Genome Size Variation in Elms (Ulmus spp.) and Related Genera

Alan T. Whittemore1
U.S. National Arboretum, 3501 New York Avenue NE, Washington, DC 20002-1938

Zheng-Lian Xia
U.S. National Arboretum, Room 124, Building 010A, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705

Additional index words. DNA content, elm, flow cytometry, Hemiptelea, Planera, Ulmus, Zelkova

Abstract. Elms (Ulmus spp.) are iconic street and landscape trees, but their use is currently limited by susceptibility to disease, especially Dutch elm disease (DED). Improved access to disease-resistant germplasm will be of great benefit for ongoing breeding and selection programs, but these programs have been limited historically by uncertain relationships among Ulmus species, especially the North American species and their putative Old World relatives. Estimates of genome size from 28 species representing both subgenera of Ulmus (subg. Ulmus and subg. Oreoptelea) and six species in the related small genera Zelkova, Hemiptelea, and Planera were estimated using flow cytometry. Genome-size estimates were calibrated using seven elms with known chromosome counts. Results strongly supported the subgeneric classification of Wiegreffe et al. Monopoloid genome size was found to be quite constant within the subgenera of Ulmus they recognized and within the small genera, and polyplody is uncommon in these plants. However, there are consistent differences in genome size between the subgenera of Ulmus and between them and the smaller genera, and these differences can be used to place species in their proper taxon, knowledge which can be useful in identifying disease-resistant germplasm that may be compatible with Ulmus americana and other North American taxa. Two Asian species that have sometimes been considered to be related to North American species now placed in subg. Oreoptelea were tested. The Himalayan Ulmus villosa has a much smaller genome than either of the subgenera, indicating that its relationship with other elms is rather remote. It may be a source of novel genes in Ulmus, but our results indicate it is not close to U. americana or other New World species. In contrast, results from the rare Chinese species Ulmus elongata support its placement in subg. Oreoptelea. It is the only close relative of the North American elms that is native to Asia, where DED is believed to have originated, and its response to DED infection should be evaluated.

The genus Ulmus L. (the elms) holds a preeminent place in North American and European horticulture. Ulmus spp. have served as iconic street and landscape trees in both of these continents (Campanella, 2003; Dunn, 2000). Elms have also served many other purposes in other Northern Hemisphere cultures (Heybroek, 2015). The genus consists of 20–40 species, widespread in the north temperate zone and extending south into tropical mountains in both hemispheres (Fu et al., 2004; Sherman-Broyles, 1997).

The closest relatives of Ulmus are three small genera native to temperate regions of the Northern Hemisphere, Zelkova Spach, Hemiptelea Planch., and Planera J. F. Gmel. (Wiegreffe et al., 1998). The genus Zelkova Spach, with five or six species disjunct across Eurasia (Denk and Grimm, 2005), has also become important in American horticulture. The other two genera have one species each, Hemiptelea davidii (Hance) Planch., native to northeastern China and Korea, is used as a small tree or clipped into a thorn hedge in China, but it is not used in American horticulture. Genotypes from Inner Mongolia, China, are noted for their red fall color (Deligen, 2006), but have not yet been introduced to the West. Planera aquatica J. F. Gmel., native to seasonally flooded river bottoms in the southeastern United States (Godfrey, 1988), has been little used outside its native range, but it grows as a small tree in gardens and may be valuable for its tolerance of heat, flooding, and poorly drained soils.

Studies of chromosome number and structure have found very limited genomic divergence in the group. All members of these four genera that have been studied have chromosome numbers based on \( n = 14 \), with no aneuploid variation (Goldblatt and Johnson, 1979; Oginuma et al., 1990). Polyplody is rare, and it is known from only two species. U. americana is known to include tetraploids with \( 2n = 56 \) (Karrfalt and Kamskos, 1975) as well as diploids (Whittemore and Olsen, 2011), and Hemiptelea davidii has two reported chromosome numbers, \( 2n = 56 \) (Fu et al., 1998) and \( 2n = 84 \) (Oginuma et al., 1990), presumably tetraploid and hexaploid numbers based on \( n = 14 \).

Elms in North America and Europe have suffered high mortality from two diseases: DED, caused by several species of fungi native to East Asia (Brasier, 1991, 2001), and elm yellows (elm phloem necrosis), caused by a phytoplasma (Mittemperger, 2000; Sinclair, 2000). Because of the horticultural importance of elms, there has been much interest in selection and breeding for disease tolerance in the genus (Dunn, 2000).

To date, elm breeding has mostly involved species native to Europe and North America, although the fungi that cause DED are native to eastern Asia; the North American and European elm species have low levels of resistance. Although Ulmus is most diverse in East Asia, with 21 species native to China alone (Fu et al., 2004), until recently, relatively little germplasm from this area has been available to Western breeders. The work of Fu et al. (2004), which reduces several of the names used in previous literature, including U. japonica (Rehder Sarg. 1907 not Sieb. 1830, U. propinqua Koidz., and U. wilsoniana C.K. Schneid, to synonyms of U. davidiana var. japonica (Rehder) Nakai, indicates that the level of diversity is even lower than what was previously thought; thus, Warren (2000) lists four Asian elm species (U. japonica, U. paryvfolia, U. pumila, and U. wilsoniana) that have contributed to commercial cultivars now available in the west, but using the taxonomy of Fu et al. (2004), this list includes only three valid species, U. davidiana var. japonica, U. paryvfolia, and U. pumila, which are now recognized.

In the 1980s and 1990s, much new elm germplasm was introduced to North America from China through the efforts of the late George Ware (Ware, 1995). Chromosome counts for many of these introductions were published, but the plants were juvenile and not flowering at the time they were studied (Santamour and Ware, 1997). Most of these trees are now producing fruit and showing adult bark characteristics, both of which are important for identification. In addition, taxonomic treatments of the Chinese species have been published (Fu et al., 1998, 2004), allowing more accurate identifications of the Asian species. It has thus been possible to correct some misidentifications in Ware’s Asian germplasm.

Unfortunately, the relevance of this material to research and breeding on U. americana is uncertain. The difficulty of crossing tetraploid U. americana with other Ulmus spp. has often been attributed to ploidy differences, but Ager and Guries (1982) and Bob et al. (1986) demonstrate that crossing...
barriers between *U. americana* and several diploid elm species are not ploidy-related. Studies of interspecific hybridization in *Ulmus* have shown that different combinations of parents show different levels of compatibility (Hans, 1981; Townsend, 1975), but the planning of controlled breeding programs was limited in the past because traditional classifications of *Ulmus* did not seem to reflect relationships adequately (Hans, 1981).

The infrageneric classification of *Ulmus* has now been placed on a more solid footing by the work of Wiegrefe et al. (1994). All elms available to those authors were placed in two well-marked subgenera, *Ulmus* subg. *Ulmus* and *Ulmus* subg. *Oreoptelea* (Spach) Planch. This has presented a problem for the American elm breeders. *U. americana* belongs to subg. *Oreoptelea*, which is predominately North American (only a single Old World species, the European *U. laevis*, was placed here by Wiegrefe et al.), whereas all of the elm species studied by Wiegrefe et al. that are native to eastern Asia (the area where the fungi causing DED are native) belong to subg. *Ulmus*. Past attempts to breed resistance to DED from other elm species into *U. americana* have not met with success, but they involved crossing *U. americana* (of subg. *Oreoptelea*) with species of subg. *Ulmus* (especially *U. pumila*). Quite a few hybrid combinations have been reported to yield at least occasional fruit (Townsend, 1975), but the only hybrid cultivars that have been released are hybrids among the Euro- pean and Asian species of subg. *Ulmus* (Warren, 2000), indicating limited genetic compatibility between the subgenera.

However, two Asian species that were not available to Wiegrefe et al. have been placed by some authors in sect. *Chaetoptelea* (Liebm.) C.K. Schneid., a group of species that falls within *Ulmus* subg. *Oreoptelea* as defined by Wiegrefe et al. (1994). If true, then sources of resistance genes that are more compatible with the North American elms may be available in the genus. The Himalayan species *U. villosa* was placed in this group by Gruzdinskaya (1974) and Richens (1983), based on the characteristics of its fruit (its inflorescence resembles that of subg. *Ulmus*), whereas Fu et al. (1979, 1998) placed the Chinese species *U. elongata* in this group, based on the characteristics of its inflorescence and fruit. If these placements are correct, *U. villosa* and *U. elongata* would fall within *Ulmus* subg. *Oreoptelea* as defined by Wiegrefe et al. (1994), and these poorly known species would be the only close relatives of *U. americana* native to Asia, where DED is believed to have originated. In this case, it would be worth investigating them as possible sources of resistance genes that would be more compatible with the genetic background of *U. americana* and other species of subg. *Oreoptelea* than the species of subg. *Ulmus*, the only DED-resistant species that have been studied to date. Santamour (1979) presented evidence that *U. villosa* shows resistance to DED, but DED resistance has never been studied in *U. elongata* (Smalley and Guries, 2000). Finding other characteristics that distinguish the subgenera could confirm or refute the placement of these two species in subg. *Oreoptelea*, which in turn could provide direction for future study of how elm species respond to these diseases and for future elm breeding.

Flow cytometry provides a measure of nuclear DNA content and a method for examining genomic diversification that is much faster and easier than chromosome counts. Flow cytometry can be carried out on most tissues of the plant, and it provides a different view of diversity in a group of plants than chromosome number. A recent flow cytometry survey of *U. americana* (Whittemore and Olsen, 2011) revealed unexpected variation in the species. It is desirable to extend this work for several reasons. First, the study of Whittemore and Olsen quantified the DNA by staining with 4',6-diamidino-2-phenylindole (DAPI) and calibrated the measurements with a single chromosome count. The staining reaction of DAPI is specific to AT bps, so it gives an accurate estimate of total DNA only if the percent AT in the genome is similar in the study organism and the internal standard (Dolezel et al., 2007a). This is not a problem if DNA is visualized using an intercalating dye such as propidium iodide (PI) rather than a stain. Carrying out flow cytometry using PI and calibrating the work using additional trees with known chromosome numbers will give us a firmer understanding of variation in genome size. In addition, a broad survey of nuclear DNA content in *Ulmus* and related genera using flow cytometry could provide more information on the distribution of natural polyploids, and reveal differences in genome size between the genera and subgenera. Knowledge of genome-size variation, in turn, can help to place species whose relationships are uncertain. In view of the importance of *Ulmus* and *Zelkova* in American horticulture, and especially the need to find disease-resistant germplasm, a broad survey of *Ulmus* and related genera using flow cytometry was conducted, emphasizing species of uncertain relationship, and germplasm that has only been recently introduced to American horticulture and not well characterized.

Materials and Methods

Fruit and leaf tissue of *Ulmus, Zelkova, Hemiptelea,* and *Planera* were collected from trees in the living collections of the U.S. National Arboretum or from wild plants in the United States, and fresh leaves for analysis were supplied by collaborators from the collections of Arnold Arboretum (Jamaica Plain, MA) and Morton Arboretum (Lisle, IL). Voucher specimens are preserved in the herbarium of the U.S. National Arboretum (Index Herbariorum acronym: NA; Thiers, 2016). The tissue for analysis was stored on ice or in the refrigerator and analyzed when fresh. Whenever possible, at least three accessions per species were sampled. Both *U. bergmanniana* C.K. Schneid. and *U. gaussenii* W. C. Cheng have been reported to be cultivated in North America, but all seen cultivated specimens that were named as these species were found to be misidentified, so the species could not be sampled. Samples included seven elms with known chromosome counts previously reported by Santamour (1969), Santamour and Ware (1997), and Sherald et al. (1994). The identity and source of all plant material are listed in Tables 1 and 2.

Table 1. DNA content estimates for accessions with known chromosome counts.

| Taxon subg. *Oreoptelea* | Individual | Reported chromosome number | Source of chromosome number | 2C | 1C |
|------------------------|------------|-----------------------------|----------------------------|----|----|
| *Ulmus americana* Jefferson | NPS 3-487, NA 62001 | 2n = 3x = 42 | Sherald et al. (1994) | 4.652 pg | 1.551 pg |
| *Ulmus elongata* | R94-6, NA 68995 | 2n = 2x = 28 | Santamour and Ware (1997) | 3.00 pg | 1.50 pg |
| *Ulmus castaneifolia* | R94-11, NA 68978 | 2n = 2x = 28 | Santamour and Ware (1997) | 3.838 pg | 1.919 pg |
| *Ulmus chenmoui* | R93-17, NA 76222 | 2n = 2x = 28 | Santamour and Ware (1997) | 3.979 pg | 1.909 pg |
| *Ulmus davidiana* var. *davidiana* | R94-8, NA 76224 | 2n = 2x = 28 | Santamour and Ware (1997) | 3.876 pg | 1.938 pg |
| (as *U. gaussenii*) | | | | | |
| *Ulmus lamellosa* (as *U. taitangshanica*) | R95-21, NA 68992 | 2n = 2x = 28 | Santamour and Ware (1997) | 3.955 pg | 1.978 pg |
| *Ulmus macrocarpa* | PI 138018, NA 1847 | 2n = 2x = 28 | Santamour (1969) | 3.987 pg | 1.993 pg |

1 Accession numbers beginning in R are Morton Arboretum research numbers (used by Santamour and Ware, 1997); beginning in PI, USDA PI numbers (used by Santamour, 1994); beginning in NPS, National Park Service numbers (used by Sherald et al., 1994); beginning in NA, National Arboretum accession numbers (current database numbers for these accessions). All samples are currently growing at the U.S. National Arboretum.

2 2C designates the measured DNA content of one nucleus (Dolezel et al., 2007b).

3 1C designates the inferred DNA content of a haploid chromosome set (Dolezel et al., 2007b).
Table 2. Nuclear genome-size estimates for 96 accessions (34 species) of *Ulmus* and three related genera. Samples are in the same order as in Fig. 1.

| Genus/subg. | Taxon | Source | ID | Provenance | Organ | N<sub>e</sub> | 2C | CV<sub>e</sub> |
|-------------|-------|--------|----|------------|-------|-------------|----|-------------|
| *Ulmus* L. subg. *Oreoptelea* Spach (mean estimated 1C: 1.55 pg) | *U. alata* Michx. | AA | 404-95-a | Franklin County, Tennessee | L | 3 | 3.008 | 0.009 |
| | *U. alata* ‘Lace Parasol’ | NA | 77773-H | Unknown | L | 3 | 3.142 | 0.008 |
| | *U. alata* | Wild | Whittemore 16-029 | Banks County, Georgia | L | 3 | 3.141 | 0.003 |
| | *U. alata* | Wild | Whittemore 16-034 | Gaston County, North Carolina | L | 3 | 3.044 | 0.011 |
| | *U. americana* L. 2x | NA | Whittemore 12-014 | Orange County, Virginia | L | 3 | 3.111 | 0.008 |
| | *U. americana* 2x | NA | Whittemore 12-040 | Etowah County, Alabama | L | 3 | 3.169 | 0.011 |
| | *U. americana* 2x | NA | Whittemore 12-049 | Cocke County, Tennessee | L | 3 | 3.161 | 0.003 |
| | *U. americana* 2x | NA | Whittemore 13-027 | Taylor County, Texas | L | 3 | 3.157 | 0.015 |
| | *U. americana* 2x | NA | Whittemore 14-048 | Montgomery County, Maryland | L | 3 | 3.196 | 0.013 |
| | *U. americana* 4x | NA | Whittemore 12-030 | Butts County, Georgia | L | 3 | 6.572 | 0.007 |
| | *U. americana* 4x | NA | Whittemore 13-018 | Hill County, Texas | L | 3 | 6.234 | 0.007 |
| | *U. americana* 4x | NA | Whittemore 13-019 | Hill County, Texas | L | 3 | 6.007 | 0.010 |
| | *U. americana* 4x | Wild | Whittemore 16-032 | Cherokee County, South Carolina | L | 3 | 6.204 | 0.014 |
| | *U. americana* 4x | MOR | 1053-28*1 | Unknown | L | 3 | 6.241 | 0.019 |
| | *U. americana* 4x | MOR | 13-058B | Jackson County, Arkansas | L | 3 | 6.296 | 0.010 |
| | *U. americana* ‘Delaware’ | NA | 66341-H | Unknown | L | 3 | 6.210 | 0.012 |
| | *U. americana* ‘New Harmony’ | AA | 57844-P | Unknown | L | 3 | 6.077 | 0.010 |
| | *U. americana* ‘Valley Forge’ | NA | 76510-L | Unknown | L | 3 | 6.216 | 0.007 |
| | *U. crassifolia* Nutt. | NA | 311-2002-a | Travis County, Texas | L | 3 | 2.223 | 0.030 |
| | *U. elongata* | AA | 758-86-a | Yunnan, China | L | 3 | 3.106 | 0.017 |
| | *U. elongata* | AA | 445-2002-b | Yunnan, China | L | 3 | 3.891 | 0.005 |
| | *U. elongata* | AA | 445-2002-c | Yunnan, China | L | 3 | 3.721 | 0.003 |
| | *U. laciniata* Planch. var. *laciniata* Rehd. Nakai ‘Prospector’ | NA | 68987 | Yunnan, China | L | 3 | 3.838 | 0.010 |
| | *U. laciniata* Planch. var. *nigrolineata* (Rehd.) Mayr | NA | 76224-006 | Anhui, China | F&L | 6 | 3.837 | 0.019 |
| | *U. laciniata* Planch. var. *nigrolineata* (Rehd.) Mayr ‘Morton’ | NA | 76234-004 | Inner Mongolia Autonomous Region, China | F | 3 | 3.908 | 0.016 |
| | *U. laciniata* Planch. var. *japonica* (Rehd.) Nakai ‘Prospector’ | NA | 55398 | Unknown | L | 3 | 3.780 | 0.017 |
| | *U. laciniata* Planch. var. *japonica* (Rehd.) Nakai ‘Morton’ | NA | 76945-H | Unknown | L | 3 | 3.633 | 0.004 |
| | *U. laciniata* Planch. var. *japonica* | NA | 76243-001 | Unknown | F&L | 6 | 3.781 | 0.016 |
| | *U. laciniata* Planch. var. *uncertain* | NA | 68990 | Shanxi, China | L | 3 | 3.649 | 0.009 |
| | *U. glabra* Huds. | AA | 391-2001-b | Republic of Georgia | L | 3 | 3.982 | 0.004 |
| | *U. glabra* | AA | 391-2001-a | Republic of Georgia | L | 3 | 3.947 | 0.007 |
| | *U. glabra* | MOR | 255-81*6 | Baden-Wurttemberg, Germany | L | 3 | 4.058 | 0.014 |
| | *U. glabra* | MOR | 553-2001*4 | Republic of Georgia | L | 3 | 4.009 | 0.011 |
| | *U. glaucescens* Franch. var. *glaucescens* | NA | 76248-001 | China | L | 3 | 3.674 | 0.005 |
| | *U. harbinensis* S.Q. Nie and K.G. Huang | NA | 76255-002 | China | L | 3 | 3.804 | 0.000 |
| | *U. laciniata* (Trautv.) Mayr var. *laciniata* | AA | 250-2001-a | Mt. Oh-dae, Korea | L | 3 | 3.759 | 0.003 |
| | *U. laciniata* var. *nikkoensis* Rehd. | MOR | 180-84*1 | Hongshu, Japan | L | 3 | 3.961 | 0.014 |

(Continued on next page)
Table 2. (Continued) Nuclear genome-size estimates for 96 accessions (34 species) of *Ulmus* and three related genera. Samples are in the same order as in Fig. 1.

| Genus/subgenus | Taxon | Source | ID | Provenance | Organ | N | 2C | CV |
|----------------|-------|--------|----|------------|-------|---|----|----|
| Ulmus subg. uncertain (mean estimated 1C: 1.11 pg) | *U. lamellosa* Wang and S.L. Chang | NA | 68915H | China | F&L | 9 | 3.771 | 0.027 |
| | *U. lamellosa* | NA | 68992 | China | L | 3 | 3.955 | 0.006 |
| | *U. lamellosa* | NA | 76230-002 | China | L | 3 | 3.872 | 0.008 |
| | *U. macrocarpa* Hance | NA | 1847T | Beijing, China | F&L | 12 | 3.987 | 0.031 |
| | var. macrocarpa | NA | 76247-001 | China | L | 6 | 5.678 | 0.010 |
| | *U. microcarpa* L.K. Fu | NA | 12852H | Unknown | F&L | 6 | 3.724 | 0.016 |
| | *U. minor* Mill. 'Christine Busman' | NA | 76240-002 | Heilongjiang, China | L | 3 | 3.732 | 0.002 |
| | *U. pseudoepingpua* Wang and Li | NA | 76252-005 | Unknown | F&L | 6 | 3.781 | 0.012 |
| | *U. pumila* L. | AA | 673-87-a | Beijing, China | L | 6 | 3.671 | 0.028 |
| | *U. pumila* | Cult | Whitmore 16-011 | Unknown | L | 3 | 3.790 | 0.015 |
| | *U. pumila* | Cult | Whitmore 16-046 | Unknown | F | 3 | 3.920 | 0.011 |
| | *U. rubra* Muhl. | Wild | Whitmore 16-005 | Prince George’s County, Maryland | F&L | 6 | 3.770 | 0.011 |
| | *U. rubra* | NA | 73233-H | Rutherford County, North Carolina | L | 3 | 4.006 | 0.004 |
| | *U. scheuchzeriana* Fang | NA | 76235-005 | China | L | 3 | 3.781 | 0.012 |
| | *U. scheuchzeriana* | NA | 76250-005 | China | F&L | 6 | 3.711 | 0.016 |
| | *U. yomatsui* Hayata | NA | Whitmore 16-047 | Unknown | F | 3 | 4.023 | 0.021 |
| | *U. wallichiana* Planch. | NA | 68981 | India | L | 3 | 4.165 | 0.013 |

*Ulmus* subg. uncertain (mean estimated 1C: 1.11 pg)

| Taxon | Source | ID | Provenance | Organ | N | 2C | CV |
|-------|--------|----|------------|-------|---|----|----|
| *U. villosa* Brandis | Cult | Bartlett 8384 | Unknown | L | 3 | 2.175 | 0.005 |
| *U. villosa* | Cult | Bartlett 8385 | Unknown | L | 7 | 2.277 | 0.030 |

*Hemiptelea* Planch. (mean estimated 1C: 0.84 pg)

| Taxon | Source | ID | Provenance | Organ | N | 2C | CV |
|-------|--------|----|------------|-------|---|----|----|
| *H. davidii* (Hance) Planch. | AA | 14698-a | Republic of Korea | L | 3 | 5.065 | 0.006 |
| *H. davidii* | AA | 382-91-a | Republic of Korea | L | 3 | 4.829 | 0.014 |
| *H. davidii* | AA | 387-92-a | Pyongyang, PDR of Korea | L | 6 | 3.486 | 0.017 |

*Planera* J.F. Gmel. (mean estimated 1C: 1.39 pg)

| Taxon | Source | ID | Provenance | Organ | N | 2C | CV |
|-------|--------|----|------------|-------|---|----|----|
| *P. aquatica* J.F. Gmel. | Wild | Whitmore 16-026 | Effingham County, Georgia | L | 3 | 2.772 | 0.006 |

*Zelkova* Spach (mean estimated 1C: 1.68 pg)

| Taxon | Source | ID | Provenance | Organ | N | 2C | CV |
|-------|--------|----|------------|-------|---|----|----|
| *Z. carpinifolia* (Pall.) K. Koch | NA | 74243-003 | Republic of Georgia | L | 3 | 3.218 | 0.018 |
| *Z. schneideriana* Hand.-Mazz. | NA | 64945-H | Hubei, China | L | 3 | 3.457 | 0.015 |
| *Z. serrata* (Thunb.) Makino | NA | 44933-H | Honshu, Japan | L | 3 | 3.478 | 0.006 |
| *Z. sinica* C.K. Schneid. | NA | 71056-H | Unknown | L | 3 | 3.304 | 0.017 |

Flow cytometry was carried out on a Sysmex CyFlow Space flow cytometer using the extraction buffer and staining buffer from the Sysmex Cystain PI Absolute P kit (Sysmex, Germany) according to the manufacturer’s instructions. Fresh leaf tissue of *Glycine max* ‘Williams 82’, with a monoploid genome size [1C-value: DNA content of the monoploid chromosome set (Greilhuber et al., 2005)] of 1.13 pg (Chesnay et al., 2007), was used as an internal standard. Accessions of *U. villosa* overlapped with *Glycine max* ‘Williams 82’, so size estimates were confirmed using a second internal standard, *Zea mays* ‘CE-177’, with a monoploid chromosome set of 2.715 pg (Dolezel et al., 2007a). About 0.5 cm² of *Ulmus* tissue was cochopped with leaf tissue of the internal standard (<0.5 cm²) with a double-sided razor blade for 30 to 60 s in 400 µL of extraction buffer, then incubated for ≈ 60 s. Suspensions were filtered through 50 µm nylon mesh filters, and nuclei were stained with 1.6 mL of staining buffer containing PI and RNAase A, then incubated for about 2 h protected from light at room temperature. The nuclear suspension was analyzed on the flow cytometer with fluorescence excitation provided by a laser emitting at 488 nm. The mean fluorescence of each sample was compared with mean fluorescence of the internal standard to determine holoploid genome size [2C-value: DNA content of the whole complement of chromosomes characteristic for the organism, irrespective of the ploidy level (Greilhuber et al., 2005)]. At
least 3000 nuclei were counted to determine the ratio of sample peak to the internal standard and, thus, nuclear DNA content \(2C\) value.

\[
(\text{sample peak}/\text{internal standard peak}) \cdot (2^{1.13} \text{ pg})
\]

A minimum of three independent preparations were run from each plant sample, and the mean and coefficient of variation (CV) were calculated for all runs with each sample. Ploidy level, thus DNA content of the monoploid chromosome set \(1C\), was determined by comparison with seven elms of known chromosome number (see above). For selected specimens, separate analyses were done on fruit and expanding leaves, or on fruit (spring) and late-summer leaves, to test whether results from different organs and different seasons are comparable.

Results and Discussion

Seven elms were available with known chromosome counts previously reported by Santamour (1969), Santamour and Ware (1997), and Sherald et al. (1994), representing two species of Ulmus subg. Oreoptelea and five species of Ulmus subg. Ulmus. Flow cytometry was used to measure nuclear DNA content \(2C\)-value for these seven plants. Monoploid DNA content \(1C\) was calculated from the \(2C\) value assuming a haploid chromosome number of \(x = 14\). Results are given in Table 1. Estimates of monoploid genome size \(1C\) were consistent within subgenera, but estimates for species of subg. Ulmus were consistently about 30% larger than they were for subg. Oreoptelea.

The genome size of one of these trees, Ulmus americana ‘Jefferson’ (NPS 3-487, NA 62001), was previously measured using DAPI rather than PI as the stain (Whittemore and Olsen, 2011). Size estimates using the two stains were similar \((2C = 4.652 \text{ pg using PI}, 2C = 4.71 \text{ pg using DAPI})\), providing preliminary indications that the proportions of AT and GC bps in the Ulmus genome are about similar to proportions in the plant used as the size standard, Glycine max ‘Williams 82’ (65.25% AT, 34.75% GC; Song et al., 2010). This provides evidence that as long as Glycine max ‘Williams 82’ is used as the internal standard, no correction needs to be applied when comparing size estimates using PI and DAPI, at least for Ulmus americana.

Estimates of nuclear DNA content were obtained from a total of 96 individuals, representing 34 species and four genera. Size estimates from multiple runs on the same sample were consistent, with among-run CVs averaging 1.2%, and 93% of the among-run CVs below 2.5%. Results obtained from fruit, expanding leaves, and late-summer leaves were similar (data not shown).

Although the base chromosome number is constant in these four genera, flow cytometry does reveal divergence in genome size among the genera and subgenera. These results provide strong support for the subgenera as defined by Wiegrefe et al. (1994). In the full data set, as in the seven species of known chromosome count, estimates of monoploid genome size \(1C\) were consistent within Wiegrefe et al.’s subgenera, but estimates for species of subg. Ulmus were consistently about 30% larger than they were for subg. Oreoptelea. Monoploid genome size also varies among the smaller genera, but it is consistent within Zelkova, the only other genus with more than one species (Table 2; Fig. 1). Flow cytometry may thus be useful in placing species in their correct genus or subgenus, checking identification of sterile elms, and checking the parentage of putative hybrids between different subgenera of Ulmus.

This preliminary survey confirms variable ploidy in the two species where it has been reported previously, Ulmus americana and Hemiptelea davidii. The ploidy variation in Hemiptelea davidii, together with the low pollen stainability reported by Fu et al. (1998), make it seem that reproduction in this species may follow some unusual genetic process; for example, apomixis is often associated with high ploidy levels and low pollen viability (Savidan et al., 2001). Tetraploid Ulmus americana, in
contrast, is well known to reproduce sexually in the normal way (Ager and Guries, 1982; Shattuck, 1905).

No evidence was found of cryptic variation in any other species, such as that recently demonstrated within *U. americana* (Whittewome and Olsen, 2011), although more extensive sampling within the species will be required to completely rule this out. The single triploid tree of *U. microcarpa* is not too surprising because rare polyploid individuals are found in other elm species (Ehrenberg, 1949; A.T. Whittewome, unpublished data). Additional study of *U. microcarpa* was not possible because tissue suitable for flow cytometry could only be obtained from the one tree.

These results confirm the uniqueness of tetraploid *U. americana* in this otherwise diploid genus, already commented on by many authors (Santamour and Ware, 1997).

The flow cytometry results from the two samples of *U. villosa* do not support the inclusion of this poorly known Himalayan species in subg. *Oreoptelea*, where it was placed by Grudzinskaya (1974) and Richens (1983). The diploid genome of *U. villosa* is only \(2C = 2.2\) pg, much smaller than any of the other diploid elms tested (3.0–4.2 pg). Morphologically, this species does not fit into either of the recognized subgenera (see above). Its genome size, which differs sharply from all other elm species, and its ambiguous morphology both provide evidence of a relatively strong overall genetic divergence from the remaining species of the genus. Further investigation will probably show that a third subgenus will be needed to accommodate this single species. Because of its remote position and strong genomic differentiation, *U. villosa* may be a potential source of novel characteristics not found in other elms.

In contrast, the genome of *U. elongata* (2C = 3.00) measured from the one available sample is similar in size to genomes of species placed in subg. *Oreoptelea*, but it is much smaller than the genomes of species in subg. *Ulmus*. This supports the conclusion of Fu et al. (1979, 1998) that *U. elongata* is related to a group of North American species, and it should be placed in *Ulmus* subg. *Oreoptelea* as defined by Wiegrefe et al. (1994). This poorly known species is thus confirmed to be the only close relative of *U. americana* native to eastern Asia, where DEL originated. As previously mentioned, study of the response of *U. elongata* to DED infection could be valuable. If it shows resistance to DED fungi, as might be expected for an elm native to eastern Asia, any resistance genes found in the species are expected to be more compatible with the genetic background of *U. americana* and other species of *Ulmus*. *Oreoptelea* is more closely related than the species of subg. *Ulmus*, the only DEL-resistant species that have been studied to date. Research into how *U. elongata* responds to inoculation with DEL fungi could improve our understanding of survival strategies that are viable in the species of *Ulmus* subg. *Oreoptelea*. Furthermore, breeding with *U. elongata* could potentially produce disease-resistant elms with characteristics of *U. americana*, something that has not been accomplished by crossing *U. americana* with members of subg. *Ulmus*.

In its native range in China, *U. elongata* is uncommon and its habitat is highly fragmented, and it is legally protected (Gao et al., 2012). Limited germplasm is available in the United States (possibly all cuttings from a single tree). This germplasm is being propagated, and it is hoped that it can be distributed to multiple sites, so the germplasm will be more secure and available for future research.

This study demonstrates consistent genome-size differences between subgenera of *Ulmus* and related genera that can be used to clarify the taxonomic placement of species whose relationships are ambiguous. It also indicates that *U. elongata* may be a valuable target for future research and breeding of North American members of *Ulmus* subg. *Oreoptelea* (most New World elms). On the other hand, *U. villosa* may be a good source of novel alleles in *Ulmus*, but should not be expected to show strong genetic compatibility with other species of the genus. At the same time, these results confirm the overall conservatism of genome-size evolution in *Ulmus* and related genera.

### Literature Cited

Ager, A.A. and R.P. Guries. 1982. Barriers to interspecific hybridization in *Ulmus americana* Euphytica. 31:909–920.

Bob, C.F., B.L. Redmond, and D.F. Karrnosky. 1986. On the nature of intra- and interspecific incompatibility in *Ulmus*. Amer. J. Bot. 73:465–474.

Brazier, C.M. 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemic. Mycopathologia 115:151–161.

Brazier, C.M. 2001. Rapid evolution of introduced plant pathogens via interspecific hybridization. Bioscience 51:123–133.

Campanella, T.J. 2003. Republic of shade: New England and the American elm. Yale Univ. Press, New Haven, CT.

Chesnay, C., A. Kumar, and S.R. Pearce. 2007. Genetic diversity of SIRE-1 retroelements in *Glycine max*. Mol. Gen. Genomics 277:449–458.

Dolezel, J., J. Greilhuber, and J. Suda. 2007b. Flow cytometry in plant genetics and cytogenetics. Kluwer Academic Publishers, Boston, MA.

Ehrenberg, C.E. 1949. Studies on asynapsis in the *Bacillus* var. *lignens*. Z. Wiss. Ver. Naturforsch. 9:129–157.

Deligen, R.J. 2005. *Rhodora* 107:5–47.

Dobzhansky, T. 1945. *Genetics and the Origin of Species*. Columbia University Press, New York.

Dolezel, J., J. Greilhuber, and J. Suda. 2007a. Flow cytometry in plant genetics and cytogenetics. Kluwer Academic Publishers, Boston, MA.

Ehrenberg, C.E. 1949. Studies on asynapsis in the *Bacillus* var. *lignens*. Z. Wiss. Ver. Naturforsch. 9:129–157.

Dolezel, J., J. Greilhuber, and J. Suda. 2007a. Flow cytometry in plant genetics and cytogenetics. Kluwer Academic Publishers, Boston, MA.

Ehrenberg, C.E. 1949. Studies on asynapsis in the *Bacillus* var. *lignens*. Z. Wiss. Ver. Naturforsch. 9:129–157.

Dolezel, J., J. Greilhuber, and J. Suda. 2007a. Flow cytometry in plant genetics and cytogenetics. Kluwer Academic Publishers, Boston, MA.

Ehrenberg, C.E. 1949. Studies on asynapsis in the *Bacillus* var. *lignens*. Z. Wiss. Ver. Naturforsch. 9:129–157.
Thiers, B. 2016. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. 20 Sept. 2016. <http://sweetgum.nybg.org/science/ih/>.

Townsend, A.M. 1975. Crossability patterns and morphological variation among elm species and hybrids. Silvae Genet. 24:18–23.

Ware, G.H. 1995. Little-known elms from China: Landscape tree possibilities. J. Arboriculture 21:284–288.

Warren, K. 2000. The return of the elm: Status of elms in the nursery industry, p. 341–348. In: C.P. Dunn (ed.). The elms: Breeding, conservation, and disease management. Kluwer Academic Publishers, Boston, MA.

Whittemore, A.T. and R. Olsen. 2011. *Ulmus americana* (Ulmaceae) is a polyploid complex. Amer. J. Bot. 98:754–760.

Wiegrefe, S.J., K.J. Sytsma, and R.P. Guries. 1994. Phylogeny of elms (*Ulmus*, Ulmaceae): Molecular evidence for a sectional classification. Syst. Bot. 19(4):590–612.

Wiegrefe, S.J., K.J. Sytsma, and R.P. Guries. 1998. The Ulmaceae, one family or two? evidence from chloroplast DNA restriction site mapping. Plant Syst. Evol. 210:249–270.