Clarifying Taxonomy and Nomenclature of *Fothergilla* (Hamamelidaceae) Cultivars and Hybrids

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Significance to Industry: *Fothergilla* L. spp. (fothergilla or witch-alder) are exceptional garden plants that display showy, white, fragrant flowers in a terminal spike that resembles a bottlebrush. Summer foliage color can be dark green to blue-green with fall foliage ranging from and including multi-colored combinations of yellow, orange, maroon, and scarlet. *Fothergilla* have few pest problems, they tolerate a broad range of climates (USDA hardiness zones 4-9), soil types, and shade. As a result, *Fothergilla* have become valuable nursery and garden plants. However, clear differentiation among *F. gardenii* Murray, *F. major* Lodd., and potential hybrids can be difficult based solely on morphological characteristics. A combination of chromosome counts and DNA contents was used to clearly differentiate among *F. gardenii* (*2n* = 4x = 48), *F. major* (*2n* = 6x = 72), and hybrids (*2n* = 5x = 60). *Fothergilla ×intermedia* Ranney and Fantz (hybrid fothergilla) is proposed as the name for these hybrids. The correct classification and nomenclature for 17 different taxa are presented.

Nature of Work: There are two species of *Fothergilla, F. gardenii* and *F. major* and both are native to the Southeastern United States. *Fothergilla gardenii* is found in wet savannas and pocosins in the Coastal Plain of North Carolina, South Carolina, Georgia, Florida, and Alabama (Weaver, Jr., 1969). This species is generally smaller in stature (3-10 dm) than *F. major,* and is distinguished sometimes by smaller leaves that are generally toothed only on the upper half and symmetric at the base. Cytology determined a chromosome number of *2n* = 4x = 48 (Weaver, Jr., 1969). In contrast, *F. major* is found on upland sites in the piedmont and mountains of North Carolina, South Carolina, Georgia, Alabama, Tennessee, and Arkansas (Weaver, Jr., 1969). This species generally is larger in stature (7-65 dm) than *F. gardenii* and is sometimes distinguished by larger leaves that generally are toothed from below the middle and conspicuously asymmetric at the base. Cytology determined a chromosome number of *2n* = 6x = 72 (Weaver, Jr., 1969). The two species of *Fothergilla* are sometimes confused and attempts to properly identify them based on morphological characteristics is often inconclusive. There has also been speculation that the two species of *Fothergilla* hybridize (Dirr, 1998). Hybrids between these species should have a chromosome number of *2n* = 5x = 60.

Microscopic determination of chromosome numbers is not a practical approach for separating species and hybrids among large numbers of cultivars. However, flow cytometry can provide a fast and accurate determination of nuclear DNA content that is related directly to ploidy level (among closely related taxa) and can be used as a taxonomic tool (Doležel, et al., 1998).

The objectives of this research were to verify the existence of hybrids between *F. gardenii* and *F. major,* and to clarify the proper taxa designations for clones of *Fothergilla* commonly grown in the nursery industry. A comparison of morphological characteristics was made among diverse clones representing both species and potential hybrids from collections of *Fothergilla* at the N.C. State University, Mountain Horticultural Crops Research and Extension Center, Fletcher, NC (NCSU) and Yew Dell Gardens, Crestwood, KY (YDG). Morphological measurements were taken on lamina length, lamina width, leaf margin dentation location (strictly above the middle, to the middle, or extending to below the middle), symmetry of leaf base (symmetrical, variable, or asymmetrical), stipule length, stamen number, and hypanthium depth and width at anthesis. Twelve measurements were taken for each leaf morphology character and six measurements were taken for each flower morphology character for each clone.

Holoploid, 2C DNA contents (i.e., DNA content of the entire non-replicated, chromosome complement irrespective of ploidy level) were determined via flow cytometry (Greilhuber et al., 2005). Nuclei isolation and staining from approximately 12 stamen filaments followed protocols provided by Partec (Partec GmbH, Münster, Germany). The mean fluorescence of each sample was compared with an internal standard of known genome size [*Pisum sativum* L. 'Ctirad', 2C = 9.09 pg; (Doležel et al., 1998)]. A minimum of 4,500 nuclei were analyzed to calculate the ratio of sample peak to the internal standard for determining genome size [2C pg = (mean fluorescence of sample peak/ mean fluorescence of internal standard peak) $\times$ 9.09 pg]. Two to six subsamples were analyzed for each taxa. Chromosome counts were conducted on root tips collected in the morning from newly rooted stem cuttings of *Fothergilla* 'Mt. Airy'.

Results and Discussion: Cytological examination of 14 mitotic cells revealed that *Fothergilla* 'Mt. Airy' was a pentaploid with *2n* = 5x = 60 (Fig. 1), thereby confirming it is a hybrid between the tetraploid *F. gardenii* and hexaploid *F. major*. *Fothergilla* 'Mt. Airy', a confirmed pentaploid, was used as a reference to compare the approximate genome sizes (DNA content) for the different ploidy levels (Table 1). Genome sizes within species and hybrids had a narrow range providing clear distinction between the three taxonomic groups consistent with variations in ploidy levels (Table 1).

Separating hybrids from parental species was particularly challenging when based strictly on morphology. Most ranges for morphological measurements of

hybrids overlapped with one or the other parent (Table 1). One exception was that the lamina width of *F. gardenii* was consistently narrower than either *F. major* or the hybrids. In general, hybrids tended to resemble *F. major* more closely, likely resulting from higher ploidy level and gene dose that was contributed from *F. major.*

It was determined that the majority of cultivars represented in commerce were hybrids. To help clarify the taxonomy and nomenclature of *Fothergilla* spp., nothospecies *Fothergilla ×intermedia* Ranney and Fantz is proposed for the hybrid species name in accordance with Article H.3-5 (Greuter et al., 2000). Based on this study, we further identified the cultivars 'Appalachia', 'Bill's True Dwarf', 'Blue Mist', 'Harold Epstein', and 'Jane Platt' as *F. gardenii.* The cultivars 'Arkansas Beauty' and 'KLMG' Mystic Harbor™ were found to be *F. major*. The cultivars 'Blue Shadow', 'Eastern Form', 'KLMtwo' Beaver Creek®, one unnamed clone (YDG 2005-323-A), 'KLMfifteen' Red Monarch™, 'KLMsixteen' May Bouquet™, 'Mt. Airy', 'Red Licorice', 'Sea Spray', and 'Windy City' were hybrids, *Fothergilla ×intermedia.*

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Table 1. Comparison of characteristics of *Fothergilla gardenii, F. ×intermedia*, and *F. major*.

Characteristic	*F. gardenii*	*F. ×intermedia*	*F. major*
Chromosome no[z]	*2n* = 4x = 48	*2n* = 5x = 60	*2n* = 6x = 72
Genome size (2C)	4.2-4.5 ρg	5.2-5.5 ρg	6.2-6.4 ρg
Lamina length (cm)	3.4-5.4 (8)[y]	5.3-11.1	6.0-11.5
Lamina width (cm)	2.1-4.0	4.3-7.8 (9.5)	6.0-11.0
Leaf dentation location	Mostly toothed above the middle	Toothed above, interm., or below the middle	Toothed from below the middle
Leaf base	Symmetrical or variable	Asymmetrical or variable	Variable
Stipule length	3.9-8.8	3.8-10.9	6.0-10.0
Stamen no.	12-28	14-30	16-27
Hypanthium depth (mm)	0.7-2.3	0.9-2.6	1.0-2.7
Hypanthium width (mm)	1.0-2.2	1.0-3.4	1.4-3.0

[z]Chromosome numbers for *F. gardenii* and *F. major* were determined by Weaver, Jr. (1969).
[y]Numbers in parentheses indicate extreme ranges, but uncommon occurrences.

Figure 1. Photomicrograph of root tip cell of *Fothergilla ×intermedia* 'Mt. Airy' in prophase with 60 somatic chromosomes.