Genome Size and Ploidy of *Phlox paniculata* and Related Germplasm in Subsections *Paniculatae* and *Phlox*

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**ABSTRACT.** *Phlox* is an important genus of herbaceous ornamental plants previously targeted for germplasm development, characterization, and enhancement by the U.S. Department of Agriculture, National Plant Germplasm System. Among *Phlox* in cultivation, *Phlox paniculata* is the most widely grown and intensively bred species, but little is known about variation in genome size and ploidy of this species or of related taxa that may be used for germplasm enhancement. The objective of this study was to assess cytotype variation in a diverse collection of cultivars and wild germplasm of *P. paniculata* (subsection *Paniculatae*) and of related taxa in subsections *Paniculatae* and *Phlox*. The collection included 138 accessions from seven species and two interspecific hybrids. Flow cytometry was used to estimate holoploid (2C) genome sizes and to infer ploidy levels. Chromosome counts were made to calibrate ploidy with genome size for a subset of taxa. Most cultivars were diploid (2\(n = 2x = 14\)) and had mean genome sizes that did not vary between subsections *Paniculatae* (14.33 pg) and *Phlox* (14.23 pg) although size variation was greater among cultivars within subsection *Phlox*. Triploid cultivars of *P. paniculata*, with a mean genome size of 21.36 pg and mitotic chromosome counts of \(2n = 3x = 21\), were identified. Such triploids suggest previous interpollen hybridization within this taxon. For the putative hybrid *Phlox* Suffruticosa Group ‘Miss Lingard’ showed an intermediate genome size of 21.21 pg supporting a triploid, hybrid origin of this taxon. Mean 2C genome sizes among wild-collected accessions were similar to values reported for cultivars (*Paniculatae* = 14.59 pg, *Phlox* = 14.23 pg), but taxa in subsection *Phlox* exhibited greater variation that included two tetraploids identified among wild-collected accessions; one, of *P. pulchra*, had a mean genome size of 26.17 pg, representing the first report of polyploidy in the taxon. This is the first report on genome size for the majority of species in the study. Although genome size could not be used to differentiate taxa in subsections *Paniculatae* and *Phlox*, the data provide further insights into cytotype variation of *Phlox* germplasm useful for plant breeders and systematists.

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*Phlox* (Polemoniaceae) is a phenotypically diverse genus with substantial interpopulation differentiation for numerous morphological characters and ploidy; it includes \(\approx 65\) species with centers of diversity in the eastern and western United States (Wherry, 1955). The most important horticultural forms of *Phlox* are found in the eastern species, which are taxonomically distributed among three sections (*Anmaeae, Occidentales,* and *Phlox*) and six subsections (*Divaricatae, Subulatae, Phlox, Paniculatae, Stoloniferae,* and *Cluteanee*) based on variations in the calyx, style length, and geographic distribution (Wherry, 1955). Subsections *Paniculatae* and *Phlox* include species having a style that exceeds 15 mm in length and that is as long as, or longer than, the corolla tube (Fig. 1) (Wherry, 1955). This feature differentiates these species from all other *Phlox* taxa that are collectively known as “long-styled” phloxes. Style length has been suggested as a possible barrier to interspecific hybridization among species of *Phlox* that differ for the trait, but this has not been rigorously tested and documented (Levin, 1966; Locklear, 2011; Symons-Jeune, 1953).

Among long-styled phloxes, *P. amplifolia* and *P. paniculata* comprise subsection *Paniculatae*, and are distinguished from taxa in subsection *Phlox* by their areolate leaf veins, white or cream-colored pollen, and pubescent calyces (Fig. 1; Table 1). Subsection *Phlox* contains up to six species, but the taxonomy of this group has been historically confused and revised several times; a well-resolved taxonomy or phylogeny does not yet exist. The taxonomic confusion is caused by extensive intra-specific variation prevalent in *Phlox carolina* and *P. glaberrima*, which has led some to refer to these as the *P. carolina–P. glaberrima* complex, but more recently referred to as the “*P. glaberrima complex*” (Ferguson and Jansen, 2002; Ferguson et al., 1999; Wherry, 1932a, 1932b, 1945, 1955). These species are phenotypically similar to those in subsection *Paniculatae*, but are differentiated by having obscure leaf veins, yellow pollen, and glabrous calyces (Fig. 1; Table 1). To some degree, the taxa can be further distinguished from each other on the basis of morphology, geographic distribution, and habitat preference, but the traditional morphological characters used...
by lack of phylogenetic and taxonomic resolution. Genome size in this group remains unrealized, and has likely been hindered specific hybrids, but the potential for further breeding and selection has resulted in the selection of cultivars and putative interspecies for each species based on morphology, but also taxa that were described on the basis of morphology, geographic distribution, and habitat preference. Molecular analysis of the “P. glaberrima complex” and related eastern taxa from other subsections using data from the internal transcribed spacer (ITS) region of ribosomal DNA and chloroplast DNA (cpDNA) restriction sites resulted in a paraphyletic grouping and included species from at least three subsections (Ferguson and Jansen, 2002; Ferguson et al., 1999; Locklear, 2011; Wherry, 1935b, 1945, 1955). The traditional morphological markers (calyx, ves- ture, and leaf size/shape) used to differentiate species and subspecies in related subsections, cannot be used to delineate taxa of this com-plex (Fig. 1; Table 1). In his final attempt to resolve historical taxonomic confusion, Wherry (1955) recognized two primary species, P. carolina and P. glaberrima, and six intraspecific taxa that were described on the basis of morphology, geographic distribution, and habitat preference. Molecular analysis of the “P. glaberrima complex” and related eastern taxa from other subsections using data from the internal transcribed spacer (ITS) region of ribosomal DNA and chloroplast DNA (cpDNA) restriction sites resulted in a paraphyletic grouping and included species from at least three subsections (Ferguson and Jansen, 2002; Ferguson et al., 1999). These studies underscore the concept of a species complex where a single, widespread, but polymorphic taxon is recognized. Previous data indicate that this polymorphism likely resulted from hybridization, but recent evolutionary divergence could produce similar results. More recently, Locklear (2011) recognized P. carolina and P. glaberrima as distinct, and described one subspecies for each species based on morphology, but also indicated P. carolina was “… an enigma … its distinctiveness as a species appears to have been more apparent in the past than it is today.” In our experience, Wherry’s intraspecific
### Table 1. Distinguishing morphological characteristics and habitat preferences of taxa from the “Phlox glaberrima complex” in subsection Phlox and from the two taxa in subsection Paniculatae, as described by Wherry (1955), used in this study to assign taxa to accessions in the germplasm collection.

| Character                  | carolina ssp. carolina | carolina ssp. alta | angusta | glaberrima ssp. glaberrima | glaberrima ssp. interior | glaberrima ssp. triloba |
|----------------------------|------------------------|--------------------|---------|---------------------------|--------------------------|-------------------------|
| Stem ht (cm)               | 40–100                 | 75–200             | 45–90   | 50–150                    | 35–120                   | 40–60                   |
| Leaves                     | Lanceolate to ovate-oblong | Elliptic        | Linear  | Linear to narrow lanceolate | Linear to lanceolate      | Linear to lanceolate-ovate |
| Leaf veins                 | Obscure                | Glabrous           | Obscure | Glabrous                  | Obscure                  | Obscure                 |
| Leaf margin                | Glabrous               | Glabrous           | Glabrous | Glabrous                  | Glabrous                 | Glabrous               |
| Leaf distribution          | Largest above midstem  | Largest above midstem | Largest above midstem | Largest above midstem | Largest above midstem | Largest above midstem  |
| Inflorescence              | Panicle of cymes       | Panicle of cymes  | Cymose  | Panicle of cymes          | Panicle of cymes         | Aggregate of 3-flowered cymes |
| Calyx                      | Subcampanulate         | Subcampanulate    | Subcampanulate | Subcampanulate | Subcampanulate      | Subcampanulate        |
| Pollen color               | Yellow                 | Yellow             | Yellow  | Yellow                    | Yellow                   | Yellow                 |
| Persistent sterile stems   | Rarely                 | No                 | No      | No                        | Yes                      | No                      |
| Habitat                    | Mesic/hydric           | Mesic              | Hydric  | Hydric                    | Hydric                   | Mesic/xeric            |

### Subsection Paniculatae

| Character                  | amplifolia             | paniculata         |
|----------------------------|------------------------|--------------------|
| Pollen color               | Subcampanulate         | Cream              |
| Calyx                      | Subcampanulate         | Subcampanulate    |
| Pollen color               | Yellow                 | Cream-white        |
| Persistent sterile stems   | No                     | No                 |
| Inflorescence              | Aggregate of 3-flowered cymes | Compound panicle of cymes |

Although genome size and ploidy has been characterized in Phlox in the past, this study provides a more comprehensive understanding of the diversity within the genus, particularly in terms of polyploidy. The information is useful for breeders as it highlights the potential to produce new breeding lines with novel traits, and for taxonomists as it aids in the clarification of species boundaries. The use of genome size and ploidy as markers in the taxonomy of Phlox may provide a more accurate resolution than traditional morphological characteristics alone.
some Phlox species, estimates for several taxa remain undocumented (Bennett and Leitch, 2012). The positive correlation generally established between genome size and ploidy in a number of genera has facilitated rapid and extensive study of cytotypic variation, not only between species but also between populations of a species (Greilhuber and Leitch, 2013). Flow cytometry provides a well-established, rapid method for estimating genome size in plants, and such estimates can be used to infer ploidy when chromosome counts are limited or difficult to generate (Doležel, 2009; Doležel et al., 1998; Parris et al., 2010). Analysis of genome size in populations of Dianthus broteri (Balao et al., 2009), P. pilosa (Worcester et al., 2012), Ranunculus pannarxisfolius (Cieres et al., 2009), and others have demonstrated the existence of intraspecific cytotypic variation between populations that may have an adaptive value (Greilhuber and Leitch, 2013; Smarda and Bureš, 2010). Thus, it cannot be assumed that individuals in different populations of a taxon necessarily have the same genome size or ploidy level.

The objective of this study was to survey the genome size in a diverse germplasm collection of Phlox that included the long-styled taxa in subsections Paniculatae and Phlox, to infer ploidy and assess cytotype variation both within and between species. Validation of inferred ploidy with chromosome counts would reinforce the characterization of the collection, and provide baseline information for use by Phlox breeders.

Materials and Methods

PHLOX COLLECTION. Living plants were obtained from nursery sources and from collection sites in the east-central United States. Emphasis was placed on gathering a diverse collection of P. paniculata cultivars readily available in the United States. Many older cultivars cited in the literature are not commercially available, or have been altogether lost to cultivation. Cultivar names were verified using a variety of sources (Bendtsen, 2009; Fuchs, 1994; Locklear, 2011; Meyer, 1944; Pridham, 1934; Symons-Jeune, 1953; Wherry, 1955). Nearly all P. paniculata cultivars were selected for unique flower colors and/or patterns, and this served as the primary means of identification. Where possible, some cultivars were obtained from more than one source to confirm identification, but most cultivars are rarely grown and could only be obtained from a single source. The identity of wild-collected material was confirmed using the taxonomic keys of Locklear (2011) and Wherry (1955), information from the phylogenetic analyses of Ferguson et al. (1999) and Ferguson and Jansen (2002), and comparison with herbarium specimens from herbaria at The Ohio State University, University of Kentucky, and West Virginia University. Herbarium vouchers of the collections are maintained at the Ornamental Plant Germplasm Center (OPGC), Columbus, OH.

PLANT CULTIVATION. Plants were grown in 16-cm-wide × 14.5-cm-deep round, plastic containers in Metro Mix 360 (Scotts Co., Marysville, OH) on greenhouse benches in a temperature regime of 22.2 ± 6 °C during the day and 18 ± 3 °C at night. Nutrients were supplied using 15N–3.9P–9.9K pelleted, slow-release fertilizer containing micronutrients (Osmocote®; Scotts Co., Marysville, OH). Samples from these plants were used for chromosome and flow cytometry analysis.

ISOLATION OF NUCLEI AND GENOME SIZE ANALYSIS. Preparation of samples for flow cytometry was done following procedures of Arugumanathan and Earle (1991) and Doležel et al. (1998). In brief, leaf tissue was collected on the day of the analysis, and consisted of fully expanded, disease-free leaves from the upper one-half of the plants. Three samples were prepared for each taxon; a minimum of one plant was used for cultivars, and up to three individuals were used for wild-collected accessions. Approximately 2 cm² of Phlox newly expanded leaves and ≈0.5 cm² of Pisum sativum ‘Citrad’ leaf tissue were placed in a plastic petri dish, 1 mL cold buffer containing propidium iodide fluorochrome [Solution A (Arugumanathan and Earle, 1991)] was added, and the leaves were chopped concurrently with a new single-edge razor blade. The nuclei suspensions were then filtered through a 70-μm nylon cell strainer (BD Falcon, Bedford, MA) into a 1.5-mL microfuge tube to remove debris, and centrifuged at 17,000 g for 20 s. The supernatant was discarded and the remaining pellets were resuspended in 200 μL of Solution B (Arugumanathan and Earle, 1991). Samples were then incubated in a 37 °C water bath for 15 min to digest RNA and subsequently kept on ice until analyzed. Flow cytometry was done with a flow cytometer (FACSCalibur; BD, Franklin Lakes, NJ). Sample runs were considered complete once 10,000 events had been reached, and a minimum of 3000 nuclei had been analyzed. Data collected from each sample was analyzed with CellQuest Pro V (BD Biosciences, San Jose, CA). Genome size was calculated as described by Doležel (2009) using a value of 8.76 pg for the 2C DNA content of the pea reference (Doležel et al., 1998; Greilhuber et al., 2007) where 2C represents the holoploid, or complete, genome size.

CYTOLOGICAL ANALYSIS. Both meiotic (flower bud) and mitotic (root tip) tissues were used to determine chromosome numbers. Root tips were initially treated with 0.002 M 8-hydroxyquinoline (Thermo Fisher Scientific, Waltham, MA) for 2 to 4 h to accumulate condensed mitotic metaphase chromosomes and then rinsed three times with distilled water. Treated root tips or freshly harvested immature flower buds were fixed in a 3:1 (v/v) solution of 100% ethanol (EtOH): glacial acetic acid for 1 to 2 h at room temperature. Samples were subsequently rinsed in distilled water and either used immediately for observation or stored in 95% EtOH (v/v) at 2 °C. Excised anthers were macerated and stained in 1% aceticarmine solution for 5 min; the cover slip was placed and slides were gently heated over a flame until the cover slip became cloudy. After cooling, the slide was squashed with direct pressure for at least 1 min before visualization. Root samples were softened in 1 M HCl for 10 to 20 min and then stained in 1% aceticarmine solution for at least 1 h at room temperature. Root tips were excised under a dissecting microscope, placed on a microscope slide in two drops of 1% acetocarmine heated, and squashed as mentioned above. Chromosomes were visualized at ×1000 in a microscope (IM-20; Zeiss United States, Peabody, MA). A minimum of 10 cells displaying metaphase chromosomes were used to confirm chromosome counts.

STATISTICAL ANALYSIS. Data for monoploid genome sizes were subjected to analysis of variance by subsection and species, and means were separated using Fisher’s least significant difference (Proc GLM, SAS version 9.3; SAS Institute, Cary, NC).

Results

A total of 414 samples representing 138 accessions of 15 Phlox taxa from two subsections (Phlox and Paniculatae) were
analyzed (Tables 2 and 3). We measured genome size in 85 accessions of wild species, cultivars, and hybrids from subsection *Paniculatae*. In subsection *Phlox*, 53 accessions from five species were analyzed: 37 were collected from natural plant populations and 16 were cultivars.

The flow cytometric analysis revealed plants with DNA content or genome size equivalent to diploid, triploid, and tetraploid levels (Tables 2 and 3). There was little variation between samples of a single accession, and none of the samples had a CV greater than 5%; the mean CV of all 414 samples was 1.65% (Tables 2 and 3). The flow cytometry histograms for every *Phlox* sample consistently displayed two peaks, and normal readouts included a well-defined G₁ peak and a reduced G₂ peak for the *Phlox*, and also a prominent G₁ peak of the internal standard *P. sativum* ‘Citrad’ (Fig. 2). To validate our methodology, four *Phlox* species with different mean genome sizes, *P. buckleyi* (21.36 pg), *P. pilosa* ssp. *pilosa* (12.00 pg), *P. paniculata* (13.92 pg), and *P. subulata* (7.44 pg), were processed concurrently and analyzed to verify the position and placement of peaks in relation to each other (Fig. 3). Such concurrent analysis of samples in the absence of a reference standard and correlates with the internal standard (Doležel, 2009). The analysis confirmed the spacing of measured peaks with the *P. sativum* standard and correlates with estimated genome sizes as shown in Tables 2 and 3.

**Genomes size in subsection *Paniculatae*.** Of the 70 cultivars of *P. paniculata* and two of *P. ×arendsii*, 68 had a mean genome size of 2C = 14.22 pg with a range of 13.70 to 15.01 pg, that varied by 9.50%; these sizes correspond to a diploid level (2n = 2x = 14) that was confirmed with chromosome counts from root tips or pollen mother cells (Fig. 2; Table 3). Cultivars described as *P. ×arendsii* (*P. paniculata × P. divaricata*), had a genome size essentially indistinguishable from that of *P. paniculata* even though the putative parental species differed in genome size. Progeny obtained from a cross between the putative progenitor species of *P. ×arendsii* at the OPGC displayed an intermediate genome size that differs from the cultivars (Zale, 2014). Four cultivars of *P. paniculata* (Blushing Bride, Dick Weaver, John Fanick, and Robert Poore) had a mean genome size of 2C = 21.36 pg, with a range of 20.95 to 21.75 pg and that varied by 3.8%. These sizes are intermediate to the diploid (14.22 pg) and calculated tetraploid (25.92 pg) levels. Analysis of chromosomes in pollen mother cells and dividing root tips at metaphase showed that chromosome number of these taxa is 2n = 3x = 21, suggesting that they are triploids (Fig. 2; Table 3).

In addition to the genome size analyzed in cultivars from subsection *Paniculatae*, a collection of 13 accessions obtained from natural populations of *P. amplifolia* (five different sources) and *P. paniculata* (eight different sources) was also examined (Table 3). Mean genome size of *P. amplifolia* (2C = 14.29 pg) was equivalent to that of *P. paniculata* (2C = 14.02 pg). Overall, the 13 accessions had a mean genome size of 14.12 pg with a range of 13.23 to 14.65 pg, and 10.80% variation (Table 3); these wild-derived collections are consistent with diploid levels (2n = 2x = 14) and the sizes equivalent to those of the diploid cultivars. Tetraploids have not yet been identified in wild-collected or cultivated accessions of taxa in *Paniculatae* although artificial tetraploids have been created (Matsiška and Vejsadová, 2010). There was no significant difference between *P. amplifolia* and *P. paniculata* in monoploid genome size, but the mean monoploid genome size of subsection *Paniculatae* was significantly different from that of subsection *Phlox* (Table 4).

**Genome size in subsection *Phlox*.** There are far fewer cultivars from taxa in subsection *Phlox* [synonym subsection *Ovatae* (sensu Wherry, 1955)] than in subsection *Paniculatae* although the number of species is greater. Sixteen cultivars of *P. carolina* (two), *P. glaberrima* (eight), *P. maculata* (three), and *P. pulchra* (one) were included, as well as two cultivars in the Suffruticosa Group, described as hybrids between *P. carolina* and *P. maculata* (Locklear, 2011). The cultivars of *P. carolina*, *P. maculata*, *P. pulchra*, and one of the Suffruticosa Group (‘Monica Lynden-Bell’) had genome size equivalent to the diploid level (2C = 14.25 pg, range = 13.83 to 15.09, 11.50% variation) (Table 2). The eight cultivars of *P. glaberrima* differed in genome size such that three of them had diploid-equivalent sizes (2C = 14.39 pg) and the remaining five had tetraploid-equivalent sizes (2C = 25.44 pg). All five polyploid cultivars could be keyed to *P. glaberrima* ssp. *triflora* (Wherry, 1955). Chromosome counts confirmed that these accessions were tetraploid (2n = 4x = 28). *Phlox* Suffruticosa Group ‘Miss Lingard’, had a mean genome size of 21.21 pg, an intermediate genome size that confirmed the triploid nature of this putative hybrid cultivar (Table 2).

Among wild-collected accessions, the five species differed in mean holoploid genome size at the diploid level: *P. carolina* (2C = 14.67 pg), *P. glaberrima* (2C = 13.57), *P. maculata* (2C = 14.15), *P. ovata* (2C = 11.99), and *P. pulchra* (2C = 14.12 (one accession only)), and there were significant differences among monoploid genome sizes among the species (Table 4). Combined, these wild-collected accessions had a mean genome size of 13.83 pg and a range of 11.71 to 15.55 pg. The 28.1% variation in genome size among the diploid taxa in this subsection is larger than that found in subsection *Paniculatae*. Among the 37 wild populations, we found two species that had genome sizes suggestive of polyploidy, most likely tetraploid, one in *P. glaberrima* ssp. *triflora* (2C = 25.68 pg) and the other in *P. pulchra* (2C = 26.17 pg) (Fig. 2; Table 3).

**Discussion**

We successfully measured nuclear genome size and ploidy of a closely related group of long-styled *Phlox* species and cultivars found in the germplasm collection at the OPGC and have been able to assess the degree of cytoype variation within and among species. Characterization of genome size within this group has been a prelude to interspecific hybridization studies that are part of a *Phlox* germplasm enhancement program that encompasses the majority of eastern species (Zale, 2014). The following observations can be highlighted. 1) Wild populations of the seven species in subsections *Paniculatae* and *Phlox* were primarily diploid, but tetraploid individuals were identified in *P. glaberrima* ssp. *triflora* and *P. pulchra*. 2) The diploid taxa in both subsections have 2C genome sizes that are similar, ranging from 13 to 15 pg. 3) Cultivars of species in the two subsections could be diploid, triploid, and tetraploid. More than 90% of the 70 *P. paniculata* cultivars examined were diploid, but four novel triploid cytotypes were identified. Cultivars of *P. glaberrima* ssp. *triflora* have been selected at the tetraploid level. 4) Although polyploidy appears to be rare in the different species of subsection *Phlox*, the identification of polyploid cultivars suggests that increased ploidy may be associated with
Table 2. Relative holoploid (2C) and monoploid (1C) genome sizes, and inferred ploidy levels for a diverse collection of cultivars and hybrids of *Phlox* (section *Phlox*, subsections *Paniculatae* and *Phlox*). Genome size was determined using flow cytometry and inferred ploidy was confirmed by chromosome counts of selected samples.

| Taxa | Accession no. | Source | Relative 2C genome size [mean ± SD (pg)] | CV (%) | Ploidy | 1C genome size (pg) |
|------|---------------|--------|----------------------------------------|--------|--------|---------------------|
| *Phlox paniculata* Subsection *Paniculatae* | | | | | | |
| ‘Amethyst’ | PZ12-001 | F | 14.06 ± 0.25 | 2.05 | 2x | 7.03 |
| ‘Anne’ | PZ10-068 | F | 14.51 ± 0.16 | 1.62 | 2x | 7.26 |
| ‘Balmoral’ | PZ10-002 | F | 13.85 ± 0.02 | 1.46 | 2x | 6.93 |
| ‘Bartwentynine’ White Flame™ | PZ10-236 | A | 14.43 ± 0.04 | 1.46 | 2x | 7.22 |
| ‘Blue Boy’ | PZ10-003 | F | 14.40 ± 0.06 | 1.63 | 2x | 7.20 |
| ‘Blue Lagoon’ | PZ10-004 | F | 14.18 ± 0.37 | 1.82 | 2x | 7.09 |
| ‘Blue Paradise’ | PZ10-170 | F | 14.98 ± 0.05 | 1.26 | 2x | 7.49 |
| ‘Blushing Bride’ | PZ10-090 | F | 21.35 ± 0.34 | 1.97 | 3x | 7.12 |
| ‘Blushing Shortwood’ | PZ10-091 | F | 14.12 ± 0.27 | 1.26 | 2x | 7.06 |
| ‘Brigadier’ | PZ10-092 | F | 14.22 ± 0.04 | 1.43 | 2x | 7.11 |
| ‘Bright Eyes’ | PZ10-005 | F | 14.16 ± 0.04 | 1.75 | 2x | 7.08 |
| ‘Cabot Pink’ | PZ10-088 | F | 13.98 ± 0.01 | 1.25 | 2x | 6.99 |
| ‘Carol Van Den Berg’ | PZ10-006 | F | 14.31 ± 0.10 | 1.52 | 2x | 7.16 |
| ‘Caspian’ | PZ10-087 | F | 14.23 ± 0.02 | 1.73 | 2x | 7.12 |
| ‘Cinderella’ | PZ10-089 | F | 14.12 ± 0.01 | 1.48 | 2x | 7.06 |
| ‘Crème de Menthe’ | PZ10-008 | F | 14.59 ± 0.09 | 1.75 | 2x | 7.30 |
| ‘Dodo Hanbury Forbes’ | PZ10-085 | F | 14.00 ± 0.22 | 1.53 | 2x | 7.00 |
| ‘Darwin’s Choice’ | PZ10-009 | F | 14.47 ± 0.21 | 1.70 | 2x | 7.24 |
| ‘David’ | PZ10-029 | C | 13.93 ± 0.28 | 1.45 | 2x | 6.97 |
| ‘David’s Lavender’ | PZ10-189 | F | 14.19 ± 0.03 | 1.33 | 2x | 7.10 |
| ‘Delta Snow’ | PZ10-027 | B | 14.36 ± 0.29 | 1.64 | 2x | 7.18 |
| ‘Dick Weaver’ | PZ10-120 | G | 21.37 ± 0.24 | 1.55 | 3x | 7.12 |
| ‘Dorffreude’ | PZ10-084 | F | 14.25 ± 0.03 | 1.71 | 2x | 7.13 |
| ‘Dunbar Creek’ | PZ10-122 | G | 14.34 ± 0.09 | 1.84 | 2x | 7.16 |
| ‘Ending Blue’ | PZ10-081 | F | 14.43 ± 0.13 | 1.79 | 2x | 7.22 |
| ‘Europa’ | PZ10-010 | F | 14.03 ± 0.28 | 1.67 | 2x | 7.02 |
| ‘Eventide’ | PZ10-082 | F | 14.26 ± 0.19 | 1.65 | 2x | 7.13 |
| ‘Fairest One’ | PZ10-083 | F | 14.12 ± 0.04 | 1.69 | 2x | 7.06 |
| ‘Hot September Pink’ | PZ10-079 | F | 14.30 ± 0.03 | 1.73 | 2x | 7.15 |
| ‘John Fanick’ | PZ10-240 | G | 21.75 ± 0.13 | 1.48 | 3x | 7.25 |
| ‘Jeana’ | PZ10-251 | G | 13.70 ± 0.87 | 1.72 | 2x | 6.85 |
| ‘Jr. Dream’ | PZ12-013 | F | 14.14 ± 0.24 | 1.64 | 2x | 7.07 |
| ‘Jules Sandeau’ | PZ12-014 | F | 14.01 ± 0.02 | 1.98 | 2x | 7.01 |
| ‘Kirmeslander’ | PZ12-015 | F | 13.83 ± 0.41 | 2.43 | 2x | 6.92 |
| ‘Leo P. Schlageter’ | PZ10-066 | F | 14.45 ± 0.62 | 1.66 | 2x | 7.23 |
| ‘Little Boy’ | PZ12-017 | F | 14.38 ± 0.13 | 1.74 | 2x | 7.19 |
| ‘Lizzy’ | PZ12-018 | F | 14.02 ± 0.12 | 1.79 | 2x | 7.01 |
| ‘Lord Clayton’ | PZ11-011 | C | 13.79 ± 0.29 | 1.63 | 2x | 6.90 |
| ‘Mile High Pink’ | PZ10-064 | F | 13.91 ± 0.22 | 1.4 | 2x | 6.96 |
| ‘Miss Ellie’ | PZ12-019 | F | 13.97 ± 0.01 | 1.75 | 2x | 6.99 |
| ‘Miss Holland’ | PZ10-067 | F | 13.81 ± 0.06 | 1.37 | 2x | 6.91 |
| ‘Miss Kelly’ | PZ10-076 | F | 15.01 ± 0.20 | 1.43 | 2x | 7.51 |
| ‘Miss Universe’ | PZ10-062 | F | 14.18 ± 0.30 | 1.57 | 2x | 7.09 |
| ‘Mother of Pearl’ | PZ12-021 | F | 14.19 ± 0.16 | 1.58 | 2x | 7.10 |
| ‘Mt. Fujiyama’ | PZ12-022 | F | 14.31 ± 0.43 | 1.50 | 2x | 7.16 |
| ‘Newbird’ | PZ10-075 | F | 14.30 ± 0.06 | 1.42 | 2x | 7.15 |
| ‘Nicky’ | PZ12-023 | F | 14.76 ± 0.58 | 1.41 | 2x | 7.38 |
| ‘Old Cellarhole’ | PZ10-072 | F | 14.68 ± 0.51 | 1.69 | 2x | 7.34 |
| ‘Orange Perfection’ | PZ12-024 | F | 14.31 ± 0.02 | 1.75 | 2x | 7.16 |
| ‘Picasso’ | PZ12-025 | F | 14.53 ± 0.09 | 1.50 | 2x | 7.27 |
| ‘Pink Bud’ | PZ10-063 | F | 13.91 ± 0.34 | 1.43 | 2x | 6.96 |
| ‘Pixie Miracle Grace’ | PZ10-178 | C | 14.02 ± 0.30 | 1.40 | 2x | 7.01 |
enhanced ornamental attributes, as evidenced by the inadvertent selection of such lines, and may have greater adaptive importance than previously described.

The mean 1Cx genome size of the eastern *Phlox* taxa in this study, 7.01 pg (Tables 2 and 3) is higher than those of other *Phlox* species that have been examined thus far. *Phlox pilosa* of subsection *Divaricatae* had a 1C value ranging from 5.49 to 5.70 pg; the *P. amabilis*–*P. woodhousei* complex of subsection *Speciosae* from 4.26 to 4.36 pg; and the *P. nana* complex from 3.49 to 3.83 pg (Fehlberg and Ferguson, 2012a; Worcester

![Table 2. Continued.](image)

| Taxa                        | Accession no. | Source | Relative 2C genome size [mean ± sd (pg)] | CV (%) | Ploidy | 1Cx genome size (pg) |
|-----------------------------|---------------|--------|------------------------------------------|--------|--------|----------------------|
| 'Red Magic'                 | PZ12-027      | F      | 14.37 ± 0.29                            | 1.60   | 2x     | 7.19                 |
| 'Red Riding Hood'           | PZ10-240      | F      | 13.89 ± 0.48                            | 2.11   | 2x     | 6.95                 |
| 'Red Super'                 | PZ12-028      | F      | 14.35 ± 0.27                            | 2.5    | 2x     | 7.18                 |
| 'Rijnstroom'                | PZ10-071      | F      | 13.98 ± 0.72                            | 1.37   | 2x     | 6.99                 |
| 'Robert Poore'              | PZ10-028      | B      | 20.95 ± 0.13                            | 1.38   | 3x     | 6.98                 |
| 'Russian Violet'            | PZ10-073      | F      | 14.14 ± 0.09                            | 2.03   | 2x     | 7.07                 |
| 'Salmon Beauty'             | PZ10-070      | F      | 14.17 ± 0.05                            | 1.41   | 2x     | 7.09                 |
| 'Sherbert Cocktail'         | PZ10-184      | C      | 13.85 ± 0.21                            | 1.5    | 2x     | 6.93                 |
| 'Shorty White'              | PZ10-030      | F      | 14.43 ± 0.08                            | 1.81   | 2x     | 7.22                 |
| 'Sir John Falstaff'         | PZ10-031      | F      | 14.61 ± 0.23                            | 1.55   | 2x     | 7.31                 |
| 'Spinners'                  | PZ10-190      | C      | 14.55 ± 0.03                            | 1.50   | 2x     | 7.26                 |
| 'Spatifire'                 | PZ12-032      | F      | 14.08 ± 0.42                            | 1.65   | 2x     | 7.04                 |
| 'Starfire'                  | PZ12-033      | F      | 14.24 ± 0.14                            | 1.86   | 2x     | 7.12                 |
| 'Starry Skies'              | PZ12-034      | F      | 14.81 ± 0.10                            | 1.55   | 2x     | 7.41                 |
| 'White Admiral’              | PZ1-035       | F      | 13.99 ± 0.39                            | 1.33   | 2x     | 6.99                 |
| 'Widar'                     | PZ12-036      | F      | 14.06 ± 0.33                            | 1.50   | 2x     | 7.03                 |
| 'Wilhelm Kesselring’        | PZ12-037      | F      | 14.45 ± 0.06                            | 1.52   | 2x     | 7.23                 |
| 'Zurstock Rose’             | PZ12-038      | F      | 14.27 ± 0.19                            | 1.73   | 2x     | 7.14                 |
| Phlox xarendsi              |               |        |                                          |        |        |                      |
| 'Hesperis’                  | PZ12-011      | F      | 13.90 ± 0.19                            | 1.95   | 2x     | 6.95                 |
| 'Ping Pong’                 | PZ10-176      | C      | 13.79 ± 0.20                            | 1.38   | 2x     | 6.90                 |
| Subsection Phlox            |               |        |                                          |        |        |                      |
| Phlox carolina              |               |        |                                          |        |        |                      |
| 'Minnie Pearl’              | PZ11-012      | C      | 14.08 ± 0.14                            | 3.08   | 2x     | 7.04                 |
| 'Kim’                       | PZ11-017      | G      | 13.86 ± 0.01                            | 2.00   | 2x     | 6.93                 |
| Phlox glaberrima            |               |        |                                          |        |        |                      |
| 'N3 Hvtke Meske’            | PZ11-017      | D      | 15.09 ± 0.03                            | 1.60   | 2x     | 7.55                 |
| 'N3 Springfall’             | PZ10-103      | D      | 14.62 ± 0.03                            | 1.83   | 2x     | 7.31                 |
| Phlox glaberrima ssp. triflora|           |        |                                          |        |        |                      |
| 'Anita Kister’              | PZ10-175      | C      | 25.73 ± 0.29                            | 0.70   | 4x     | 6.43                 |
| 'Bill Baker’                | PZ11-031      | H      | 25.41 ± 0.03                            | 0.79   | 4x     | 6.35                 |
| 'Morris Berd’               | PZ10-061      | H      | 24.91 ± 0.30                            | 0.85   | 4x     | 6.23                 |
| 'Spring Delight’            | PZ12-136      | H      | 13.45 ± 0.01                            | 1.13   | 2x     | 6.73                 |
| 'Triple Play’               | PZ10-130      | G      | 24.79 ± 0.10                            | 0.97   | 4x     | 6.20                 |
| Growild, Inc. selection     | PZ10-032      | B      | 26.36 ± 0.12                            | 0.89   | 4x     | 6.59                 |
| Phlox maculata              |               |        |                                          |        |        |                      |
| 'Natascha’                  | PZ10-035      | B      | 14.23 ± 0.02                            | 1.74   | 2x     | 7.12                 |
| 'Flower Power’              | PZ10-235      | A      | 14.31 ± 0.09                            | 1.42   | 2x     | 7.16                 |
| 'Rosalinde’                 | PZ12-029      | F      | 14.63 ± 0.57                            | 2.03   | 2x     | 7.32                 |
| Phlox pulchra               |               |        |                                          |        |        |                      |
| 'Eco Pale Moon’             | PZ10-102      | C      | 13.83 ± 0.01                            | 1.19   | 2x     | 6.92                 |
| Phlox Suffruticos Group     |               |        |                                          |        |        |                      |
| 'Miss Lingard’              | PZ10-245      | F      | 21.21 ± 0.04                            | 1.00   | 3x     | 7.07                 |
| 'Monica Lynden-Bell’        | PZ12-020      | F      | 14.28 ± 0.26                            | 1.67   | 2x     | 7.14                 |

A = Forestfarm at Pacifica (Williams, OR), B = Growild, Inc. (Fairview, TN), C = Millcreek Gardens, (Ostrander, OH), D = Nearly Native Nursery, Inc. (Fayetteville, GA), E = Ornamental Plant Germplasm Center (Columbus, OH), F = Perennial Pleasures Nursery (East Hardwick, VT), G = Plant Delights Nursery (Raleigh, NC), H = The Primrose Path (Scottsdale, PA), I = Proven Winners (Sycamore, IL).

Holoploid genome sizes were determined with propidium iodide as the fluorochrome.

Coefficient of variation.

2x = diploid, 3x = triploid, 4x = tetraploid.

1Cx values were calculated as 2C value/ploidy level.

Ploidy levels were confirmed by chromosome counts in our laboratory.
Table 3. Relative holoploid (2C) and monoploid (1C\textsubscript{x}) genome sizes, and inferred ploidy levels, for a wild-collected accession of *Phlox* taxa (section *Phlox*, subsections *Paniculatae* and *Phlox*). Genome size was determined using flow cytometry and inferred ploidy was confirmed by chromosome counts of selected samples.

| Taxa\textsuperscript{a,y} | Accession no. | Relative 2C genome size [mean ± sd (pg)\textsuperscript{b}] | cv (%)\textsuperscript{c} | Ploidy\textsuperscript{d} | 1C\textsubscript{x} genome size (pg)\textsuperscript{e} |
|--------------------------|---------------|-----------------------------------------------------------|-----------------|-----------------|----------------------------------|
| **Subsection *Paniculatae*** | | | | | |
| *Phlox* af. *amplifolia* | | | | | |
| Campbell County, TN | PZ11-010 | 14.58 ± 0.12 | 1.94 | 2\textsuperscript{x} | 7.29 |
| Tucker County, WV | PZ12-106 | 14.24 ± 0.73 | 1.61 | 2\textsuperscript{x} | 7.12 |
| *Phlox* *amplifolia* | | | | | |
| Claiborne County, TN | PZ12-109 | 14.22 ± 0.02 | 1.20 | 2\textsuperscript{x} | 7.11 |
| Cocke County, TN | PZ11-050 | 14.65 ± 0.23 | 3.72 | 2\textsuperscript{x} | 7.33 |
| Woodford County, KY | PZ11-022 | 13.74 ± 0.33 | 3.28 | 2\textsuperscript{x} | 6.87 |
| *Phlox* *paniculata* | | | | | |
| Adams County, OH | PZ10-201 | 13.94 ± 0.13 | 1.68 | 2\textsuperscript{x} | 6.97 |
| Erie County, OH | PZ10-231 | 14.08 ± 0.16 | 2.65 | 2\textsuperscript{x} | 7.04 |
| Gallia County, OH | PZ11-061 | 14.43 ± 0.14 | 1.17 | 2\textsuperscript{x} | 7.22 |
| Highland County, OH | PZ10-204 | 14.26 ± 0.22 | 1.35 | 2\textsuperscript{x} | 7.13 |
| Claiborne County, TN | PZ12-110 | 13.98 ± 0.37 | 1.22 | 2\textsuperscript{x} | 6.99 |
| Carrol County, AR | PZ12-141 | 13.23 ± 1.52 | 1.41 | 2\textsuperscript{x} | 6.62 |
| Lumpkin County, GA | PZ10-109 | 14.21 ± 0.28 | 1.62 | 2\textsuperscript{x} | 7.11 |
| Preston County, WV | PZ11-040 | 14.00 ± 0.05 | 1.19 | 2\textsuperscript{x} | 7.00 |
| **Subsection *Phlox*** | | | | | |
| *Phlox* carolina ssp. *carolina* | | | | | |
| Clay County, MS | PZ11-036 | 14.68 ± 0.38 | 4.31 | 2\textsuperscript{x} | 7.34 |
| Gadsden County, FL | PZSH2011-005 | 15.55 ± 0.26 | 1.79 | 2\textsuperscript{x} | 7.78 |
| *P. carolina* ssp. *alta* | | | | | |
| Union County, GA | PZ11-048 | 14.11 ± 0.11 | 4.75 | 2\textsuperscript{x} | 7.06 |
| Union County, GA | PZ11-049 | 14.21 ± 0.24 | 4.32 | 2\textsuperscript{x} | 7.11 |
| Haywood County, NC | PZ11-045 | 15.10 ± 0.17 | 1.97 | 2\textsuperscript{x} | 7.55 |
| Transylvania County, NC | PZ11-072 | 14.93 ± 0.10 | 1.89 | 2\textsuperscript{x} | 7.47 |
| *P. carolina* ssp. *angusta* | | | | | |
| Bibb County, AL | PZ11-067 | 14.64 ± 0.11 | 3.38 | 2\textsuperscript{x} | 7.32 |
| Marshall County, KY | PZ12-047 | 13.52 ± 0.03 | 4.43 | 2\textsuperscript{x} | 6.76 |
| Cumberland County, TN | PZ10-093 | 14.96 ± 0.20 | 2.7 | 2\textsuperscript{x} | 7.48 |
| A | PZ10-034 | 15.02 ± 0.06 | 1.66 | 2\textsuperscript{x} | 7.51 |
| *Phlox* glaberrima ssp. *glaberrima* | | | | | |
| Polk Co. TN | PZ12-131 | 14.04 ± 0.11 | 1.45 | 2\textsuperscript{x} | 7.02 |
| *P. glaberrima* ssp. *interior* | | | | | |
| Lewis County, TN | PZ12-055 | 14.68 ± 0.08 | 1.55 | 2\textsuperscript{x} | 7.34 |
| White County, IN | PZ12-063 | 13.47 ± 0.42 | 2.38 | 2\textsuperscript{x} | 6.74 |
| *P. glaberrima* ssp. *triflora* | | | | | |
| Blount County, AL | PZ11-057 | 13.88 ± 0.52 | 1.68 | 2\textsuperscript{x} | 6.94 |
| Bullitt County, KY | PZ11-027 | 13.46 ± 0.34 | 2.45 | 2\textsuperscript{x} | 6.73 |
| Adams County, OH | PZ10-193 | 12.91 ± 0.18 | 2.31 | 2\textsuperscript{x} | 6.46 |
| Lyon County, KY | PZ12-046 | 12.89 ± 0.04 | 2.10 | 2\textsuperscript{x} | 6.45 |
| Bath County, VA | PZ11-019 | 25.68 ± 0.08 | 0.99 | 4\textsuperscript{x} | 6.42 |
| Hawkins County, TN | PZ12-135 | 13.20 ± 0.01 | 1.45 | 2\textsuperscript{x} | 6.60 |
| *Phlox* maculata | | | | | |
| Campbell County, TN | PZ12-103 | 14.38 ± 0.56 | 1.33 | 2\textsuperscript{x} | 7.19 |
| Campbell County, TN | PZ12-105 | 14.05 ± 0.12 | 1.21 | 2\textsuperscript{x} | 7.03 |
| Campbell County, TN | PZ12-107 | 14.34 ± 0.12 | 1.58 | 2\textsuperscript{x} | 7.17 |
| Campbell County, TN | PZ12-108 | 13.68 ± 0.21 | 1.56 | 2\textsuperscript{x} | 6.84 |
| Champaign County, OH | PZ10-198 | 13.88 ± 0.08 | 1.64 | 2\textsuperscript{x} | 6.94 |
| Adams County, OH | PZ10-208 | 13.94 ± 0.16 | 1.93 | 2\textsuperscript{x} | 6.97 |
| Scioto County, OH | PZ13-004 | 14.40 ± 0.26 | 1.96 | 2\textsuperscript{x} | 7.20 |
| McCreaey County, KY | PZ11-033 | 14.55 ± 0.49 | 2.91 | 2\textsuperscript{x} | 7.28 |
| *Phlox* ovata | | | | | |
| Lucas County, OH | PZ11-014 | 11.71 ± 0.21 | 3.51 | 2\textsuperscript{x} | 5.86 |
| Greenbrier County, WV | PZ12-086/089 | 11.80 ± 0.28 | 1.83 | 2\textsuperscript{x} | 5.90 |

*Continued next page*
Within the P. carolina and P. pulchra complex phylogeny using ITS and cpDNA restriction sites resulted from North Creek Nursery (Landenburg, PA) from a wild source in eastern Georgia. Other western species such as P. aculeata, P. caryophylla, P. cluteana, P. longifolia, and P. stansburyi had a 1C value ranging from 3.66 to 5.48 pg (Fehlberg and Ferguson, 2012b). This nearly 2-fold range of 1C values applies to diploid, tetraploid, pentaploid, and hexaploid cytotypes found among some of these species. Within other genera of the Polemoniaceae, the only genome size estimates we could find include Navarretia squarrosa, 1C = 1.32 pg, and Collomia grandiflora, 1C = 2.08 pg (Bennett and Leitch, 2012). The genome size for Phlox is within the same order of magnitude for the size of the majority of angiosperms whose average 1C value is 5.9 pg, although there is a 2400-fold range in size within the clade (Bennett and Leitch, 2012).

Genome size differences have been proposed as one feature that can distinguish species in a large genus like Penstemon (Broderick et al., 2011), but we could not use this measure to distinguish the species in our survey. Mean genome sizes of the two species in subsection Paniculatae were indistinguishable (2C = 14.02 pg for P. paniculata, 2C = 14.29 pg for P. amplifolia) whereas size variation in subsection Phlox was more pronounced. While the average 2C values at the diploid level (2n = 2x = 14) for P. carolina, P. glaberrima, P. maculata, and P. pulchra ranged from 13.57 to 14.67 pg, the variation within the P. carolina–P. glaberrima complex, approaching 20.5%, represents considerable within-species variation and may be related to subtle morphological differences and ecotypic divergence seen in these and other taxa (Ceccarelli et al., 2011; Cires et al., 2009). Such variation underscores the taxonomic uncertainty in this group and precludes use of the estimates of genome size to distinguish between the members of the complex. Comparable variation in genome size was also found among taxa in the P. pilosa complex, but not among other taxa analyzed in this study (Tables 2 and 3; Worcester et al., 2012).

Among diploids, variation in genome size (28.1%) of subsection Phlox far exceeds the “normal” range of variation (3%) that is exhibited by other angiosperm species (Bennett and Leitch, 2005; Gurushidze et al., 2012; Solis et al., 2003). Initial molecular reconstructions of the P. carolina-glaberrima complex phylogeny using ITS and cpDNA restriction sites resulted in a paraphyletic group that involved members of subsections Divaricatae, Paniculatae, and Phlox. Hence, these studies failed to provide resolution and taxonomic stability of these species. Hybridization was implicated as the cause of the paraphyletic grouping; however, it is also possible that Phlox taxa have recently diverged and that the markers used were not sufficient to capture a strong phylogenetic signal. The variation in genome size suggested that recent treatments of this complex, that have reduced the number of subspecies, might not accurately reflect the evolutionary and taxonomic history of the morphologically variable and geographically widespread complex of taxa.

The analysis of genome size of P. paniculata cultivars revealed that four of 66 cultivars (6%) had genome sizes larger than diploid, and the chromosome counts indicated they were triploid. Such triploid cytotypes have not been previously described for this species, but evidence from related species and from hybridization experiments suggests they are derived from crosses between diploid and tetraploid cytotypes (Fehlberg and Ferguson, 2012a; Flory, 1934; Zale, 2014). Tetraploid P. paniculata were not identified among the wild-collected accessions analyzed in this study, but population sampling was not exhaustive, and large portions of the species’ geographic range were not covered. The recent discovery of polyploid populations of other eastern Phlox taxa in related subsections, at the edges of their respective geographic distribution, suggests that targeted sampling along the perimeter of the geographic range of P. paniculata may reveal polyploid populations (Fehlberg and Ferguson, 2012a; Worcester et al., 2012; Wright, 2014; Zale, 2014). The east–west geographic distribution of P. paniculata stretches from the eastern Pennsylvania–New Jersey border to northwestern Arkansas; this species can be abundant in certain localities, and extensive phenotypic variation has been noted in wild populations (Locklear, 2011; Symons-Jeune, 1953; Wherry, 1955). Wild-sourced polyploid germplasm, should it be found, could be used to verify the products of F1 interpoll cross and have the potential to benefit Phlox breeding programs. Introduction of wild-collected germplasm can also result in genotypes not currently in cultivation and can be useful in systematic studies to discern the evolutionary history of the species.

Table 3. Continued.

| Taxa                  | Accession no. | Relative 2C genome size [mean ± SD (pg)] | cv (%) | Ploidy | 1Cx genome size (pg) |
|-----------------------|---------------|----------------------------------------|--------|--------|----------------------|
| Montgomery County, VA | PZ12-082      | 12.29 ± 0.04                          | 1.83   | 2x     | 6.15                 |
| Roanoke County, VA    | PZ12-080      | 12.09 ± 0.08                          | 1.73   | 2x     | 6.05                 |
| Union County, TN      | PZ12-112      | 11.72 ± 0.20                          | 2.05   | 2x     | 5.86                 |
| Claiborne County, TN  | PZ12-111      | 12.04 ± 0.15                          | 1.77   | 2x     | 6.02                 |
| Hamblen County, TN    | PZ12-115      | 12.29 ± 0.05                          | 1.85   | 2x     | 6.15                 |

Collections were made from wild populations by the first author from 2010 to 2013. The county of origin is provided where known; A = plant obtained from North Creek Nursery (Landenburg, PA) from a wild source in eastern Georgia.

Positive identifications were made using the taxonomic keys of Locklear (2011) and Wherry (1955) and comparison with herbarium specimens.

Holoploid genome sizes were determined with propidium iodide as the fluorochrome.

Coefficient of variation.

1Cx values were calculated as 2C value/ploidy level.

Ploidy levels were confirmed by chromosome counts in our laboratory.
Fig. 2. Flow cytometric determination of holoploid genome size and ploidy level of diploid ($2n = 2x = 14$), triploid ($2n = 3x = 21$), and tetraploid ($2n = 4x = 28$) cultivars of *Phlox paniculata* and *P. amplifolia* using *Pisum sativum* ‘Ctirad’ as the internal standard. (A) Histogram and meiotic metaphase chromosome counts for the diploid *P. paniculata* ‘Delta Snow’ (2C = 14.36 pg). (B) Histogram and mitotic metaphase chromosome counts for the diploid *P. amplifolia* PZ11-050 (2C = 14.65 pg); (C) Histogram and mitotic metaphase chromosome count of the triploid *P. paniculata* ‘John Fanick’ (2C = 21.75 pg); (D) Histogram and mitotic metaphase chromosome count of the tetraploid *P. glaberrima* ssp. *triflora* PZ11-019 (2C = 25.68 pg).
An interesting observation about the triploid cultivars is that ‘Robert Poore’ and ‘John Fanick’ are consistently rated among the *P. paniculata* cultivars most resistant to powdery mildew (Hawke, 1999, 2011; Taylor et al., 2002). Susceptibility to powdery mildew (*Erysiphe cichoracearum*) is the foremost factor limiting more widespread usage of *P. paniculata* in landscapes, but despite the popularity of this species, a definitive mode of resistance has yet to be identified (Hawke, 1999; Jorosz et al., 1982). There are highly mildew-resistant diploid cultivars such as Shortwood, David, and Delta Snow (Bir 1999, 2003; Hawke, 1999, 2011), so resistance in this species may have different pathways that potentially involve morphological adaptations, physiological alterations, specific R-gene action, or environmental interactions (Jorosz et al., 1982). While no studies directly link polyploidy to disease resistance in plants, genetic variation generated through inter-specific crosses and failure to set seed in hybrid crosses (Wherry, 1935a). Our analysis of genome size for this cultivar shows that the 21.21-pg 2C size is consistent with a triploid level, supporting a hybrid origin from a cross of a tetraploid and a diploid parent. While no tetraploids were found among our survey of accessions from wild populations and cultivars of plants keyed to *P. carolina* and *P. maculata*, the morphological similarity and historical taxonomic confusion of taxa of the *P. glaberrima* complex suggests that ‘Miss Lingard’ may have resulted from a cross between a tetraploid *P. glaberrima* and diploid *P. maculata*. Tetraploid cytotypes were found among *P. glaberrima* cultivars tested in this study and are known to occur in natural populations in the southern Appalachian Mountains; it is possible that early horticultural introductions originated in this area (Ferguson, 1998; Wherry, 1955). The hybrid origin of ‘Miss Lingard’ is also supported by pollen sterility in the tested clones and failure to set seed in hybrid crosses (Wherry, 1935a).

The presence of pentaploids among populations of taxa in the *P. amabilis–P. woodhousei* complex suggests that crosses between individuals of different ploidy may be more widespread among *Phlox* than previously known (Fehlberg and Ferguson, 2012a).

**Table 4.** Monoploid (1Cx) genome sizes of *Phlox* taxa from subsections *Paniculatae* and *Phlox* determined using flow cytometry, grouped by subsection and species.

| Subsection | Species      | 1Cx [mean ± sd (pg)] |
|------------|--------------|----------------------|
| *Paniculatae* | *amplifolia* | 7.01 ± 0.36 a       |
|            | *paniculata* | 7.10 ± 0.15 a       |
|            | *carolinae*  | 7.28 ± 0.30 a       |
|            | *glaberrimae*| 6.71 ± 0.40 b       |
|            | *maculata*   | 7.11 ± 0.15 a       |
|            | *ovata*      | 5.99 ± 0.13 c       |
|            | *pulchra*    | 6.84 ± 0.27 b       |
| *Phlox*    |              | 6.85 ± 0.49 b       |

Values separated within columns using the Fishers least significant difference with *P* < 0.05.

Includes all botanical varieties described for these species listed in Table 4.

**Fig. 3.** Concurrent comparison of four *Phlox* species from three sections and four different subsections of the genus: (A) diploid *P. subulata*, (B) diploid *P. pilosa ssp. pilosa*, (C) diploid *P. paniculata*, and (D) tetraploid *P. buckleyi*. G, peak placement and values support data from analyses of the same individual samples with an internal standard.
or are backcross progeny of *P. paniculata*; a conclusion further supported by the observation that viable seed was produced after open pollination in such cultivars (Wherry, 1955). However, only a small subset of *P. arenardsii* cultivars was tested. Many more exist, and it is possible that some may represent interspecific hybrids derived from multiple origins.

There has been one previous report of polyploidy in taxa of subsection *Phlox*, a tetraploid individual of *P. glaberrima* collected from the southern Appalachians (Ferguson, 1998). We have now identified six tetraploid accessions in subsection *Phlox*: five in *P. glaberrima* ssp. *triflora* and one in *P. pulchra*. This is the first report of polyploidy among cultivars of subsection *Phlox*, and the first report of a tetraploid cytotype of *P. pulchra* (Table 3). The tetraploid *P. pulchra* has the largest genome size among all *Phlox* taxa measured to date (2C = 26.17 pg) that were part of a large survey of such genomes in wild and cultivated *Phlox* (Zale, 2014). The tetraploid *P. glaberrima* cultivars ‘Anita Kistler’ and ‘Morris Berd’ are known to have been collected directly from natural populations, and the remaining tetraploid cultivars are not likely to be more than one generation removed from wild-collected progenitors (Bendtsen, 2009; Fuchs, 1994). All of these selections were probably made without the knowledge of ploidy, and suggest inadvertent selection for polyploid plants because of novel phenotypic traits or increased adaptation and persistence in a range of environmental conditions. Furthermore, all of the *P. glaberrima* cultivars can be attributed to the morphologically distinct taxon *P. glaberrima* ssp. *triflora*. Together with *P. ovata* and *P. pulchra*, this taxon displays midseason (May–June) phenology; a proliferation of sterile, evergreen stems; and a preference for xeric habitats (Table 1). These morphological similarities between the three species distinguish them from others in the subsection *Phlox*. The presence of polyploidy among these, but not other taxa in the subsection, suggests that ploidy may be an additional differentiating factor between these and other species (Michaux, 1803; Ferguson et al., 1999; Smith and Levin, 1967). Such observations highlight the importance of introducing wild-collected *Phlox* germplasm to increase the genetic diversity of available ornamental crops and identify avenues for polyploid breeding of *Phlox*.

Identification of triploid *P. paniculata* cultivars indicates that ploidy manipulation has already occurred in *Phlox*, either deliberately or inadvertently. Induced tetraploids have been developed in *P. drummondii* (Raghuvanshi and Pathak, 1975; Tiwari and Mishra, 2012; Vyas et al., 2007), *P. paniculata* (Matiska and Vejsadov, 2010), and *P. subulata* (Zhang et al., 2008), but the extent to which these have contributed to new cultivars is unclear. There is room for further experimentation and manipulation of polyploidy in this important genus, perhaps to alter flowering characteristics (flower size, longevity, and flowering period) and size and vigor (Dar et al., 2013). Sterile interspecific hybrids may also be restored to fertility by allopolyploidization (Leus et al., 2012).

The analysis of genome size in cultivars and wild forms of *P. paniculata* and its long-styled relatives provides further insight into the natural diversity and the horticultural manipulation of this group of ornamental plants. Although most accessions were diploid, the presence of triploid cultivars that likely originated from crosses between diploid and tetraploid parental taxa suggests that ploidy manipulation could be useful in breeding *Phlox*. Tetraploids were more abundant among cultivars than wild-collected accessions indicating that polyploids probably have been inadvertently selected for horticultural purposes, and thus a more deliberate effort to alter ploidy may be warranted. This information is likely to be of use to plant breeders, systematists, and plant biologists.

**Literature Cited**

Arends, J. 1912. *Phlox arenardsii*. Proc. Royal Hort. Soc. 38:151.

Arugumanathan, K. and E.D. Earle. 1991. Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol. Biol. Rpt. 9:229–241.

Balao, F., R. Casimiro-Soriguier, M. Talaver, J. Herrera, and S. Talaver. 2009. Distribution and diversity of cytotypes in *Dianthus broteri* as evidenced by genome size variations. Ann. Bot. (Lond.) 104:965–973.

Bendtsen, B.H. 2009. *Phlox*: Phlox fur den garten. Forlaget Geranium Press, Viborg, Denmark.

Bennett, M.D. and I.J. Leitch. 2005. Plant genome size research: A field in focus. Ann. Bot. (Lond.) 95:1–6.

Bennett, M.D. and I.J. Leitch. 2012. Plant DNA C-values Database (release 6.0, Dec. 2012), 20 Aug. 2014. <http://www.kew.org/cvalues/>.

Bir, R.E. 1999. *Phlox* without fail. Organic Gardening 46:52–55.

Bir, R.E. 2003. *Phlox* get humidity test. Amer. Gardener 82:19.

Broderick, S.R., M.R. Stevens, B. Geary, S.L. Love, E.N. Jellen, R.B. Dockter, S.L. Daley, and D.T. Lindgren. 2011. A survey of *Penstemon*’s genome size. Genome 54:160–173.

Ceccarelli, M., V. Sarri, M.E. Caceres, and P.G. Cionini. 2011. Intraspecific genotypic diversity in plants. Genome 54:701–709.

Cires, E., C. Cuesta, E.L. Peredo, M. Ai. Revilla, and J.A.F. Prieto. 2009. Genome size and morphological differentiation within *Ranunculus parnassifolius* group (*Ranunculaceae*) from calcareous scree in the northwest of Spain. Plant Syst. Evol. 281:193–208.

Cohen, R., A. Hanan, and H.S. Paris. 2003. Single-gene resistance to powdery mildew in zucchini squash (*Cucurbita pepo*). Euphytica 130:433–441.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Nat. Rev. Genet. 6:836–846.

Contreras, R.N., J.M. Ruter, and W.W. Hanna. 2009. An oryzalin-induced autoallooctoploid of *Hibiscus acetosella* ‘Panama Red’. J. Amer. Soc. Hort. Sci. 134:553–559.

Dar, T.H., S.N. Raina, and S. Goel. 2013. Molecular analysis of genomic changes in synthetic autotetraploid *Phlox drummondii* Hook. Bot. J. Linn. Soc. 110:591–605.

Deam, C.C. 1940. The flora of Indiana. Div. Forestry, Indiana Dept. Conservation, Indianapolis, IN.

Doležel, J. 2009. Determination of nuclear genome size. 10 Sept. 2011. <http://olomouc.ubc.cas.cz/book/determination-nuclear-genome-size/>.

Doležel, J., J. Greilhuber, S. Lucrettii, A. Meister, M.A. Lysák, L. Nardi, and R. Obermayer. 1998. Plant genome size estimation by flow cytometry: Interlaboratory comparison. Ann. Bot. (Lond.) 82:17–26.

Eeckhaut, T., K. van Laere, J. De Riek, and J. van Huylenbroek. 2006. Overcoming barriers in floriculture, ornamental and plant biotech- nology, Vol. I. Global Science Books. Cambridge Univ. Press, Cambridge, UK.

Fehlberg, S.D. and C.J. Ferguson. 2012a. Intraspecific cytotypic variation and complex genetic structure in the *Phlox amabilis*–*P. woodhouseii* (Polemoniaceae) complex. Amer. J. Bot. 99:865–874.

Fehlberg, S.D. and C.J. Ferguson. 2012b. Intraspecific cytotypic variation and conservation: An example from *Phlox* (Polemoniaceae). Calochortiana 1:189–195.

Ferguson, C.J. 1998. Molecular systematics of eastern *Phlox* species. PhD Thesis, Univ. Texas, Austin, TX.

Ferguson, C.J. and R.K. Jansen. 2002. A chloroplast DNA phylogeny of eastern *Phlox* (Polemoniaceae): Implications of congruence and incongruence with the ITS phylogeny. Amer. J. Bot. 89:1324–1335.

Ferguson, C.J., F. Krämer, and R.K. Jansen. 1999. Relationships of eastern North American *Phlox* (Polemoniaceae) based on ITS sequence data. Syst. Bot. 24:616–631.
