BOTTINI *et al.,* 2000).

Acacia is another example which shows wide variation at diploid and higher ploidy levels as depicted by 43 taxa studied (BUKHARI, 1997b). Six taxa from subgenus *Heterophyllum* differ in 2C values from 1.41 pg in *A. mearnsii* (2n=2x=26) to 3.3 pg in *A. holosericae* (2n=4x=52), 21 taxa from subgenus *Acacia* vary from 1.056 pg in *A. tortilis* (2n=4x=52) to 2.2 pg in *A. nilotica* var. *adstringens* (2n=8x=104), while in subgenus *Aculeiferum* 16 taxa studied show a variation from 1.09 pg in *A. polycantha* (2n=4x=52) to 1.17 pg in *A. mellifera* (2n=2x=26). The mean 1Cx values of subgenera *Heterophyllum*, *Acacia* and *Aculeiferum* are 0.78 pg, 0.29 pg and 0.56 pg respectively which shows that the subgenera differ greatly in the size of their chromosomes while abrupt differences in DNA content can also occur by polyploidy (BUKHARI, 1997). An interesting case of proportionate increase of holoploid genome size in an intraspecific polyploid series is shown in Acacia senegal with ploidy levels of 2x,3x,4x and 6x corresponding to 2C values of 1.47, 2.12, 2.89 and 4.51 pg respectively (ODEE *et al.,* 2015). Although the species is predominantly diploid, the occurrence of diploid-polyploid complexes in northern range of Sub-Saharan Africa suggests the role of polyploidy in colonization and expansion in these regions (ODEE *et al.,* 2015). Similarly, 31 species of genus *Camellia* belonging to section *Thea* show a 1.5-fold variarion in 2C values ranging from 4.45 pg in *C. gymnogyna* to 6.51 pg in *C. ptilophylla*. The section *Camellia* is more complicated because of frequent interspecific hybridization and polyploidy. The ploidy level in 53 species studied show a range of 2x,4x,6x,8x,10x,and 12x. The 2C values varied

8.9-fold from 2.86 pg in *C. delicata* to 25.35 pg in *C. lanosituba* (HUANG *et al.,* 2013). In addition to this the 2C DNA amount also varies 2-fold in diploid species which shows that DNA amount is varying both at diploid and polyploid levels (HUANG *et al.,* 2013).

Some studies are available which allow the comparison of genome size between woody and herbaceous taxa within certain narrow taxonomic groups or even among congeneric species. The genus *Cassia* is a fine example which comprises arboreal, shrubby and herbaceous species. Ten species studied are all diploid with 2n=28 except *C. tora* which has 2n = 26. The 2C DNA values in herbaceous species vary from 1.30 pg (*C. absus*) to 1.36 pg (*C. occidentalis*), shrubby species vary from 1.43 pg (*C. auriculata*) to 1.47 pg (*C. biflora*) while arboreal species studied show 1.39 pg (*C. fistula*), 1.40 pg (*C. siamea*) and 2.54 pg (*C. excelsa*). Therefore, interestingly one of the arboreal species *C. excelsa* has 1.9-fold bigger genome size than the average value of the herbaceous species (Ohri et al. 1986). Similarly in *Medicago* the genome size is known for 8 species. The sole arboreal species *M. arborea* (2n = 4x = 32) with 2C = 3.60 pg is 2.26-fold greater than the average 2C value (2C = 1.58 pg) of 5 diploid (2n = 2x = 16) herbaceous species while rest of the two tetraploid (2n = 32) herbaceous species *M. sativa* (2C = 3.5 pg) and *M. glutinosa* (2C = 3.9 pg) have similar values as the arboreal species (BENNETT and LEITCH, 2010). In the family Asteraceae genome sizes have been reported to range from 0.8 pg in *Leontodon longirostris* to 56.56 pg in *Coreopsis nuecensis* (VALLES *et al.,* 2013). Later a sample of 167 taxa of Asteraceae studied by GARCIA *et al.* (2013), showing a range from 1.12 pg in Inula heleniodes to 29.38 pg in *Santolina chamaecyparissus*, also included a woody species *Dasyphyllum villosum* which shows a genome size (2C = 8.44 pg) much higher than most of the herbaceous species in the family (GARCIA *et al.,* 2013). Similarly 10 species of *Cornus* show variation in 1Cx from 1.07 pg in *C. canadensis* (2n = 4x = 44) to 5.08 pg in *C. eydeana* (2n = 2x = 18) showing a 4.7-fold difference. Remarkably, the average 1Cx value (2.60 pg) of 9 arboreal species is 2.42-fold greater than *C. canadensis* which is a creeping, rhizomatous perennial herbaceous subshrub growing to 20 cm tall (SHEARER and RANNEY, 2013). These examples show that woody taxa within certain taxonomic groups in fact possess larger genome sizes than their closely related herbaceous taxa.

Insular and secondary woodiness

It is now known that many insular woody species have evolved, following adaptive change, from their continental herbaceous colonizer species. This is substantiated by recent molecular studies in which the insular woody species of some genera have been shown to be closely related to their herbaceous relatives on the mainland some of the examples are: *Aeonium* (BOHLE, 1996), *Echium* (MES AND 'T HART, 1996), *Viola* (BALLARD and SYTSEMA, 2000), *Eryngium* (CALVINO *et al.,* 2010), *Senecio* (PELSER *et al.,* 2010), *Fitchia* (HUFFORD *et al.,* 2003). LENS *et al.* (2013) have listed a number of species belonging to the families Crassulaceae, Fabaceae, Euphorbiaceae, Brassicaceae, Resedaceae, Malvaceae, Amaranthaceae, Caryophyllaceae, Polygonaceae, Gentianaceae, Rubiaceae, Lamiaceae, Plantaginaceae, Convulvulaceae, Boraginaceae, Apiaceae, Asteraceae, Caprifoliaceae showing insular woodiness on the Canary Islands. The question which arises here, in the present context, is how do the genome sizes of these insular woody species compare with that of their herbaceous relatives or does the change to woody habit lead to some alterations in genome size.

The 2C values of 13 *Echium* (2n = 16) species from Canary Islands show a range from 0.64 pg (*E. bonnetii*) to 1.01 pg (*E. aculeatum* and *E. hierrense*) (SUDA *et al.,* 2005). Interestingly, the average genome size of 12 woody species (2C = 0.84–1.01 pg) is 1.5-fold more than that of the only herbaceous species *E. bonnetii* (2C = 0.64 pg) (SUDA *et al.,* 2005). Another interesting case is of secondarily woody sunflower (MOYERS and RIESEBERG, 2013). The woody perennial winteri ecotype showed early development of vascular cambium and late flowering as compared to the typical *Helianthus annuus*. However, no difference in genome size was seen between the typical and mutant (woody) sunflower types (MOYERS and RIESEBERG, 2013).

Conclusions

The foregoing account shows that the genome size of hardwoods is not as constrained and limited in size as has been considered. Hardwoods have been shown to be genomically and kary-

ologically as diverse as their herbaceous counterparts. The genome size can show dramatic changes at homoploid levels as well as by the development of polyploidy series, natural triploidy and by induced polyploidy. These changes have been shown to have effect on their adaptability and geographic distribution. Moreover, the genome size, in certain groups having both herbaceous and woody taxa, is not correlated with the habit as woody taxa may be showing bigger genome size than their related herbaceous taxa. This anomaly is further exemplified by the insular or secondarily woody species where the insular woody species in some genera have on an average bigger genome size than the herbaceous species and secondary woodiness does not lead to any changes in the genome size. It can be concluded that while hardwoods may not have developed massive genome sizes like conifers but their genome sizes may not be as small and constrained as has been generally considered as they show remarkable variation both at holoploid and at 1Cx levels.

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