Ploidy and Genome Size in Lilac Species, Cultivars, and Interploid Hybrids

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ABSTRACT. Genome size variation can be used to investigate biodiversity, genome evolution, and taxonomic relationships among related taxa. Plant breeders use genome size variation to identify parents useful for breeding sterile or improved ornamentals. Lilacs (Syringa) are deciduous trees and shrubs valued for their fragrant spring and summer flowers. The genus is divided into six series: Syringa (Vulgares), Pinnatifoliae, Ligustrea, Ligustrina, Pubescentes, and Villosae. Reports conflict on genome evolution, base chromosome number, and polyploidy in lilac. The purpose of this study was to investigate genome size and ploidy variation across a diverse collection. Flow cytometry was used to estimate monoploid (1Cx) and holoploid (2Cx) genome sizes in series, species, cultivars, and seedlings from parents with three ploidy combinations: 2x, 2x x 2x, and 3x x 2x. Pollen diameter was measured to investigate the frequency of unreduced gametes in diploid and triploid Syringa vulgaris cultivars. Three triploids of S. vulgaris were observed: ‘Aucubaefolia’, ‘Aginecourt Beauty’, and ‘President Grévy’. Across taxa, significant variations in 1Cx genome size were discovered. The smallest and largest values were found in the interspecific hybrids S. ×lacinuata (1.32 ± 0.04 pg) and S. ×hyacinthiflora ‘Old Glory’ (1.78 ± 0.05), both of which are in series Syringa. Series Syringa (1.68 ± 0.02 pg) had a significantly larger 1Cx genome size than the other series. No significant differences were found within series Pubescentes (1.47 ± 0.01 pg), Villosae (1.55 ± 0.02 pg), Ligustrea (1.49 ± 0.05 pg), and Pinnatifoliae (1.52 ± 0.02 pg). For S. vulgaris crosses, no significant variation in 2C genome size was discovered in 2x x 2x crosses. Interploid crosses between ‘Blue Skies’ (2x) and ‘President Grévy’ (3x) produced an aneuploid population with variable 2C genome sizes ranging from 3.41 ± 0.03 to 4.35 ± 0.03 pg. Only one viable seedling was recovered from a cross combination between ‘President Grévy’ (3x) and ‘Sensation’ (2x). This seedling had a larger 2C genome size (5.65 ± 0.02 pg) than either parent and the largest 2C genome size currently reported in lilac. ‘Sensation’ produced 8.5% unreduced pollen, which we inferred was responsible for the increased genome size. No unreduced pollen was discovered in the other diploids examined. Increased ploidy may provide a mechanism for recovering progeny from incompatible taxa in lilac breeding.

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size (C-value) and ploidy level within Syringa would contribute to the call for a global census of angiosperm C-values (Galbraith et al., 2011). Although genome sequencing is a powerful tool for studying gene function, C-values calculated from sequencing data tend to underestimate true genome size (relative to flow cytometry) because of misassembly and the inability to sequence through repetitive regions of the genome (Bennett and Leitch, 2011). Flow cytometry measurements of genome size have proven useful for the identification of species, hybrids, polyploids, and polyploid series (Galbraith et al., 2011).

In genera such as lilac with a long history of breeding and cultivation, variation in genome size and chromosome number can occur from interspecific hybridization, unreduced gametes, and the induction of autopolyploids. Interspecific hybridization has been a valuable tool for producing many new cultivars of lilac (Table 1). Two reports on genome size estimates in lilac focused on two European species, S. vulgaris and S. josikae. Siljak-Yakovlev et al. (2010) reported S. vulgaris to have a 2C genome size of 2.4 pg based on propidium iodide flow cytometry. Olszewska and Osiecka (1984) reported S. josikae to have a 2C genome size of 2.6 pg based on Feulgen cytometry. Despite the paucity of genome size estimates in lilac, much effort has been dedicated to studying chromosome number variation in lilac and the Oleaceae.

Phylogenetic analysis has determined the ancestral state of the Oleaceae to be diploid (Taylor, 1945). Cyto-taxonomy divides the Oleaceae into two groups according to basic chromosome number with the first group consisting of Mendora (x = 11), Jasminum (x = 13), Fontanesia (x = 13), Forsythia (x = 14), and Abelophyllum (x = 14). The second group (originally designated as subfamily Oleoideae) consists of genera with x = 23, including Olea, Syringa, Ligustrum, Fraxinus, Osmanthus, Forestiera, Phillyrea, Osmarea, and Chionanthus (Taylor, 1945). Lilacs are primarily diploids with basic chromosome number in lilac to be x = 12 (Taylor, 1945). Lilacs are primarily diploids with basic chromosome number reported at x = 11 and x = 12. Therefore, the variation in chromosome number observed in common lilac is likely the result of aneuploidy over centuries of plant collection and wide hybridization.

Aside from theories of ancestral allopolyploidy, no reports exist to confirm polyploidy in wild or cultivated lilac populations. In addition, no reports of natural polyploidy exist for the closely related genus Ligustrum. However, natural polyploidy has been discovered in other related genera in Oleaceae. Taylor (1945) reported tetraploids in Mendora, tetraploids and triploids in Jasminum, tetraploids and hexaploids in Fraxinus, and hexaploids in Osmanthus. In white ash (Fraxinus), the tetraploid F. smilii and hexaploids, such as F. bitimoreana and F. profunda, are hypothesized to have allopolyploid origins (Miller, 1955; Nesom, 2010; Santamour, 1962).

Early efforts producing artificial polyploids in lilac were reported to be successful. In the middle of the 20th century, Karl Sax produced colchicine-induced autopolyploids of S. vulgaris at the Arnold Arboretum (Fiala and Vrugtman, 2008). Fiala reportedly produced tetraploid forms of S. julianae, S. komarowii, S. ×prestoniae, S. wolffi, S. yunnanensis, S. vulgaris, S. oblata, and S. ×hyacinthiflora (Fiala and Vrugtman, 2008).

Despite these previous reports of induced polyploidy, no cytological evidence exists to support these claims. Lilacs have been bred for centuries, yet polyploid lilac breeding remains a largely unexplored field (Fiala and Vrugtman, 2008). Few modern studies have confirmed successful induction of auto-polyploid lilacs. Rose et al. (2000) created mixoploid and tetraploid lilacs from colchicine-treated cuttings of an interseries hybrid, S. vulgaris × S. pinnatifolia. Rothleutner (2014) recovered diplids, mixoploids, tetraploids, and octoploids from oryzalin-treated seedlings of S. reticulata cultivars. Both Rose et al. (2000) and Rothleutner (2014) used flow cytometry to confirm autopolyploids. In many crops, hybridization between tetraploid and diploid populations has been useful for creating sterile triploid progeny due to meiotic irregularities. Where some fertility exists in triploids, they can provide an important bridge in wide crosses and their range of gametes can be used in the production of high copy number polyploids such as tetraploids, pentaploids, and hexaploids (Wang et al., 2010). Aneuploid progeny have been produced in other woody plants through diploid-triploid hybridization including Pyrus (Phillips et al., 2016), Ulmus (Santamour, 1971), and Populus (Wang et al., 2010).

The purpose of this study was to explore the genome size, ploidy variation, and presence of unreduced gametes in a diverse collection of lilacs including representative species and cultivars from five lilac series and inter series hybrids in series Syringa.

### Methods and Materials

**Plant Material.** Lilac taxa were acquired from gardens, arboreta, and nurseries. Representative taxa were obtained from five of the six series within genus Syringa including Syringa, Pubescentes, Villosae, Ligustrina, and

| Interspecific hybrid | ♂ parent | ♂ series | ♀ parent | ♀ series |
|----------------------|----------|----------|----------|----------|
| Syringa ×chinensis   | S. protolaciniata | Syringa | S. vulgaris | Syringa |
| Syringa ×diversifolia | S. pinnatifolia | Pinnatifolae | S. oblata ss. oblata | Syringa |
| Syringa ×henryi      | S. josikae   | Villosae  | S. villosa | Villosae |
| Syringa ×hyacinthiflora | S. oblata | Syringa | S. vulgaris | Syringa |
| Syringa ×josiflexa   | S. josikae   | Villosae  | S. reflexa | Villosae |
| Syringa ×laciniata   | Unknown     | Unknown  | Unknown  | Unknown  |
| Syringa ×nanceiana   | S. henryi    | Villosae  | S. xsweginzowii | Villosae |
| Syringa ×persica     | Unknown     | Unknown  | Unknown  | Unknown  |
| Syringa ×prestoniae | S. villosa  | Villosae  | S. komarowii | Villosae |
| Syringa ×sweginzowii | S. komarowii | Villosae  | S. xsweginzowii | Villosae |

The single named interspecific hybrid resulting from an interseries cross in lilacs.
Results and Discussion

Genome sizes. Holoploid 2C genome sizes ranged from 2.64 ± 0.08 pg in *S. xalaciniata* to 4.94 ± 0.06 pg in *S. vulgaris*.
Table 2. Taxonomic, trademark, accession, and source information for *Syringa* source material used in the current study.

| Series* | Taxon* | Cultivar (trademark name) | Accession no.* | Source* |
|---------|--------|--------------------------|----------------|---------|
| Syringa | *S.* oblata | 09-0058 | Arboretum Mlyňany |
| | *S.* oblata var. *alba* | 09-0059 | Arboretum Mlyňany |
| | *S.* vulgaris | | |
| | | Agincourt Beauty | 13-0036 | Briggs Nursery |
| | | Angel White | 10-0043 | Blue Heron Farm |
| | | Arcubaeoflia | 13-0039 | Briggs Nursery |
| | | Charles Joly | 14-0127 | Dennis’ 7 Dees |
| | | E.J. Gardner | 15-0014 | Blue Heron Farm |
| | | Miss Ellen Willmott | 14-0215 | Portland Nursery |
| | | Monore (Blue Skies®) | 13-0076 | Monrovia |
| | | Katherine Havemeyer | 15-0014 | Blue Heron Farm |
| | | Krasavitsa Moskvy | 13-0043 | Briggs Nursery |
| | | Lavender Lady | 13-0078 | Monrovia |
| | | Ludwig Spaeth | 10-0042 | Blue Heron Farm |
| | | Madame Lemoine | 14-0122 | Portland Nursery |
| | | Prairie Pette | 13-0035 | Briggs Nursery |
| | | President Grevy | 10-0040 | Blue Heron Farm |
| | | President Lincoln | 13-0080 | Monrovia |
| | | Primrose | 13-0040 | Briggs Nursery |
| | | Sensation | 13-0081 | Monrovia |
| | | Elsdancer (Tiny Dancer) | 13-0001 | Heritage Seedlings |
| | *S.* × *hyacinthiflora* | | |
| | | Betsy Ross | 13-0034 | Briggs Nursery |
| | | Maiden’s Blush | 14-0123 | Dennis’ 7 Dees |
| | | Old Glory | 13-0085 | Monrovia |
| | | Pocahontas | 13-0084 | Monrovia |
| | *S.* × *chinensis* | Lilac Sunday | 13-0041 | Briggs Nursery |
| | *S.* × *laciniata* | LS | OSU campus |
| Pubescentes | *S.* meyeri | Palabin | 10-0209 | Bailey Nurseries |
| | *S.* pubescens | Penda (Bloomerang® Purple) | 12-0026 | Garland Nursery |
| | | SMSJBP7 (Bloomerang® Dark Purple) | 13-0071 | Monrovia |
| | | MORj0s 060F (Josee) | 10-0039 | Blue Heron Farm |
| | | Bailbelle (Tinkerbelle®) | 12-0027 | Bailey Nurseries |
| | | Bailsugar (Sugar Plum Fairy®) | 14-0190 | Select Plus |
| | | Colby’s Wishing Star | 14-0191 | Select Plus |
| | | SMSXPM (Scent and Sensibility) | 13-0074 | Monrovia |
| | | Red Pixie | 16-0013 | Forest Farm |
| | | SMSMPRZ1 (Rhythm & Bloom®) | 15-0018 | Kraemer’s Nursery |
| | *S.* pubescens ssp. *patula* | Miss Kim | 13-0072 | Monrovia |
| | *S.* pubescens ssp. *patula* | | 13-0073 | Monrovia |
| Villosae | *S.* emodi | | |
| | *S.* josikaeae | 09-0038 | Hohenheim Gardens |
| | *S.* julianae | 09-0057 | Hohenheim Gardens |
| | *S.* sveginzowii | 11-0021 | NBG Dublin |
| | *S.* tigerstedtii | 09-0040 | Hohenheim Gardens |
| | *S.* villosa | 09-0061 | Arboretum Mlyňany |
| | *S.* wolfii | | 13-0038 | Briggs Nursery |
| | *S.* × *prestoniae* | Miss Canada | 13-0037 | Briggs Nursery |
| | | Donald Wyman | 13-0086 | Monrovia |
| | | Redwine | 13-0088 | Monrovia |
| | *S.* yunnanensis | | 09-0063 | Arboretum Mlyňany |
| Ligustrina | *S.* pekinensis | Morton (China Snow®) | LS | Carlton Nursery |
| | *S.* pekinensis | DTR 124 (Summer Charm®) | LS | Carlton Nursery |
| | *S.* reticulata | | 09-0060 | Arboretum Mlyňany |
| Pinnatifoliae | *S.* pinnatifolia var. *alashanensis* | | 13-0026 | Briggs Nursery |

*Series designation based on Li et al. (2012).*

*Individual taxon in *Syringa* based on (Li et al., 2012) and revisions (Chen et al., 2009).*

*Accession number in research population; LS = nonaccessioned leaf samples for flow cytometry.*

*Container plants, seeds, and leaf samples collected from the following sources: Arboretum Mlyňany (Slepcany, Slovakia), Bailey Nurseries (Yamhill, OR), Blue Heron Farm (Corvallis, OR), Briggs Nursery (Elma, WA), Carlton Plants (Dayton, OR), Dennis’ 7 Dees Landscaping & Garden Centers (Portland, OR), Garland Nursery (Corvallis, OR), Heritage Seedlings & Liners (Salem, OR), Hohenheim Gardens (Stuttgart, Germany), Knemer’s Nursery (McMinnville, OR), Monrovia (Dayton, OR), National Botanic Gardens [NBG Dublin (Glasnevin, Ireland)]; Oregon State University [OSU campus (Corvallis, OR)], Portland Nursery (Portland, OR), Select Plus International Lilac Nursery (Mascouche, Canada).
‘Aucubaefolia’. All 2C relative genome sizes were larger than the two previously reported genome sizes of European lilacs (Olszewska and Osiecka, 1984; Siljak-Yakovlev et al., 2010). Previous reports have shown similar variation due to different binding properties of fluorochrome stains (Lattier, 2016; Parris et al., 2010). Only three taxa of *S. vulgaris*, nested within series *Syringa*, had a 2C relative genome size larger than 4.00 pg, including *S. vulgaris* ‘Aucubaefolia’ (4.94 ± 0.06 pg), *S. vulgaris* ‘Agincourt Beauty’ (4.90 ± 0.03 pg), and *S. vulgaris* ‘President Grévy’ (4.85 ± 0.00 pg). Chromosome counts of *S. vulgaris* ‘Aucubaefolia’ revealed this group to be triploids (Fig. 1). The presence of triploids in our collection supports early reports of polyploid induction experiments and interpollen hybridization (Fiala and Vrugtman, 2008) but surprisingly, no tetraploids were observed. All other root tip cells investigated were diploid, including *S. ×hyacinthiflora* ‘Maiden’s Blush’, *S. ×hyacinthiflora* ‘Old Glory’, *S. ×prestoniae* ‘Miss Canada’, *S. reticulata*, and *S. pubescens* Bloomerang*®* Purple (Fig. 1). Chromosome counts in the current study provided no evidence for base chromosome number other than *x* = 23 (Fig. 1), in contrast to previous reports that varied from *x* = 22 to 24 (Darlington and Wylie, 1956).

Significant differences were found among taxa for 1C genome size (*P* < 0.0001). Values ranged from 1.32 ± 0.04 pg in *S. ×laciniata* to 1.78 ± 0.05 pg in *S. ×hyacinthiflora* ‘Old Glory’ (Table 3). Series *Syringa* had a significantly larger average 1C genome size (1.68 ± 0.02 pg) than the other four series investigated (Table 3). There were no significant differences among series *Pubescentes* (1.47 ± 0.01 pg), *Villosae* (1.55 ± 0.02 pg), *Ligustrina* (1.49 ± 0.05 pg), and *Pinnatifoliae* (1.52 ± 0.02 pg) (Table 3).

Within series *Syringa*, *S. ×laciniata* had a significantly smaller genome size compared with other tested taxa in series *Syringa*. No reports exist on the pedigree of *S. ×laciniata* (Table 1), although Fiala and Vrugtman (2008) hypothesize it to be a cross of the Afghan lilac, *S. protolaciniata*, and another unknown parent. *Syringa ×laciniata* has a heavily dissected leaf, much like *S. pinnatifolia*, while *S. protolaciniata* produces heterophyllous leaves with margins varying from lobed to entire (Fiala and Vrugtman, 2008; Green, 1995). In addition, the only other heavily dissected lilac, *S. pinnatifolia*, has also proven to be the only species successfully used in interseries crosses (Pringle, 1981). If *S. ×laciniata* is from an interseries hybridization, then aneuploidy concomitant with wide hybridization could explain the significant reduction in genome size compared with other taxa in series *Syringa*. Further chromosome counts need to be performed on this hybrid.

Most 1C genome sizes within series *Syringa* were above 1.60 pg (Table 3). Although *S. oblata* is native to Asia and *S. vulgaris* is native to southeastern Europe, their different geographical origins are not reflected in significant genome size variation. Wild-type *S. oblata* and the white-flowered, *S. oblata* var. *alba*, both had a 1C genome size of 1.73 ± 0.03 pg (Table 3). The smallest and largest monoploid genome sizes in *S. vulgaris* were from two white, double-flowered taxa, *S. vulgaris* ‘Miss Ellen Willmott’ (1.61 ± 0.01 pg) and *S. vulgaris* ‘Madame Lemoine’ (1.76 ± 0.05 pg) (Table 3). Taxa representing hybrids between *S. oblata* and *S. vulgaris* had a monoploid genome size range from *S. ×hyacinthiflora* ‘Betsy Ross’ (1.70 ± 0.02 pg) to *S. ×hyacinthiflora* ‘Old Glory’ (1.78 ± 0.05 pg); however, there were no differences among the four hybrid cultivars included (Table 3). One additional interspecific hybrid, *S. ×chinensis* (1.74 ± 0.07 pg), representing a cross between *S. protolaciniata* and *S. vulgaris* was found to have a similar 1C genome size to *S. vulgaris* (Table 3).

Within series *Pubescentes*, most 1C genome sizes were below 1.50 pg and there were no significant differences among the 12 taxa included. The smallest genome size was *S. pubescens* Rhythm & Bloom*®* (1.43 ± 0.01 pg), whereas the largest was in *S. pubescens* ssp. *patula* ‘Miss Kim’ (1.54 ± 0.01 pg). Within series *Villosae*, most 1C genome sizes were above 1.50 pg and ranged from *S. tigerstedtii* (1.38 ± 0.01 pg) to *S. villosa* ‘Aurea’ (1.62 ± 0.03 pg) (Table 3). *Syringa villosa* exhibited a 1C genome size similar to cultivars of *S. ×prestoniae*, which has *S. villosa* along with *S. komarowii* in its pedigree (Table 1). *Syringa tigerstedtii* had a significantly smaller 1C genome size compared with all other taxa except *S. ×prestoniae* ‘Donald Wyman’ (1.50 ± 0.00 pg).
Table 3. Ploidy and relative genome size in *Syringa* determined using flow cytometry analysis of DAPI-stained nuclei with *Pisum sativum* ‘Cirrad’ (8.76 pg/2C) as an internal standard.

| Series | 1Cx genome size [mean ± se (pg)] | Taxa | Ploidy | 1Cx genome size [mean ± se (pg)] |
|--------|----------------------------------|------|--------|----------------------------------|
| *Syringa* | 1.68 ± 0.02 a | *S.* *oblata* | 2x | 1.73 ± 0.03 a–d |
| | | *S.* *oblata* var. *alba* | 2x | 1.73 ± 0.03 a–c |
| | | *S.* *vulgaris* ‘Agincourt Beauty’ | 3x | 1.63 ± 0.01 a–m |
| | | *S.* *vulgaris* ‘Angel White’ | 2x | 1.67 ± 0.05 a–k |
| | | *S.* *vulgaris* ‘Aucubaefolia’ | 3x | 1.65 ± 0.02 a–l |
| | | *S.* *vulgaris* Blue Skies | 2x | 1.72 ± 0.02 a–c |
| | | *S.* *vulgaris* ‘Charles Joly’ | 2x | 1.69 ± 0.02 a–h |
| | | *S.* *vulgaris* ‘E.J. Gardner’ | 2x | 1.66 ± 0.01 a–k |
| | | *S.* *vulgaris* ‘Miss Ellen Willmott’ | 2x | 1.61 ± 0.01 b–o |
| | | *S.* *vulgaris* ‘Katherine Havemeyer’ | 2x | 1.71 ± 0.03 a–e |
| | | *S.* *vulgaris* ‘Krasavitsa Moskvy’ | 2x | 1.70 ± 0.00 a–h |
| | | *S.* *vulgaris* ‘Lavender Lady’ | 2x | 1.69 ± 0.02 a–h |
| | | *S.* *vulgaris* ‘Ludwig Spaeth’ | 2x | 1.74 ± 0.03 a–c |
| Pubescentes | 1.47 ± 0.01 b | *S.* meyeri ‘Palabin’ | 2x | 1.47 ± 0.03 n–u |
| | | *S.* *pubescens* Bloomerang® Purple | 2x | 1.46 ± 0.02 o–u |
| | | *S.* *pubescens* Bloomerang® Dark Purple | 2x | 1.49 ± 0.04 l–t |
| | | *S.* *pubescens* ‘Colby’s Wishing Star’ | 2x | 1.52 ± 0.02 k–t |
| | | *S.* *pubescens* Josee | 2x | 1.45 ± 0.04 p–u |
| | | *S.* *pubescens* ‘Red Pixie’ | 2x | 1.49 ± 0.01 l–t |
| | | *S.* *pubescens* Rhythm & Bloom® | 2x | 1.43 ± 0.01 q–u |
| | | *S.* *pubescens* Scent and Sensibility | 2x | 1.47 ± 0.00 n–u |
| | | *S.* *pubescens* Sugar Plum Fairy® | 2x | 1.47 ± 0.03 n–u |
| | | *S.* *pubescens* Tinkerbelle® | 2x | 1.40 ± 0.01 s–u |
| | | *S.* *pubescens* ssp. *patula* | 2x | 1.48 ± 0.01 m–t |
| | | *S.* *pubescens* ssp. *patula* ‘Miss Kim’ | 2x | 1.54 ± 0.01 h–s |
| Villosae | 1.55 ± 0.02 b | *S.* emodi | 2x | 1.55 ± 0.01 g–s |
| | | *S.* josikaea | 2x | 1.57 ± 0.01 e–r |
| | | *S.* julianae | 2x | 1.59 ± 0.03 c–p |
| | | *S.* sweginzowii | 2x | 1.55 ± 0.02 g–s |
| | | *S.* tigerstedtii | 2x | 1.38 ± 0.01 t–u |
| | | *S.* *villosa* | 2x | 1.56 ± 0.03 f–r |
| | | *S.* *villosa* ‘Aurea’ | 2x | 1.62 ± 0.03 b–n |
| | | *S.* wolfii | 2x | 1.57 ± 0.02 d–q |
| | | *S.* x*pretostanica* ‘Donald Wyman’ | 2x | 1.50 ± 0.00 l–t |
| Ligustrina | 1.49 ± 0.05 b | *S.* pekinesis China Snow® | 2x | 1.41 ± 0.02 r–u |
| | | *S.* pekinesis Summer Charm® | 2x | 1.47 ± 0.03 n–u |
| | | *S.* reticulata | 2x | 1.59 ± 0.03 c–q |
| | | *S.* *sinensis* | 2x | 1.53 ± 0.02 i–t |
| | | *S.* *sinensis* | 2x | 1.58 ± 0.01 c–q |
| | | *S.* *yunnanensis* | 2x | 1.53 ± 0.02 i–t |
| Pinnatifolia | 1.52 ± 0.02 b† | *S.* *pinnatifolia* var. *alashanensis* | 2x | 1.52 ± 0.02 j–t |

*Series designation based on phylogeny by Li et al. (2012).*  
*Series means based on average of taxa means; letters represent Tukey–Kramer test for unequal sample sizes (α = 0.05); † = three samples of same accession were used to calculate mean.  
*Taxa grouped within series; species and market name (cultivar or trademark) presented.  
*Means separated using Tukey’s honest significant test (HSD) (α = 0.05); means followed by same letter are not significantly different; dash between letters indicate complete series of letters; minimum significant difference = 0.158.  
*Ploidy confirmed with root tip cytology.
and ‘Redwine’ (1.53 ± 0.02 pg) (Table 3). However, no other significant differences were found throughout series Villosoae, even though this diverse series was the most species-rich in the collection.

Within the tree lilacs (series Liguistrina) only two species, S. reticulata and S. pekinensis, and few cultivars exist. Green and Chang (1995) previously reported only one species, S. reticulata, with other species circumscribed to the rank of subspecies. The 1Cx genome sizes of S. pekinensis China Snow® and Summer Charm® were not significantly different. However, a significant difference was detected between S. pekinensis China Snow® (1.41 ± 0.02 pg) and S. reticulata (1.59 ± 0.03 pg) (Table 3). Within the monotypic series Pinnatifoliae, S. pinnatifolia var. alashanensis had a 1Cx genome size of 1.52 ± 0.02 (Table 3).

**Hybrid genome sizes.** Based on genome size estimates of parent taxa, hybrids from a previous cross-compatibility study on lilacs (Lattier and Contreras, 2017) were evaluated for genome size variation. As a seed parent, more than 800 flowers on lilacs (Lattier and Contreras, 2017) were evaluated for parent taxa, hybrids from a previous cross-compatibility study with other species circumscribed to the rank of sub-series. The 1C genome sizes of S. vulgaris ‘President Grévy’ (2C = 3.33 ± 0.04 pg) (Table 3). Within the monotypic series Pinnatifoliae, S. pinnatifolia var. alashanensis had a 1Cx genome size of 1.52 ± 0.02 (Table 3).

| Parent ploidy | Cross | Accession no. | Relative 2C genome size [mean ± se (pg)] |
|--------------|-------|---------------|-------------------------------------------|
| 3x x 2x      | S. vulgaris ‘President Grévy’ × S. vulgaris ‘Sensation’ | H2013-150-01 | 3.65 ± 0.02 a |
| 2x x 3x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-01 | 4.35 ± 0.03 b |
| 2x x 2x      | S. vulgaris Tiny Dancer × S. vulgaris ‘Sensation’ | H2014-032-08 | 3.30 ± 0.05 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-025-13 | 3.30 ± 0.03 h |
| S. vulgaris Blue Skies® × S. vulgaris Tiny Dancer | H2014-022-01 | 3.27 ± 0.04 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris ‘Angel White’ | H2014-022-02 | 3.23 ± 0.06 h |
| S. vulgaris ‘Sensation’ × S. vulgaris Tiny Dancer | H2014-022-04 | 3.16 ± 0.04 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris ‘Angel White’ | H2014-024-16 | 3.27 ± 0.08 h |
| S. vulgaris ‘Sensation’ × S. vulgaris Tiny Dancer | H2014-027-08 | 3.22 ± 0.03 h |

| Parent ploidy | Cross | Accession no. | Relative 2C genome size [mean ± se (pg)] |
|--------------|-------|---------------|-------------------------------------------|
| 2x x 3x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-01 | 4.35 ± 0.03 b |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-08 | 4.28 ± 0.05 bc |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-04 | 4.25 ± 0.06 bc |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-05 | 4.25 ± 0.03 bc |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-12 | 4.07 ± 0.03 cd |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-09 | 4.02 ± 0.13 cde |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-02 | 3.86 ± 0.05 def |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-03 | 3.80 ± 0.06 def |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-07 | 3.74 ± 0.05 ef |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-10 | 3.74 ± 0.04 f |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-06 | 3.58 ± 0.08 fg |
| 2x x 2x      | S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ | H2014-033-11 | 3.41 ± 0.03 gh |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-022-01 | 3.27 ± 0.04 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-022-02 | 3.23 ± 0.06 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-022-04 | 3.16 ± 0.04 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-024-16 | 3.27 ± 0.08 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-024-25 | 3.23 ± 0.04 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-024-27 | 3.23 ± 0.02 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-024-22 | 3.22 ± 0.04 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-024-03 | 3.20 ± 0.03 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-027-08 | 3.22 ± 0.03 h |
| S. ×hyacinthiflora ‘Old Glory’ × S. vulgaris Tiny Dancer | H2014-027-03 | 3.19 ± 0.06 h |

Table 4. Comparison of hybrid genome size from interploid and intraploid crosses in Syringa.

*Ploidy of parent taxa including tripliod by diploid (3x × 2x), diploid by tripliod (2x × 3x), and diploid by diploid (2x × 2x) crosses.

*Crosses among cultivars in series Syringa; seed parent listed first and pollen parent listed second.

*Individual accessions in research population.

*Relative 2C holoploid genome sizes; means separated using Tukey’s honest significant difference (hSD) test at (α = 0.05; minimum significant difference = 0.286); means followed by the same letter are not significantly different.
S. vulgaris ‘Sensation’ (discussed below) and an aneuploid gamete from S. vulgaris. Fig. 2. Flow cytometry histogram of three taxa of parents, S. vulgaris hybrid seedlings varied in 2C genome size between the two parents, S. vulgaris and S. vulgaris ‘President Grévy’ used in crosses with S. vulgaris ‘Tiny Dancer’ failed to contribute unreduced gametes to produce triploid seedlings (Table 4).

As a pollen parent, the triploid S. vulgaris ‘President Grévy’ was used in crosses with S. vulgaris Blue Skies®, S. vulgaris ‘President Lincoln’, and S. vulgaris ‘Sensation’ totaling 459 pollinations (Lattier and Contreras, 2017). Crosses with S. vulgaris Blue Skies® produced the only viable seed with 12 seedlings recovered from 135 pollinations (Lattier and Contreras, 2017). With the exception of one seedling (H2014-033-11), all hybrid seedlings varied in 2C genome size between the two parents, S. vulgaris Blue Skies® (3.44 ± 0.03 pg) and S. vulgaris ‘President Grévy’ (4.85 ± 0.00 pg) (Table 4). Relative 2C genome sizes of seedlings varied significantly from 3.41 ± 0.03 pg (H2014-033-11) to 4.35 ± 0.03 pg (H2014-033-01) (Table 4), the majority representing aneuploid genome sizes.

The estimations of chromosome numbers across aneuploid populations can be performed with knowledge of holoploid 2C genome sizes using Eq. [1]. Previous studies have produced models based on a holoploid genome size of a theoretical average, single chromosome based on parent genome sizes, and chromosome counts. Although some estimates of aneuploid chromosomes have been based solely on hypothetical chromosome size (Palmer et al., 2009), several studies have tested this model with root squashes and found most of their predictions to be concurrent with the true chromosome number or accurate within two to three chromosomes in Primula (Hayashi et al., 2009), Lilium (Lim et al., 2003), and Calluna (Behrend et al., 2015). Considering these previous studies and the relatively uniform chromosome size observed in lilac (Fig. 1), a simple linear model was used to predict chromosome number in the 2x × 3x aneuploid population (Fig. 3).

Based on a linear model with an average chromosome size of 0.06 pg (Fig. 3), the chromosome numbers in our aneuploid seedlings from S. vulgaris Blue Skies® × S. vulgaris ‘President Grévy’ varied from 46 to 61 with an average of 54.3 ± 1.4 chromosomes. Chromosome numbers of triploid gametes can be deduced by subtracting the euploid chromosome number from the seedlings somatic chromosome numbers (Iorizzo et al., 2012). Assuming that S. vulgaris Blue Skies® consistently contributed haploid gametes with 23 chromosomes, S. vulgaris ‘President Grévy’ produced a range of aneuploid pollen from 23 to 38 chromosomes to progeny from this cross. Previous research in other crops has shown that triploids produce a higher percentage unreduced and/or aneuploid gametes than their diploid or tetraploid counterparts (Burton and Husband, 2001; Herben et al., 2016; Ramsey and Schemske, 1998). Viable aneuploid gametes have been described in plants such as C. sativus (Diao et al., 2009), Brassica (Brassica; Lu and Kato, 2001), and Tulipa (Marasek-Ciolakowska et al., 2014), yet other plants only tolerate euploid gametes as in Vaccinium corymbosum (Vorsa and Ballington, 1991). The resulting aneuploids from the 2x × 3x lilac crosses were skewed slightly to the diploid cytotype compared with a theoretical bimodal distribution with an average of 57.5 chromosomes (Fig. 3). In the 3x × 2x cross, S. vulgaris ‘President Grévy’ × S. vulgaris ‘Sensation’, the triploid parent contributed 39 chromosomes (2n = 7) as a seed parent. It is unclear if this slightly higher contribution from the triploid is due to combining with an unreduced gamete, the direction of the cross, or chance. Brandham (1982) reported a greater prevalence of aneuploidy over the range between diploid and triploid when the latter are females—presumably associated with endosperm balance number (discussed in later sections). However, with only a single seedling it is impossible to draw conclusions.

Although our seedling cytotypes varied from a random distribution of aneuploid cytotypes, lilac aneuploid segregation conflicts with the limited number of similar studies on 2x × 3x crosses by being less concentrated at either euploid level (diploid or triploid). In lilies, these crosses resulted in all triploid or near-triploid seedlings derived from viable 2n gametes from triploid male parents (Marasek-Ciolakowska et al., 2014). In Tulipa, 2x × 3x crosses yielded a majority of diploid and near-diploid progeny with a small percent of near-triploids (Mizuochi et al., 2009). This same study found that the reciprocal cross in Tulipa yielded a binomial distribution of aneuploids, with the female triploid parent producing a wide range of fertile aneuploids (Mizuochi et al., 2009). Similar to lilies, 2x × 3x crosses in Allium schoenoprasum (Levan, 1936) and C. sativus (Diao et al., 2009) resulted in diploids or near-diploids with a small percent of near-triploids; the reciprocal crosses yielded a wider range of aneuploids. Brandham (1982) reviewed a number of studies on interploid crosses and found that with very few exceptions the triploid parent generally produced gametes that were either haploid or diploid based on...
the ploidy of the other parent. *Populus* was a notable exception from other examples given, namely a considerably higher chromosome number than other taxa discussed. *Populus* (x = 19) is similar to *Syringa* (x = 23) in chromosome number and both are almost certainly of polyploid origin. This high chromosome number indicates that there is redundancy present that likely allows the survival of aneuploid gametes produced by triploids that are inviable in taxa with fewer base chromosomes.

The importance of embryo and endosperm cytotypes often plays a role in seedling cytotype segregation and has been studied at length in diploid and triploid crosses of potato (*Solanum*). A 2:1 maternal to paternal endosperm balance ratio must be maintained for successful hybridization in potato, preventing 2x x 3x crosses and yielding progeny from 3x x 2x that are skewed to near-triploid cytotypes (Carputo, 1999; Iorizzo et al., 2012). The origin of *S. vulgaris* ‘President Grévy’ could play a role in its fertility as a triploid parent. Allotriploids are rarely used in breeding because of their difficulty in chromosome pairing during meiosis; however, autotriploids can overcome problems with meiotic pairing to produce haploid to triploid gametes (Brandham, 1982; Hayashi et al., 2009; Kato et al., 2001). The history of wide hybridization and polyploid induction in lilac leaves the question open to the origins of triploid cytotypes. In addition, it remains unclear if meiotic abnormalities in gamete formation, preferential fertilization, or preferential embryo/endosperm survival skewed the distribution of aneuploid cytotypes. Our results may simply be due to the small sampling population of aneuploid seedlings resulting from the 2x x 3x and 3x x 2x crosses.

Despite the numerous pollinations and few resulting seedlings, all aneuploid lilacs appear to be healthy and vigorous after their first 2 years of growth (J.D. Lattier, personal observation). This conflicts with some studies which reported that aneuploid seedlings from diploid-triploid crosses were nonviable past initial germination exhibiting abnormal, stunted growth (Behrend et al., 2015; Osuji et al., 1997), and sometimes reverting to euploids after more than a year (Behrend et al., 2015). Although our aneuploids appear to grow as vigorously as their diploid counterparts, female fertility and pollen viability in the aneuploid population has not been investigated as the plants have yet to reach maturity during this study. However, flow cytometry was performed on the aneuploid population for more than 2 years after germination. While it cannot be assured that this aneuploid series will not stabilize at a euploid level (diploid or triploid), the fact that these plants have maintained aneuploid chromosome complements for more than 2 years contrasts with previous studies and suggests that they may be stable.

**Unreduced pollen.** Stained pollen grains from four taxa of *S. vulgaris* were scored as viable and were measured for variability in diameter. Unstained pollen grains were negligible in all taxa, and pollen germination was not investigated. There were significant differences among taxa for pollen diameter (*P* < 0.0001) and every pairwise comparison between taxa was significant (*P* < 0.01). The largest average pollen grains were detected in *S. vulgaris* ‘Sensation’ (35.74 ± 0.16 µm), a measure likely overinflated by the presence of 8.5% unreduced pollen grains identified because of their increased volume (Fig. 4). This is the first report of unreduced (2n) pollen in lilac, but is not the first report in the Oleaceae. B-chromosomes and unreduced pollen have been reported in cultivars of *Oleae europaea* (Sheidai et al., 2008). *Syringa vulgaris* ‘Sensation’ was also the only diploid observed to produce unreduced pollen, as *S. vulgaris* ‘Ludwig Spaeth’ and *S. vulgaris* ‘Miss Ellen Willmott’ produced only 1n pollen (Fig. 4).

Because of its low fertility in crosses with the triploid seed parent *S. vulgaris* ‘President Grévy’ and the presence of an inflated genome size in the single viable seedling recovered (H2013-150-01), an unreduced pollen grain from *S. vulgaris* ‘Sensation’ likely contributed to the production of this single

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Fig. 3. Linear model of lilac 2x x 3x aneuploid progeny with predicted chromosome number based on theoretical chromosome size of 0.061 pg [(4.85 – 3.45 pg)/23 chromosomes]. Parent taxa of aneuploid progeny: diploid female parent *Syringa vulgaris* ‘Blue Skies’ (2n = 2x = 46) and triploid male parent *S. vulgaris* ‘President Grévy’ (2n = 3x = 69). Linear model follows the formula: y = 16.224x – 9.7743.
polyploid/aneuploid progeny. It remains unclear whether the rare picotee flower mutation is in some way related to the production of an unreduced pollen grain, or if other diploids of *S. vulgaris* that were not included in the current study produce unreduced pollen at a similar rate as *S. vulgaris ‘Sensation*. Since an unreduced pollen grain resulted in the only viable seedling between these two cultivars, this may indicate the utility of an increased ploidy level for improving cross-compatibility in lilac.

Pollen from the double-flowering, triploid *S. vulgaris ‘President Grévy’* proved difficult to obtain as many flowers simply did not produce viable anthers. Unreduced pollen grains (0.6%) were detected in *S. vulgaris ‘President Grévy’* out of 1689 grains measured. Although at a much lower percentage, *S. vulgaris ‘President Grévy’* was the only parent besides *S. vulgaris ‘Sensation’* to produce unreduced pollen in the current study, indicating some level of meiotic irregularities. Triploids are more likely to undergo irregularities during meiosis such as irregular chromosome pairing, supernumerary B chromosomes, laggard chromosomes, chromatin bridges, cytomixis, and out of plate chromosomes during metaphase I (Farco and Dematteis, 2014; Lavia et al., 2011). Triploids and resulting aneuploid progeny may prove to be sterile, yielding cultivars with reduced weediness and extended bloom times. Irregular meiosis during microspore development likely contributed to the poor performance of *S. vulgaris ‘President Grévy’* as a seed parent and the subsequent aneuploidy seen in its viable seedlings (Fig. 3). Based on its aneuploid offspring when used as a male parent, *S. vulgaris ‘President Grévy’* likely produces a range of aneuploid pollen. The average pollen grain diameter of *S. vulgaris ‘President Grévy’* (35.28 ± 0.07 μm) was significantly larger than the two diploids that exhibited normal meiosis, *S. vulgaris ‘Ludwig Spaeth’* (33.96 ± 0.05 μm) and *S. vulgaris ‘Miss Ellen Willmott’* (34.32 ± 0.06 μm) from which no unreduced gametes were observed in a combined 2866 pollen grains (Fig. 4).

This study provides valuable information for future lilac breeding and informs a previous study on cross-compatibility among elite cultivars of lilac (Lattier and Contreras, 2017). In addition, this study contributes genome size and ploidy information to the growing database of angiosperm genome sizes, recommended by Galbraith et al. (2011). The discovery of three triploid lilacs, *S. vulgaris ‘Aucubaefolia’, S. vulgaris ‘Agincourt Beauty’, and S. vulgaris ‘President Grévy’* lends evidence to previous reports of artificial tetraploid development and subsequent hybridization (Fiala and Vrugtman, 2008). However, no tetraploids were discovered among the research population. The discovery of high levels of aneuploidy in

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**Fig. 4.** Frequency distribution of viable pollen grain diameters of four cultivars of *Syringa vulgaris*. Regions to the right of asterisks were measured to be 26% larger than the mean and indicate unreduced gametes: (A) ‘Ludwig Spaeth’ (0% unreduced gametes); (B) ‘Sensation’ (8.5% unreduced gametes) [insert: unreduced (left) and reduced (right) pollen grains stained with 2% acetocarmine and viewed at ×630 magnification (scale bar = 10 μm)]; (C) ‘Miss Ellen Willmott’ (0% unreduced gametes); (D) ‘President Grévy’ (0.6% unreduced gametes).

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interplloid hybrids indicates meiotic irregularities in pollen development of polyploid lilacs. Further cytological studies of pollen mother cells and meiotic analyses could contribute to understanding the complexities within developing gametes of taxa in the heavily hybridized series Syringa.

The development of an aneuploid series in 2x × 3x crosses provides an avenue to develop a model for cytotype prediction in seedlings of interplloid lilac hybrids. Future efforts to confirm initial predictions of aneuploid chromosome numbers will include chromosome counts on parent taxa, chromosome counts on a subset of aneuploids, and repollination of the parent genotypes to increase the number of seedlings in the aneuploid population. Further, aneuploids can be highly variable in morphology, including reduced vigor and can have greatly reduced fertility. This may be a detriment in breeding most crops, but could be an avenue for ornamental breeders to recover more compact, longer-blooming, and sterile cultivars. As the aneuploid population matures, plants will be compared for differences in gross morphology, and flowers will be compared for pollen viability and female fertility. Reanalyzing the genome sizes of this population will be necessary over subsequent years in light of previous reports of euploidization of woody aneuploids (Behrend et al., 2015).

The discovery of unreduced pollen in S. vulgaris ‘Sensation’ and subsequent production of a seedling from a 3x × 2x cross with a larger genome than either parent, indicate that unreduced gametes or polyploidy may contribute to cross-compatibility in wide hybridization of lilac. Future work using high-throughput pollen screening by flow cytometry may reveal other cultivars with high levels of unreduced gametes. Wide hybridization with polyploids may reduce the impact of chromosome loss, which has been reported in previous cytological studies on lilac (Taylor, 1945) and the smallest genome recorded in the current study was a dissected-leaved, interspecific hybrid, S. ×laciniflora. Identifying parents with unreduced pollen or generating autoployploids in each lilac series may prove a valuable method for recovering viable progeny from wide hybridization in lilac. Interplloid hybrids continue to be the most elusive quarry for lilac breeders, with only the pinnately compound S. ×hyacinthiflora (Pringle, 1981). Using S. ×pinnatifolia as well as an induced autoployploids of cultivars proven to produce fruit and seed in interseries crosses (Lattier and Contreras, 2017) may spark a renaissance in the storied history of lilac breeding.

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