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

Peter J. Zale^{1,3} and Pablo Jourdan²

Ornamental Plant Germplasm Center, 670 Vernon L. Tharp Street, Columbus, OH 43210; Department of Horticulture and Crop Science, The Ohio State University, 2021 Coffey Road, Columbus, OH 43210; and Longwood Gardens, P.O. Box 501, Kennett Square, PA 19348

ADDITIONAL INDEX WORDS. flow cytometry, chromosome number, accession, diploid, tetraploid, triploid, phylogeny

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 ($2n = 2x = 14$) 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 suggests previous interploidy hybridization within this taxon. Five tetraploid ($2n = 4x = 28$) cultivars were found in subsection *Phlox*; all were selections of *P. glaberrima* ssp. *triflora*, and had a mean genome size of 25.44 pg; chromosome counts in one of these confirmed they were tetraploid. 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.

Phlox (Polemoniaceae) is a phenotypically diverse genus with substantial interpopulation differentiation for numerous morphological characters and ploidy; it includes ≈ 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 (*Annuae*, *Occidentales*, and *Phlox*) and six subsections (*Divaricatae*, *Subulatae*, *Phlox*, *Paniculatae*, *Stoloniferae*, and *Cluteanae*) 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

Received for publication 20 Mar. 2015. Accepted for publication 2 July 2015. Salaries and research support were provided by state and federal funds appropriated to the Ohio Agriculture Research and Development Center, The Ohio State University, as well as by the Floriculture and Research Initiative in support of the Ornamental Plant Germplasm Center.

We thank Dr. D.W.H. Robarts and the staff of the Ornamental Plant Germplasm Center for assistance with flow cytometry. Dr. D. Struve and Dr. L. McHale provided valuable comments and Dr. J.J.N. Campbell and A. Floden assisted in field work and accession collection.

¹Breeder and Curator of Plants at Longwood Gardens.

²Associate Professor.

³Corresponding author. E-mail: zale.6@buckeyemail.osu.edu.

Fig. 1. (A–L) Leaf, pollen, and style length variation of *Phlox* taxa. (A–E) Differences in leaf shape between *P. paniculata* and the “*P. glaberrima* complex”: (A) *P. paniculata* PZ10-209, note the areolate leaf veins; (B) *Phlox carolina* ssp. *alta* PZ10-045, note obscure leaf veins; (C) *P. glaberrima* ssp. *triflora* PZ10-193; (D) *P. glaberrima* ssp. *triflora* PZ11-019; (E) *P. maculata* PZ12-107. (F–I) Differences in pollen color between subsection *Paniculatae* and *Phlox*: (F) yellow pollen of *P. glaberrima* ssp. *triflora* PZ11-019; (G) yellow pollen of *P. maculata* PZ12-107; (H) cream-colored pollen of *P. paniculata* PZ10-209; (I) cream-colored pollen of *P. amplifolia* PZ11-050. (J–L) Differences in style length among *Phlox* subsections: (J) subsection *Paniculatae* and *Phlox*, (K) subsection *Subulatae*, (L) subsection *Divaricatae*; scale bar = 1 cm.

to differentiate them overlap and render identification of wild-collected germplasm difficult, if not impossible (Table 1). Extensive phenotypic variation within these two subsections has resulted in the selection of cultivars and putative interspecific hybrids, but the potential for further breeding and selection in this group remains unrealized, and has likely been hindered by lack of phylogenetic and taxonomic resolution. Genome size and ploidy estimation provide an additional criterion for assessing phylogenetic relationships among these phloxes, and to identify potential breeding barriers to hybrid cultivar development.

The taxonomy of subsection *Paniculatae* has remained stable since formal recognition, but phenotypic variation in *P. paniculata* has resulted in selection of over 800 cultivars (Bendtsen, 2009; Fuchs, 1994; Lierval, 1866; Locklear, 2011; Pridham, 1934; Symons-Jeune, 1953; Wherry, 1933, 1935a). Far more cultivars have been selected from this species than any other in the genus. Many of these are still grown, but several have also been lost to cultivation, reintroduced under different names, or have not been introduced in some regions, like the United States (Bendtsen, 2009; Hawke, 2011, 2013; Locklear,

2011; Symons-Jeune, 1953; Wherry, 1955). Cultivars have been primarily selected for variations in flower color, duration of flowering period, and variegated foliage. One cultivar, David, was chosen as the 2002 “Perennial Plant of the Year” by the Perennial Plant Association because of its adaptability to different climatic regions, soil types, and disease resistance. *Phlox paniculata* continues to be the focus of breeding programs, and cultivars are still introduced annually. The closely related *P. amplifolia* has rarely been introduced into cultivation, although European nurseries have reputedly developed cultivars (Deam, 1940; Wherry, 1935a). However, because of gross morphological similarity of *P. amplifolia* to *P. paniculata*, the true identity of these cultivars has yet to be demonstrated, and the breeding potential of *P. amplifolia* remains largely unknown.

In contrast to the taxonomic stability of subsection *Paniculatae*, taxa within the morphologically divergent “*P. glaberrima* complex” of subsection *Phlox* have been the subject of debate, frequent change, and taxonomic confusion (Ferguson and Jansen, 2002; Ferguson et al., 1999; Locklear, 2011; Wherry, 1935b, 1945, 1955). The traditional morphological markers (calyx, vesture, 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	Subsection <i>Phlox</i> , the “ <i>P. glaberrima</i> complex”									
	<i>carolina</i> ssp. <i>carolina</i>	<i>carolina</i> ssp. <i>alta</i>	<i>carolina</i> ssp. <i>angusta</i>	<i>glaberrima</i> ssp. <i>glaberrima</i>	<i>glaberrima</i> ssp. <i>interior</i>	<i>glaberrima</i> ssp. <i>triflora</i>	Subsection <i>Paniculatae</i>			
							<i>amplifolia</i>	<i>paniculata</i>		
Stem ht (cm)	40–100	75–200	45–90	50–150	35–120	40–60	50–150	75–200		
Leaves	Lanceolate to ovate-oblong	Elliptic	Linear	Linear to narrow lanceolate	Linear to lanceolate	Linear to lanceolate-ovate	Rhombic-ovate	Elliptic to elliptic-ovate		
Leaf veins	Obscure	Obscure	Obscure	Obscure	Obscure	Obscure	Areolate	Areolate		
Leaf margin	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Ciliate	Ciliate		
Leaf distribution	Largest above midstem	Largest above midstem	Largest below 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	Panicle of cymes	Compound panicle of cymes		
Calyx	Subcampanulate	Subcampanulate	Subcampanulate	Subcylindrical	Subcylindrical	Subcylindrical	Subcampanulate	Subcylindrical		
Pollen color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Cream	Cream-white		
Persistent sterile stems	Rarely	No	No	No	No	Yes	No	No		
Habitat	Mesic/hydric	Mesic	Hydric	Hydric	Hydric	Mesic/xeric	Mesic	Hydric		

taxonomy based on morphological features does not consistently separate taxa, complicating germplasm collection and characterization efforts within these polymorphic taxa. Thus, the taxonomy or phylogeny is not resolved for morphologically similar taxa in this group. As these taxa reportedly share a close phylogenetic relationship with subsection *Paniculatae*, there is a need to clarify the relationships. This confusion may adversely affect breeding efforts, and highlights the need for a resolved molecular phylogeny and the use of additional markers, such as genome size and ploidy.

A potentially confounding factor in the taxonomic relationship of these species is the recent evidence for extensive cytotype variation in populations of other *Phlox* species, including tetraploids in *Phlox pilosa*; tetraploids, pentaploids, and hexaploids in *Phlox amabilis* and *Phlox woodhousei*; tetraploids and hexaploids in *Phlox nana*; and tetraploids in *Phlox subulata* (Fehlberg and Ferguson, 2012a; Flory, 1934; Smith and Levin, 1967; Worcester et al., 2012; Wright, 2014). We have also identified polyploid populations of *P. pilosa* and *P. subulata* (Zale, 2014). In all cases where polyploid *Phlox* have been identified, the plants did not exhibit obviously altered overall morphology (e.g., the “gigas” effect), and could thus be classified as cryptic polyploids (Fehlberg and Ferguson, 2012a; Worcester et al., 2012). Both the uncertain taxonomic status of *P. carolina* and *P. glaberrima* coupled with the likelihood that cytotype variation is more common in *Phlox* than previously thought, suggest that an analysis of genome size and inferred ploidy in other *Phlox* species could further enhance the understanding of the breeding potential in the genus.

Polyploidy has played an important role in the development of ornamental plant cultivars (Contreras et al., 2009; Parris et al., 2010; Ranney, 2006), but the extent to which it has been used in *Phlox* is uncertain. We could find no published reports about genome sizes for any *Phlox* cultivars and comparatively few for *Phlox* species although chromosome counts have been reported for the principal species (Flory, 1931, 1934; Meyer, 1944). A chromosome number survey of over 100 cultivars of *P. paniculata* using meristematic tissue isolated from branch tips determined that all had the diploid ($2n = 2x = 14$) number, but that certain cultivars exhibited varying numbers of chromosomal fragments (Flory, 1931, 1934; Meyer, 1944). The number of chromosomal fragments varied between squash preparations and was not repeatable, which makes the origin, confirmation, and significance of these fragments unclear. Polyploidy in *P. paniculata* is unknown, but this observation is based on a limited number of cultivars. Observations and comments by various growers and gardeners indicate that fruit and seed production in some cultivars is absent to very low, which could be a consequence of different factors including alterations in ploidy (Bendtsen, 2009; Wherry, 1935a). Determination of genome size and ploidy level in a wide range of cultivars would be of benefit to *P. paniculata* breeders and may have the potential to produce new breeding lines with novel, useful traits (Hawke, 2011; Locklear, 2011).

Differences in ploidy between species are a potent prezygotic barrier to hybridization and are known to affect speciation and evolution by contributing to ecotypic isolation, novel gene expression, and divergence (Eckhaut et al., 2006; Hogenboom and Mather, 1975; Parris et al., 2010; Ranney, 2006). Knowledge of ploidy is crucial for characterization of germplasm collections and for directing interspecific hybridization efforts. Although genome size and ploidy has been characterized in

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 parnassifolius* (Cires 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; Šmarda 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 tested for wild-collected accessions. Approximately 2 cm² of *Phlox* newly expanded leaves and ≈0.5 cm² of *Pisum sativum* ‘Ctirad’ 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*_n 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% acetocarmine 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% acetocarmine 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 G_1 peak and a reduced G_2 peak for the *Phlox*, and also a prominent G_1 peak of the internal standard *P. sativum* 'Ctirad' (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 genome reduces variation that may be found in individual samples and reinforces calibration 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 (Matiska 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 cytotype 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 (1Cx) 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 ^z	Relative 2C genome size [mean $\pm$ sd (pg)] ^y	cv (%) ^x	Ploidy ^w	1Cx genome size (pg) ^v
Subsection <i>Paniculatae</i>						
<i>Phlox paniculata</i>						
‘Amethyst’	PZ12-001	F	14.06 $\pm$ 0.25	2.05	2x	7.03
‘Anne’	PZ10-068	F	14.51 $\pm$ 0.16	1.62	2x	7.26
‘Balmoral’	PZ12-002	F	13.85 $\pm$ 0.02	1.46	2x	6.93
‘Bartwentyne’	PZ10-236	A	14.43 $\pm$ 0.04	1.46	2x	7.22
White Flame ^{TM}						
‘Blue Boy’	PZ12-003	F	14.40 $\pm$ 0.06	1.63	2x	7.20
‘Blue Lagoon’	PZ12-004	F	14.18 $\pm$ 0.37	1.82	2x	7.09
‘Blue Paradise’	PZ10-170	F	14.98 $\pm$ 0.05	1.26	2x	7.49
‘Blushing Bride’	PZ10-090	F	21.35 $\pm$ 0.34	1.97	3x ^u	7.12
‘Blushing Shortwood’	PZ10-091	F	14.12 $\pm$ 0.27	1.26	2x	7.06
‘Brigadier’	PZ10-092	F	14.22 $\pm$ 0.04	1.43	2x	7.11
‘Bright Eyes’	PZ12-005	F	14.16 $\pm$ 0.04	1.75	2x	7.08
‘Cabot Pink’	PZ10-088	F	13.98 $\pm$ 0.01	1.25	2x	6.99
‘Carol Van Den Berg’	PZ12-006	F	14.31 $\pm$ 0.10	1.52	2x	7.16
‘Caspian’	PZ10-087	F	14.23 $\pm$ 0.02	1.73	2x	7.12
‘Cinderella’	PZ10-089	F	14.12 $\pm$ 0.01	1.48	2x	7.06
‘Crème de Menthe’	PZ12-008	F	14.59 $\pm$ 0.09	1.75	2x	7.30
‘Dodo Hanbury Forbes’	PZ10-085	F	14.00 $\pm$ 0.22	1.53	2x	7.00
‘Darwin’s Choice’	PZ12-009	F	14.47 $\pm$ 0.21	1.70	2x	7.24
‘David’	PZ10-029	C	13.93 $\pm$ 0.28	1.45	2x ^u	6.97
‘David’s Lavender’	PZ10-189	F	14.19 $\pm$ 0.03	1.33	2x	7.10
‘Delta Snow’	PZ10-027	B	14.36 $\pm$ 0.29	1.64	2x ^u	7.18
‘Dick Weaver’	PZ12-120	G	21.37 $\pm$ 0.24	1.55	3x ^u	7.12
‘Dorffreude’	PZ10-084	F	14.25 $\pm$ 0.03	1.71	2x	7.13
‘Dunbar Creek’	PZ12-122	G	14.34 $\pm$ 0.09	1.84	2x	7.17
‘Ending Blue’	PZ10-081	F	14.43 $\pm$ 0.13	1.79	2x	7.22
‘Europa’	PZ12-010	F	14.03 $\pm$ 0.28	1.67	2x	7.02
‘Eventide’	PZ10-082	F	14.26 $\pm$ 0.19	1.65	2x	7.13
‘Fairest One’	PZ10-083	F	14.12 $\pm$ 0.04	1.69	2x	7.06
‘Hot September Pink’	PZ10-079	F	14.30 $\pm$ 0.03	1.73	2x	7.15
‘John Fanick’	PZ10-240	G	21.75 $\pm$ 0.13	1.48	3x ^u	7.25
‘Jeana’	PZ10-251	G	13.70 $\pm$ 0.87	1.72	2x	6.85
‘Jr. Dream’	PZ12-013	F	14.14 $\pm$ 0.24	1.64	2x	7.07
‘Jules Sandeau’	PZ12-014	F	14.01 $\pm$ 0.02	1.98	2x	7.01
‘Kirmeslander’	PZ12-015	F	13.83 $\pm$ 0.41	2.43	2x	6.92
‘Leo P. Schlageter’	PZ10-066	F	14.45 $\pm$ 0.62	1.66	2x ^u	7.23
‘Little Boy’	PZ12-017	F	14.38 $\pm$ 0.13	1.74	2x	7.19
‘Lizzy’	PZ12-018	F	14.02 $\pm$ 0.12	1.79	2x	7.01
‘Lord Clayton’	PZ11-011	C	13.79 $\pm$ 0.29	1.63	2x	6.90
‘Mile High Pink’	PZ10-064	F	13.91 $\pm$ 0.22	1.4	2x	6.96
‘Miss Ellie’	PZ12-019	F	13.97 $\pm$ 0.01	1.75	2x	6.99
‘Miss Holland’	PZ10-067	F	13.81 $\pm$ 0.06	1.37	2x	6.91
‘Miss Kelly’	PZ10-076	F	15.01 $\pm$ 0.20	1.43	2x	7.51
‘Miss Universe’	PZ10-062	F	14.18 $\pm$ 0.30	1.57	2x	7.09
‘Mother of Pearl’	PZ12-021	F	14.19 $\pm$ 0.16	1.58	2x	7.10
‘Mt. Fujiyama’	PZ12-022	F	14.31 $\pm$ 0.43	1.50	2x	7.16
‘Newbird’	PZ10-075	F	14.30 $\pm$ 0.06	1.42	2x	7.15
‘Nicky’	PZ12-023	F	14.76 $\pm$ 0.58	1.41	2x	7.38
‘Old Cellarhole’	PZ10-072	F	14.68 $\pm$ 0.51	1.69	2x	7.34
‘Orange Perfection’	PZ12-024	F	14.31 $\pm$ 0.02	1.75	2x	7.16
‘Picasso’	PZ12-025	F	14.53 $\pm$ 0.09	1.50	2x	7.27
‘Pink Bud’	PZ10-063	F	13.91 $\pm$ 0.34	1.43	2x	6.96
‘Pixie Miracle Grace’	PZ10-178	C	14.02 $\pm$ 0.30	1.40	2x	7.01

Continued next page

Table 2. Continued.

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

^zA = 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).

^yHoloploid genome sizes were determined with propidium iodide as the fluorochrome.

^xCoefficient of variation.

^w2x = diploid, 3x = triploid, 4x = tetraploid.

^v1Cx values were calculated as 2C value/ploidy level.

^uPloidy levels were confirmed by chromosome counts in our laboratory.

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 1Cx 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 3. Relative holoploid (2C) and monoploid (1Cx) 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 ^{z,y}	Accession no.	Relative 2C genome size [mean $\pm$ SD (pg)] ^x	cv (%) ^w	Ploidy ^v	1Cx genome size (pg) ^u
Subsection <i>Paniculatae</i>					
<i>Phlox</i> aff. <i>amplifolia</i>					
Campbell County, TN	PZ11-010	14.58 $\pm$ 0.12	1.94	2x ^t	7.29
Tucker County, WV	PZ12-106	14.24 $\pm$ 0.73	1.61	2x	7.12
<i>Phlox</i> <i>amplifolia</i>					
Claiborne County, TN	PZ12-109	14.22 $\pm$ 0.02	1.20	2x ^t	7.11
Cocke County, TN	PZ11-050	14.65 $\pm$ 0.23	3.72	2x ^t	7.33
Woodford County, KY	PZ11-022	13.74 $\pm$ 0.33	3.28	2x	6.87
<i>Phlox</i> <i>paniculata</i>					
Adams County, OH	PZ10-201	13.94 $\pm$ 0.13	1.68	2x	6.97
Erie County, OH	PZ10-231	14.08 $\pm$ 0.16	2.65	2x	7.04
Gallia County, OH	PZ11-061	14.43 $\pm$ 0.14	1.17	2x	7.22
Highland County, OH	PZ10-204	14.26 $\pm$ 0.22	1.35	2x	7.13
Claiborne County, TN	PZ12-110	13.98 $\pm$ 0.37	1.22	2x	6.99
Carroll County, AR	PZ12-141	13.23 $\pm$ 1.52	1.41	2x	6.62
Lumpkin County, GA	PZ10-109	14.21 $\pm$ 0.28	1.62	2x	7.11
Preston County, WV	PZ11-040	14.00 $\pm$ 0.05	1.19	2x ^t	7.00
Subsection <i>Phlox</i>					
<i>Phlox</i> <i>carolina</i> ssp. <i>carolina</i>					
Clay County, MS	PZ11-036	14.68 $\pm$ 0.38	4.31	2x ^t	7.34
Gadsden County, FL	PZSH2011-005	15.55 $\pm$ 0.26	1.79	2x	7.78
<i>P. carolina</i> ssp. <i>alta</i>					
Union County, GA	PZ11-048	14.11 $\pm$ 0.11	4.75	2x	7.06
Union County, GA	PZ11-049	14.21 $\pm$ 0.24	4.32	2x	7.11
Haywood County, NC	PZ11-045	15.10 $\pm$ 0.17	1.97	2x ^t	7.55
Transylvania County, NC	PZ11-072	14.93 $\pm$ 0.10	1.89	2x	7.47
<i>P. carolina</i> ssp. <i>angusta</i>					
Bibb County, AL	PZ11-067	14.64 $\pm$ 0.11	3.38	2x ^t	7.32
Marshall County, KY	PZ12-047	13.52 $\pm$ 0.03	4.43	2x	6.76
Cumberland County, TN	PZ12-093	14.96 $\pm$ 0.20	2.7	2x	7.48
A	PZ10-034	15.02 $\pm$ 0.06	1.66	2x	7.51
<i>Phlox</i> <i>glaberrima</i> ssp. <i>glaberrima</i>					
Polk Co. TN	PZ12-131	14.04 $\pm$ 0.11	1.45	2x	7.02
<i>P. glaberrima</i> ssp. <i>interior</i>					
Lewis County, TN	PZ12-055	14.68 $\pm$ 0.08	1.55	2x	7.34
White County, IN	PZ12-063	13.47 $\pm$ 0.42	2.38	2x	6.74
<i>P. glaberrima</i> ssp. <i>triflora</i>					
Blount County, AL	PZ11-057	13.88 $\pm$ 0.52	1.68	2x	6.94
Bullitt County, KY	PZ11-027	13.46 $\pm$ 0.34	2.45	2x	6.73
Adams County, OH	PZ10-193	12.91 $\pm$ 0.18	2.31	2x ^t	6.46
Lyon County, KY	PZ12-046	12.89 $\pm$ 0.04	2.10	2x	6.45
Bath County, VA	PZ11-019	25.68 $\pm$ 0.08	0.99	4x ^t	6.42
Hawkins County, TN	PZ12-135	13.20 $\pm$ 0.01	1.45	2x	6.60
<i>Phlox</i> <i>maculata</i>					
Campbell County, TN	PZ12-103	14.38 $\pm$ 0.56	1.33	2x	7.19
Campbell County, TN	PZ12-105	14.05 $\pm$ 0.12	1.21	2x	7.03
Campbell County, TN	PZ12-107	14.34 $\pm$ 0.12	1.58	2x	7.17
Campbell County, TN	PZ12-108	13.68 $\pm$ 0.21	1.56	2x	6.84
Champaign County, OH	PZ10-198	13.88 $\pm$ 0.08	1.64	2x	6.94
Adams County, OH	PZ10-208	13.94 $\pm$ 0.16	1.93	2x ^t	6.97
Scioto County, OH	PZ13-004	14.40 $\pm$ 0.26	1.96	2x	7.20
McCreary County, KY	PZ11-033	14.55 $\pm$ 0.49	2.91	2x	7.28
<i>Phlox</i> <i>ovata</i>					
Lucas County, OH	PZ11-014	11.71 $\pm$ 0.21	3.51	2x ^t	5.86
Greenbrier County, WV	PZ12-086/089	11.80 $\pm$ 0.28	1.83	2x	5.90

Continued next page

Table 3. Continued.

Taxa ^{z,y}	Accession no.	Relative 2C genome size [mean $\pm$ SD (pg)] ^x	cv (%) ^w	Ploidy ^v	1Cx genome size (pg) ^u
Montgomery County, VA	PZ12-082	12.29 $\pm$ 0.04	1.83	2x	6.15
Roanoke County, VA	PZ12-080	12.09 $\pm$ 0.08	1.73	2x	6.05
Union County, TN	PZ12-112	11.72 $\pm$ 0.20	2.05	2x	5.86
Claiborne County, TN	PZ12-111	12.04 $\pm$ 0.15	1.77	2x ^t	6.02
Hamblen County, TN	PZ12-115	12.29 $\pm$ 0.05	1.85	2x	6.15
<i>Phlox pulchra</i>					
Bibb County, AL	PZ12-129	26.17 $\pm$ 0.03	1.05	4x ^t	6.54
Jefferson County, AL	PZ11-058	14.12 $\pm$ 0.54	1.68	2x ^t	7.06

^zCollections 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.

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

^xHoloploid genome sizes were determined with propidium iodide as the fluorochrome.

^wCoefficient of variation.

^v2x = diploid, 4x = tetraploid.

^u1Cx values were calculated as 2C value/ploidy level.

^tPloidy levels were confirmed by chromosome counts in our laboratory.

et al., 2012; Wright, 2014). Other western species such as *P. aculeata*, *P. caryophylla*, *P. cluteana*, *P. longifolia*, and *P. stansburyi* had a 1Cx value ranging from 3.66 to 5.48 pg (Fehlberg and Ferguson, 2012b). This nearly 2-fold range of 1Cx 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*, 1Cx = 1.32 pg, and *Collomia grandiflora*, 1Cx = 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 1Cx 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; Soltis 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 interpollid crosses 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.

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).

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*. G₁ peak placement and values support data from analyses of the same individual samples with an internal standard.

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-ploid crosses may provide a strategy to develop mildew-resistant

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

Subsection	1Cx		1Cx	
	[mean ± SD (pg)]	Species	[mean ± SD (pg)]	
<i>Paniculatae</i>	7.01 ± 0.36 a ^z	<i>amplifolia</i>	7.10 ± 0.18 a	
		<i>paniculata</i>	7.10 ± 0.15 a	
<i>Phlox</i>	6.85 ± 0.49 b	<i>carolina</i> ^y	7.28 ± 0.30 a	
		<i>glaberrima</i> ^y	6.71 ± 0.40 b	
		<i>maculata</i>	7.11 ± 0.15 a	
		<i>ovata</i>	5.99 ± 0.13 c	
		<i>pulchra</i>	6.84 ± 0.27 b	

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

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

plants. Data from a variety of ornamental and agronomic crops indicate that resistance to this obligate pathogen is controlled by one to several *R*-genes or alleles and that genetic buffering found in some polyploids may be responsible for resistance of different plant organs (Cohen et al., 2003; Comai, 2005; Kessler et al., 2010). This finding is highly speculative, and further testing for modes of mildew resistance is needed for *P. paniculata*, but the results presented here suggest that polyploid breeding may offer an option.

Triploids and pentaploids are rare in *Phlox* (Fehlberg and Ferguson, 2012a; Flory, 1934; Meyer, 1944). Only the hybrid *Phlox* Suffruticosa Group ‘Miss Lingard’, reportedly the product of a cross between *P. carolina* × *P. maculata*, has been shown to be triploid, although the parentage has not been confirmed (Locklear, 2011; Meyer, 1944; 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).

Genome size measurements of *Phlox* × *arendsii* lead us to question the identification of this hybrid in the trade. Cultivars listed as *Phlox* × *arendsii* had genome size and phenology that was the same as other *P. paniculata* cultivars. This hybrid species is considered to have originated from a cross of *P. paniculata* with *P. divaricata* (Arends, 1912; Wherry, 1955). These species differ in genome size, with *P. divaricata* having a 2C size of ≈9 to 10 pg which is 30% lower than *P. paniculata* (Zale, 2014). Controlled hybridization between these two species done at the OPGC shows the hybrids to have a genome intermediate between the parents, and pollen sterility; the hybrids also flowered much earlier than the *P. paniculata* parent (Zale, 2014). Thus, it appears that current cultivars labeled as *P. ×arendsii* may either be variants of *P. paniculata*

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. ×arendsii* 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 midspring (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 Vejsadová, 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 arendsii*. 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-Soriguer, M. Talavera, J. Herrera, and S. Talavera. 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*: Phloxer 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 screes 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.ueb.cas.cz/book/determination-nuclear-genome-size>>.
- Doležel, J., J. Greilhuber, S. Lucretti, 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 biotechnology, 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. woodhousei* (Polemoniaceae) complex. Amer. J. Bot. 99:865–874.
- Fehlberg, S.D. and C.J. Ferguson. 2012b. Intraspecific cytotype 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.

- Flory, W.S., Jr. 1931. Chromosome numbers in *Phlox*. Amer. Nat. 65:473–476.
- Flory, W.S., Jr. 1934. A cytological study on the genus *Phlox*. Cytologia (Tokyo) 6:1–18.
- Fuchs, H. 1994. *Phlox* stauden und polsterphloxe. Eugen Ulmer, Stuttgart, Germany.
- Greilhuber, J. and I.J. Leitch. 2013. Genome size and the phenotype. Plant Genome Diversity 2:320–344.
- Greilhuber, J., E.M. Temsch, and J.C.M. Loureiro. 2007. Nuclear DNA content measurements, p. 67–101. In: J. Doležal, J. Greilhuber, and J. Suda (eds.). Flow cytometry with plant cells: Analysis of genes, chromosomes and genomes. Wiley-Verlag, Weinheim, Germany.
- Gurushidze, M., J. Fuchs, and F.R. Blattner. 2012. The evolution of genome size variation in drumstick alliums (*Allium* subgenus *Melannocrommyum*). Syst. Bot. 37:96–104.
- Hawke, R.G. 1999. Plant evaluation notes: An evaluation report of selected *Phlox* species and hybrids. Chicago Hort. Soc. 13:1–4.
- Hawke, R.G. 2011. A comparative study of *Phlox paniculata* cultivars. Plant Evaluation Notes. Chicago Botanic Garden 35:1–10.
- Hawke, R.G. 2013. Fabulous phlox. 29 Oct. 2013. <<http://greenhousemag.com/gm1113-fabulous-garden-phlox.aspx>>.
- Hogenboom, N.G. and K. Mather. 1975. Incompatibility and incongruity: Two different mechanisms for the non-functioning of intimate partner relationships (and comments). Proc. R. Soc. Lond. 188:361–375.
- Jorosz, A.M., M. Sheets, and M. Levy. 1982. Cuticle thickness in *Phlox* and resistance to powdery mildew: An unreliable line of defense. Amer. J. Bot. 69:824–828.
- Kessler, S.A., H. Shimamoto-Asano, N.F. Keinath, S.E. Wuest, G. Ingram, R. Panstruga, and U. Grossniklaus. 2010. Conserved molecular components for pollen tube reception and fungal infection. Science 330:968–971.
- Leus, L., T. Eeckhaut, E. Dhooghe, M.C. van Labeke, K. van Laere, and J. van Huylenbroeck. 2012. Polyploidy breeding in vitro: Experiences with ornamentals. Acta Hort. 961:235–238.
- Levin, D.A. 1966. The *Phlox pilosa* complex: Crossing and chromosome relationships. Brittonia 18:142–162.
- Lierval, M. 1866. Culture pratique des *Phlox*, par Lierval. E. Dounnaud, Paris, France.
- Locklear, J.H. 2011. *Phlox*: A natural history and gardener's guide. Timber Press, Portland, OR.
- Matiska, P. and H. Vejsadová. 2010. Polyploidy induction in *Phlox paniculata* under in vitro conditions. Acta Univ. Agr. Silv. Mendel. Brun. 58:101–106.
- Meyer, J.R. 1944. Chromosome studies of *Phlox*. Genetics 29:199–216.
- Michaux, A. 1803. Flora boreali-americana, Sistens Characteres Plantarum quas in America septentrionali collegit et detexit. Levrault, Paris, France.
- Parris, J.K., T.G. Ranney, H.T. Knap, and W.V. Baird. 2010. Ploidy levels, genomes sizes, and base pair composition in *Magnolia*. J. Amer. Soc. Hort. Sci. 135:533–547.
- Pridham, A.M.S. 1934. History, culture, and varieties of summer-flowering phloxes. Cornell Univ. Agr. Expt. Sta. Bul. 588.
- Raghuvanshi, S.S. and C.S. Pathak. 1975. Polyploid breeding and possibility of raising double varieties in *Phlox drummondii*. Hook. Cytologia 40:355–363.
- Ranney, T.G. 2006. Polyploidy: From evolution to new plant development. Proc. Intl. Plant Prop. Soc. 56:137–142.
- Šmarda, P. and P. Bureš. 2010. Understanding intraspecific variation in genome size in plants. Preslia 82:41–61.
- Smith, D.M. and D.A. Levin. 1967. Karyotypes of eastern North American *Phlox*. Amer. J. Bot. 54:324–334.
- Soltis, D.E., P.S. Soltis, M.D. Bennett, and I.J. Leitch. 2003. Evolution of genome size in the angiosperms. Amer. J. Bot. 90:1596–1603.
- Symons-Jeune, B.H.B. 1953. *Phlox*: A flower monograph. D. Van Nostrand, New York, NY.
- Taylor, E., R. Cartwright, J. Robbins, G. Klingaman, and J. Lindstrom. 2002. Evaluation of *Phlox paniculata* L. cultivars for susceptibility to powdery mildew. Proc. Southern Nursery Assn. Res. Conf. 47:194–198.
- Tiwari, A.K. and S.K. Mishra. 2012. Effect of colchicine on mitotic polyploidization and morphological characteristics of *Phlox drummondii*. Afr. J. Biotechnol. 11:9336–9342.
- Vyas, P., M.S. Bisht, S. Miyazawa, S. Yano, K. Noguchi, I. Terashima, and S. Funayama-Noguchi. 2007. Effects of polyploidy on photosynthetic properties and anatomy in leaves of *Phlox drummondii*. Funct. Plant Biol. 34:673–682.
- Wherry, E.T. 1932a. The eastern long-styled phloxes, part 1. Bartonica 13:18–37.
- Wherry, E.T. 1932b. The eastern long-styled phloxes, part 2. Bartonica 14:14–26.
- Wherry, E.T. 1933. The eastern veiny-leaved phloxes. Bartonica 15:14–26.
- Wherry, E.T. 1935a. Our native phloxes and their horticultural derivatives. National Hort. Mag. 14:209–231.
- Wherry, E.T. 1935b. A new variety of *Phlox ovata* from the Alabama Mountains. Bartonica 16:37–39.
- Wherry, E.T. 1945. The *Phlox carolina* complex. Bartonica 23:1–9.
- Wherry, E.T. 1955. The genus *Phlox*. Morris Arboretum Monogr., Philadelphia, PA.
- Worcester, L., M.H. Mayfield, and C.J. Ferguson. 2012. Cytotypic variation in *Phlox pilosa* ssp. *pilosa* (Polemoniaceae) at the western edge of its range in the central United States. J. Bot. Res. Inst. Texas 6:443–451.
- Wright, B.A. 2014. Systematic studies in the genus *Phlox* (Polemoniaceae): Cytotypic variation in *Phlox nana* Nutt. And utility of a low copy nuclear gene region (IDBH) for phylogeny development. Master's Thesis, Kansas State Univ., Manhattan, KS.
- Zale, P.J. 2014. Germplasm development, characterization, and enhancement of eastern *Phlox* species. Ph.D. Diss., Ohio State Univ., Columbus, OH.
- Zhang, Z., H. Dai, M. Xiao, and X. Liu. 2008. In vitro induction of tetraploids in *Phlox subulata* L. Euphytica 159:59–65.