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GREEN/RED AND WHITE ASHES (*FRAXINUS* SECT. *MELIOIDES*) OF EAST-CENTRAL NORTH AMERICA: TAXONOMIC CONCEPTS AND POLYPLOIDY

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ABSTRACT

Taxonomic problems among the green/red and white ashes (*Fraxinus* sect. *Melioides*) of east-central North America are reviewed. Within green/red ashes (*F. pennsylvanica* sensu lato), a distinction is made between largely southern/eastern plants (referable to var. *subintegerrima* and var. *pennsylvanica**) and largely northern/western plants (probably referable to var. *campestris* and var. *austinii**). The latter include the common cultivar "Marshall's Seedless." They tend to have smaller leaves, shorter petiolules, more leaflet serration, larger buds relative to leaf scars, and smaller samaras. Within both groups, relatively pubescent variants (*) tend to have larger samaras and may also be usefully segregated. It has been generally assumed that pumpkin ash (*F. profunda*) is a distinct hexaploid species, but there is only one reported chromosome count, and even with fruits there is uncertainty in distinguishing some collections from *F. pennsylvanica*. White ashes have been interpreted by Nesom in 2010 as a polyploid series (*americana* = 2x, *smallii* = 4x, *biltmoreana* = 6x), based largely on cytological studies during 1947–1983, but there have been few reliable chromosome-counts indicating polyploids and these lack supporting collections. Recent results from flow cytometry with plants referable to *F. smallii* indicate only hexaploids. Relatively reliable reports of tetraploids are more restricted to southern regions than indicated by Nesom. The only obvious difference between *F. smallii* and *F. biltmoreana* is that the latter is more densely pubescent, especially on rachises and young twigs. It is suggested that most plants referable to *F. smallii* should be treated as **F. biltmoreana** var. **subcoriacea**, J.J.N. Campbell, **comb. nov**. Diploid *F. americana* (sensu stricto) remains variable in pubescence and in samara size, which displays a bimodal tendency, but there is no obvious basis yet for recognition of further segregates. This taxonomic scheme is supported by keys, state-distribution maps, principal components analysis of morphometric data from 160 collections, and patterns in frequency distributions of samara sizes.

During recent decades, most ashes (*Fraxinus*) in east-central North America—centered on the Ohio River watershed—have generally been referred to three species: blue ash (*F. quadrangulata*), green or red ash (*F. pennsylvanica*) and white ash (*F. americana*). While it is generally agreed that blue ash is relatively uniform, variants within the other species have been recognized or rejected in somewhat inconsistent fashion by botanists, foresters, and horticulturalists (e.g., Little 1952; Miller 1955; Gleason & Cronquist 1991; Burns & Honkala 1990; Dirr 1997; Ward 2011). Distinction between green/red ash and white ash is considered difficult enough in some cases, and different treatments of segregates within each of these species have tended to confuse the situation further. There has, however, been a resurgence of interest in these problems given Nesom's (2010a-f, 2014) recent review of the genus in North America, which recognized three species within *F. americana* sensu lato: typical *F. americana*, all diploid (2n = 46); *F. smallii*, reportedly tetraploid; and *F. biltmoreana*, reportedly hexaploid. Variation within the green/red ash complex has also been problematic. Morphological distinction of the supposed hexaploid *F. profunda* from diploid *F. pennsylvanica* is sometimes difficult, even with samaras. There is considerable remaining variation within *F. pennsylvanica*, although tetraploids have not been documented. The search for cryptic polyploid species within traditional "species" of vascular plant is a reasonable goal, given that such segregates tend to be reproductively isolated (e.g., Soltis et al. 2008).

The advent of Emerald Ash Borer, now devastating most ashes across northern sections of this region (Herms & McCullough 2014), now makes it urgent to resolve the taxonomic issues in defining ash species, to recognize meaningful intraspecific entities, and to document even local genetic variants. As well as the need for better assessment of native trees, it is important to determine what kinds of ash have been cultivated or planted in restoration projects. There will be an effort by the USDA to breed EAB-resistant ashes for future uses (Koch et al. 2012). The full diversity of native ash germplasm should obviously be sampled as part of this effort, avoiding potential confusion with cultivated material that has been widely distributed (Knight et al. 2010).

Most eastern species of *Fraxinus*—all except *F. nigra* and *F. quadrangulata*—belong in the largely North American sect. *Melioides* (Jeandroz et al. 1997; Wallander 2008; Hinsinger et al. 2013). This section has the following characteristics: plants dioecious, the flowers strictly unisexual, with persistent calyx, female with one pistil, male with two stamens; samaras with length/width usually 5–9, distinctly narrowed to little or no wing at base, the seed cavity usually terete; terminal buds apiculate or acute, often blackish but (at least on more exposed scales) proximally to completely covered with usually reddish-brown glands (sometimes orange, golden or purplish); leaflets 3–9, the terminal one usually at least as large as adjacent pair, the laterals on petiolules 0–15(20+) mm long, serrulate to subentire on flowering shoots, largely glabrous to densely pubescent below but without distinct basal tufts, the hairs stramineous to whitish, short and straight or long and slightly curling; young twigs when dried usually pale olive-greenish to brownish or purplish (especially at nodes), terete; trees up to 10–35 m tall. Plants have diverse flavones but lack coumarins, in marked contrast to sect. *Fraxinus* (Black-Schaefer & Beckmann 1989; Lee et al. 2012; Whitehill et al. 2012).

Sect. *Melioides* appears to contain two major subgroups (Nesom 2014): the Pennsylvanica group or “green / red ashes” and the Americana group or “white ashes.” Hybrids between these groups are apparently rare to absent in the wild (Nesom 2010a-f; Ward 2011). Santamour (1962) reported one possible hybrid out of the 46 trees sampled (“NEG-789” from southern Indiana). Taylor (1972) found only one apparent wild hybrid during three years of study. Miller (1955) suggested that the following taxa originated from hybrids: (1) plants now known as *Fraxinus pauciflora* from *F. caroliniana* and *F. americana*; (2) *F. profunda* (as *F. tomentosa*) from *F. pennsylvanica*; and (3) *F. biltmoreana* (as a variety) from *F. americana* and *F. pennsylvanica*. However, no definitive evidence has emerged to support these three hypotheses (Black-Schaefer & Beckmann 1989; Hardin & Beckmann 1982; Nesom 2010f).

Hybrids between these two subgroups of sect. *Melioides* have been produced artificially (Taylor 1972; J. Koch, pers. comm.) and some cultivars are suggestive of hybrid origin. *Fraxinus americana* “Rosehill” has clear white-waxy reticulation between ultimate veinlets but the veinlets are relatively wide and remain bright green when dried. *Fraxinus pennsylvanica* (or *F. americana*) “Cimarron” (or “Cimmzam”) also has suggested hybrid origin (US Plant Patent 8077 in 1992 by J.W. Zampini). But, again, no definitive evidence of such hybridity in cultivars has been presented.

Within *Fraxinus pennsylvanica* as generally circumscribed, Miller (1955) and Nesom (2010c) did not recognize formal segregates, but Nesom noted the potential for further study to support variants. Britton (1908) had described *F. campestris*, centered in the northern Great Plains, and Peterson (1923) used this name for all green or red ash in Nebraska. Gates (1938) treated this taxon as *F. pennsylvanica* var. *campestris* (Britton) F.C. Gates (or “prairie ash”), and he provided maps of it as well as var. *subintegerrima* and var. *pennsylvanica* in Kansas. Britton (1908) and Small (1933) also distinguished *F. darlingtonii* Britt., based on its longer more linear samaras, smaller leaf blades with entire margins, less general twig pubescence, and more southern range. Fernald (1950) treated less pubescent plants as var. *subintegerrima*, apparently including *F. darlingtonii*, and he added var. *austinii* as a name for more pubescent plants with relatively short and broad samaras, more serrate leaves, and relatively northern range. Gleason (1952) and Farrar (1995) also made useful comments on some of these variants.

Nesom (2010a) has recently improved knowledge of the enigmatic "pumpkin-ash"—*Fraxinus profunda* (= *F. tomentosa* and probably *F. michauxii*). This taxon has been interpreted as a hexaploid derivative from the *pennsylvanica* complex, usually with distinctively larger samaras and often with larger leaves. But variation within this species is poorly understood and is addressed further below.

Within *Fraxinus americana* sensu lato, Nesom's (2010f) recent division into three species (*americana*, *smallii*, *biltmoreana*) does concur somewhat with the treatments of Britton (1908), Sargent (1922), and Small (1933). Sargent's (1919) *F. americana* var. *subcoriacea* appears to be the same taxon as *F. smallii*. However, Fernald (1950) and Gleason (1952) recognized *biltmoreana* only as a more pubescent variety with relatively large samaras, centered in Appalachian to Ozark regions, and *F. smallii* has been generally not been recognized at all after Small (1933). Nesom's rationale was largely based on cytological work of Wright (1944a, 1957), Santamour (1962), Schaefer and Miksche (1977), Leser (1978), Armstrong and Funk (1980), Armstrong (1982), and Blake and Beckman (1983). Yet detailed review of this literature reveals some uncertainty in the supposed association of chromosome number with morphological features, as discussed below. And variation in samara size is considerable, even within each of Nesom's three species. Some authors (e.g., Fernald 1950) have recognized plants with unusually small samaras as *F. americana* var. *microcarpa* Gray (= var. *curtisii* (Vasey) Small), but there has been no quantitative assessment of variation in samara size.

In summary, this study is an investigation of green/red ashes (*Fraxinus pennsylvanica* sensu lato) and white ashes (*F. americana* sensu lato) in east-central North America. The distribution of each potential taxon is outlined. Confusion among some common cultivars and wild plants is addressed, with recognition of "Marshall's Seedless" and similar native plants as a distinct variety of *F. pennsylvanica* that has a relatively northwestern range. Within the *F. americana* complex, a transect of collections across central Kentucky transect is reported, with estimates of ploidy from flow cytometry. A modified version of Nesom's (2010a-f, 2014) treatment is developed, with a brief key (a) plus a more detailed version (b). Authors for names are listed in key (b) and not repeated elsewhere in the text; see Wallander (2008) for authors of other taxa. The more southern *F. caroliniana* complex is left for a subsequent paper.

Supplementary material is posted at bluegrasswoodland.com, including an extended report with details of samara size distributions and their statistics (Campbell 2017). Also posted at that website are selected images of all recognized taxa (Campbell 2015), and a provisional analysis of differences in habitat between ash taxa (Campbell 2011).

METHODS

Herbarium survey

Specimens were examined at several herbaria to improve understanding of the green/red ash and white ash complexes, based on visible characters of stems, leaves, and fruits. References below to these herbaria use standard acronyms (Thiers 2014). Characters in the existing keys of Fernald (1950), Gleason and Cronquist (1991), Nesom (2010a-f), and Weakley (2015) were used initially to review identifications. But more precise keys were developed, with elaboration or rejection of some characters where difficulties were encountered. Many specimens lack mature samaras, limiting their utility in taxonomic descriptions. Identification of such material remains problematic in some cases, despite efforts to refine descriptions of key characters. To explore variation in samara production, the percentages of specimens with samaras were determined for each suggested taxon at each herbarium. Provisional distribution maps were assembled for each suggested taxon, showing presence/absence in each state or Canadian province. Images of selected herbarium specimens and living plants were assembled to illustrate characteristics of each taxon, but presented elsewhere (Campbell 2015).

For insight to covariance among visible morphometric characters in herbaria, an exploratory principal components analysis (PCA) was conducted with data taken from 160 collections at US during 23-26th Nov 2014. It is anticipated that a more extensive and rigorous morphometric analysis will be performed in the future, in order to test for correlations with DNA-based phylogenetic divisions. This initial analysis just establishes general patterns for development of future hypotheses. In *Fraxinus profunda, F.* cf. *smallii,* and *F. biltmoreana,* all collections with well-formed samaras were recorded. In *F. pennsylvanica* variants and *F. americana* (sensu stricto), only one random collection per state was recorded. The 15 scored characters are listed in Table 2. Within each collection, an average leaf and an average samara were subjectively selected for measurement. Quantitative characters were log-transformed, and then all characters were converted to a scale of 0 (minimum value) to 1 (maximum value). Data were analyzed using the PCA routine available online in R code (Revelle 2010; Wessa 2014).

Ploidy estimation

A transect across central Kentucky was driven on 21st Sep 2011 in order to collect fresh samples of the *americana-smallii-biltmoreana* complex for flow cytometry and for herbarium collections at NA. Within each of the seven counties crossed, an effort was made to find and collect each of the three suggested taxa along roadsides, resulting in a total of 24 collections. Leaf tissue for analysis was refrigerated, shipped overnight to the National Arboretum, and then analyzed with flow cytometry 2-3 days after arrival.

Flow cytometry was carried out by Craig Carlson under supervision of R.T. Olsen at the USDA, Beltsville, Maryland, on a Partec PA II flow cytometer using the Partec CyStain UV precise P kit (Partec GmbH, Münster, Germany) according to the manufacturer's instructions. Fresh leaf tissue of *Pisum sativum* 'Citrad', with absolute 2C = 8.75 pg, was used as the internal standard based on its common use as a reference standard (Doležel and Greilhuber 2010). [2C-value = nuclear DNA content of the whole chromosome complement that is characteristic for the organism.] Approximately 0.5 cm^2 of *Fraxinus* tissue was co-chopped with leaf tissue of the internal standard (<0.5 cm^2) using a double-sided razor blade in 400 µL of extraction buffer. Suspensions were filtered through 30-µm nylon mesh filters, and nuclei were stained with 1.6 mL of staining buffer containing 4',6-diamidino-2-phenylindole (DAPI). The nuclear suspension was analyzed on the flow cytometer with fluorescence excitation provided by a mercury arc lamp. The mean fluorescence of each sample was compared to that a known diploid with 46 chromosomes, and to the internal standard for determination of relative ploidy level and holoploid genome size, respectively. At least 3000 nuclei were counted for determining the ratio of sample peak to the internal standard, and thus nuclear DNA content: 2C pg = [sample peak/internal standard peak] × 8.75 pg.

RESULTS

Current taxonomic rationale and new combination for *Fraxinus smallii*

As detailed below, typical pubescent *Fraxinus biltmoreana* is often mixed with more glabrous plants in populations, and there is little obvious ecological difference between pubescent and glabrous plants in Kentucky. Thus *F. smallii* may be regarded merely a less pubescent variant of hexaploid *F. biltmoreana*, with some geographic segregation but no discontinuity. Formal taxonomic recognition of the less pubescent form may have little evolutionary significance, but it is adopted here with the following provisional name, under which to organize observations. This allows some internal consistency between nomenclature for the *F. americana* complex and for *F. pennsylvanica,* which also has pubescent-versus-glabrous varieties. The nomenclature adopted here for varieties of *F. pennsylvanica* is provisional as well, partly since more checking of all potentially relevant types is needed to be sure of their appropriate application.

FRAXINUS BILTMOREANA Beadle var. **SUBCORIACEA** (Sargent) J.J.N. Campbell, **comb. nov.** *Fraxinus americana* var. *subcoriacea* Sargent, Bot. Gaz. 67: 241. 1919. **SYNTYPES**: **USA**. **Massachussetts**. Suffolk Co.: Boston, cultivated at the Arnold Arboretum in Jamaica Plain, 10 Oct 1905, collector not specified (AA 283679!, AA 73800!).

Fraxinus smallii Britton, N. Amer. Trees, 805, f. 735. 1908. **TYPE: USA. Georgia**. Gwinnett Co.: Yellow River near McGuire's Mill, 750 ft, 2 Aug 1895, *J.K. Small s.n.* (holotype: NY!; isotype: GH!).

Notes on characters for identification of taxa

The white waxy "papillose" or "corniculate" reticulation of Couplet 1 below is a diagnostic character of white ashes (Miller 1955; Hardin & Beckman 1982; Nesom 2010f; Williams & Nesom 2010). However, it can be difficult to assess even when viewed with at least ×40 magnification, as recommended here. The character is not generally apparent in seedlings a few years old, as noted by Taylor (1972). It is also less developed in immature leaves or in deep shade, and it may become less apparent after hotter drying. Identification of seedlings and shade leaves as white ashes (*Fraxinus americana* sensu lato) versus green/red ashes (*F. pennsylvanica* sensu lato) may still be possible from examination of lower leaflet surfaces, but more definitive analysis is needed. In white ashes from forest understories, the areolae appear to have a relatively uniform pale green to bluish-white (or 'silvery') waxy covering with fine texture (granules < apparent cell width). The fine veinlets are usually pale green with an exposed width of up to ca. 0.05 mm. In green/red ashes from the understory, lower leaflet surfaces are generally deeper green than the white ashes, and the areolae appear less uniform in color, with small patches (perhaps cells) of yellow-green to orange-brown (or 'golden') and white (perhaps waxy cells). Fine veinlets of green/red ashes appear up to ca. 0.1 mm wide and are usually deeper yellowish- or brownish-green. Relatively large, distinct, orange-brownish "peltate glands" or "scales" often appear scattered to frequent in the green/red ashes, usually near the centers of areolae. Hardin and Beckmann (1982) reported no clear differences in frequency of such "scales" between white and green/red ashes, but in the white ashes these structures tend to be obscured by the more uniform waxy surface. Stomata can also appear relatively dense or clustered in green/red ash (Taylor 1972).

Stomata, leaflet shape, petiolules, buds, twigs and wood anatomy all need more study in shade versus sun, on mature branches versus or juvenile leaves, and on slow versus fast-growing shoots, in order to determine how taxonomically useful their variation might be. White ashes usually have leaf bases 'truncate to rounded' with angles of 45–90°; green/red ashes usually have 'cuneate to acute' bases with angles of 30–45°, but often up to 60° in *Fraxinus profunda*. Petiolule length has some value, but there is overlap; Steyermark (1963) used "3–20 mm" in *F. americana* (sensu lato), versus "1–5(9) mm" in *F. pennsylvanica*. Other characters were suggested by Taylor (1972) but based only on typical *F. americana* versus typical *F. pennsylvanica* in Michigan. She noted as follows: that *F. americana* has bud scars more angled out from the twig surface (ca. 20–30^{o} versus 10–20^{o}) and strictly opposite (versus often subopposite); that twig surfaces in second year are usually greenish to purplish (versus grayish), with waxy exfoliation (versus not so); that bark remains smooth for many years and strictly gray to blackish (versus soon becoming corky in later years and often olive- to reddish-brown); and that styles are red to purple (versus greenish). She reported that bud-scar and twig characters are most distinctive on shoots growing at ca. 3–6 cm per year.

Within the green/red ash complex, there are some statistical differences in mean leaf and leaflet dimensions between taxa, based on collections at US, and these are used in the keys below. In particular, mean terminal leaflet width (± standard error) in *Fraxinus pennsylvanica* var. *subintegerrima* is 3.96 ±0.16 cm (n = 20); it is 4.50 ±0.21 cm (n = 16) in var. *pennsylvanica* (P = 0.046 with T test). However, there is no significant difference in petiolule length. *F. profunda* has generally larger leaf dimensions, including a strong difference in petiolule length: 5.94 ±0.64 mm (n

= 18) versus 3.18 ±0.27 mm in south/eastern variants of *F. pennsylvanica* (n = 36); P <0.0001 with T test. But there is again some overlapping variation within both of these species.

Pubescence of lower leaf surfaces varies too much across ranges of whole species to be a reliable character in most taxonomic distinctions. But some useful observations can be derived from local populations. Within Kentucky, *Fraxinus americana* (sensu stricto) is usually glabrous except on larger veins and it rarely if ever has dense hairs across the surface, while *F. biltmoreana* (including *F. smallii*) usually has widespread hairs that are often dense across the surface. Within *F. americana* (sensu stricto) pubescence appears generally denser in states to the west and south of Kentucky, as also indicated by Wright (1944a). However, within *smallii*-like plants pubescence appears generally less dense in states to the west and perhaps south of Kentucky.

Although samara size and shape are useful characters in several parts of the key, it is important not to overstate their value, given the initial analysis of variation in overall size presented elsewhere (Campbell 2017). Variation in anther size and shape remains largely unexplored; Fernald (1950) made a few notes on anthers but no general survey of taxa has been reported.

(a) Brief version of the key (for inital use to estimate identifications)

1. Lower leaflet surface not whitish waxy-papillose-reticulate or bluish-silvery [viewed at ×40]; samara wings decurrent to 20–100% below apex of bodies, these with L/W [length/width] ca. 5.5–12

  2. Leaflets mostly 7.5–11× 2.5–5 cm with petiolules 1–5 mm; samaras mostly 25–50 × 3–8 mm, the bodies 12–22 × 1–2.5 mm, yellowish to brown

    3. Leaves on fertile shoots averaging 15–20 cm long; lateral leaflets with petiolules mostly 1.5–3 mm, distinctly serrate; lateral buds mostly 50–150% as wide as leaf scar; samaras with L/W mostly 5.5–8

      4. Leaves and fresh stems largely glabrous; samaras mostly 25–40 × 3.5–6 mm .... **Fraxinus pennsylvanica** var. **campestris**
      4. Leaves and fresh stems densely pubescent; samaras mostly 30–45 × 4–8 mm .... **Fraxinus pennsylvanica** var. **austinii**

    3. Leaves on fertile shoots averaging 20–25 cm long; lateral leaflets with petiolules mostly 3–5 mm long, entire to weakly serrate; lateral buds mostly 30–90% as wide as leaf scars; samaras with L/W mostly 7–11

      5. Fresh twigs, rachises and lower leaflets surfaces largely glabrous; leaflets often weakly serrate .... **Fraxinus pennsylvanica** var. **subintegerrima**
      5. Fresh twigs, rachises and lower leaflets surfaces densely pubescent; leaflets usually subentire .... **Fraxinus pennsylvanica** var. **pennsylvanica**

  2. Leaflets mostly 9–15 × 3.5–7 cm with petiolules 4–14 mm; samaras mostly 42–65 × 6–11 mm, the bodies 18–30 × 2.5–4.5 mm, often reddish .... **Fraxinus profunda**

1. Lower leaflet surface whitish waxy-papillose-reticulate or bluish-silvery, at least between veinlets [less clear in juvenile or shade leaves]; samara wings decurrent to 10–30% below apex of bodies, these with L/W ca. 4–5.5

  6. Petiole bases deeply notched around buds, the margins sharp; rachises and fresh twigs usually glabrous; samaras mostly 25–37.5 × 3–5.5 mm, the bodies 6–11 × 1.5–2.5 mm [some northern populations have samaras 35–45 mm long] .... **Fraxinus americana** (sensu stricto)

  6. Petiole bases unnotched to slightly notched around buds, the margins blunt; rachises and fresh twigs densely pubescent to almost glabrous; samaras mostly 30–50 × 5–7 mm, the bodies 10–15 × 2–4 mm

7. Rachises and fresh twigs largely glabrous; leaflets subentire to weakly serrulate
.... **Fraxinus biltmoreana** var. **subcoriacea**

7. Rachises and fresh twigs usually covered with dense minute hairs; leaflets subentire
.... **Fraxinus biltmoreana** var. **biltmoreana**

(b) More detailed version of the key (for deeper examination and checking of identifications)

1. Lower leaflet surfaces green, without whitish minutely waxy-papillose reticulation or uniformly fine-textured bluish-silvery appearance [viewed at ×40], with complex texture [mosaic of cells and glands] and diverse colors from whitish to green to yellowish-brown [especially glands and veinlets], the ultimate veinlets usually prominent; upper surfaces darker than lower but both usually deep green to [especially in herbaria] dull orange-brownish or reddish green; leaves turning yellowish or orange-brown in fall, their hairs often slightly yellowish to reddish; leaflets often subsessile or with winged petiolules only 1–5 mm long [except in *profunda*], their shape and serration varied; bud scars with little [0–20%] or no notch; terminal buds brown to reddish-brown, usually acute and longer than wide, often narrower than twig when viewed on edge; twigs without flaking waxy surface; wing of samaras decurrent to ca. 20–100% below apex of body and gradually narrowed, usually acute at apex, the mature bodies [enclosing seeds] with L/W ca. 5.5–12, often distinctly ridged
.... **Pennsylvanica group**

2. Leaflets mostly 7.5–11× 2.5–5 cm [L × W 30–70 cm^2], usually cuneate, the petiolules (0)1–5(9) mm [distal pair]; pubescence largely absent or widespread on fresh twigs and leaves; female flowers with calyx 0.5–2(2.5) mm long; anthers with apiculate tip 0.2–0.4 mm long; samaras 25–50(60) × 3–8(11) mm, the wing decurrent to 20–50% below apex of body, the apex usually rounded to slightly emarginate [with notch rarely 1 mm deep], the mature bodies usually 12–22 × 1–2.5 mm, not plump [with distinctive ridges and channels], pale yellowish-brown to dark brown but rarely reddish

3. Leaves on fertile shoots usually averaging 15–20 cm long; lateral leaflets with petiolules (0.5)1.5–3(4) mm [rachis to inflexion], numbering 4–6(8), distinctly serrate [teeth mostly 0.4–0.8 mm deep], often with concentration of denser or longer hairs at base; terminal leaflet blades mostly 6–12 cm long, about as large as adjacent laterals; well-developed lateral buds mostly 50–150% as wide as leaf scar; samaras mostly 25–45 × 3.5–8 mm wide, with L/W 5.5–8, often oblanceolate to spathulate

4. Leaves and fresh stems largely glabrous except along midrib on lower leaf surface; samaras mostly 25–40 × 3.5–6 mm [or longer to east], L × W mostly 110–220 mm^2
.... **F. pennsylvanica** Marsh. var. **campestris** (Britt.) F.C. Gates

4. Leaves and fresh stems densely pubescent; samaras mostly 30–45 × 4–8 mm [or sometimes longer to east], L × W mostly 150–330 mm^2
.... **F. pennsylvanica** Marsh. var. **austinii** Fern.

3. Leaves on fertile shoots usually averaging 20–25 cm long; lateral leaflets with petiolules (2)3–5(9) mm long, numbering 6–8, entire to weakly serrate [teeth mostly 0–0.4 mm deep], without distinct concentration of hairs at base; terminal leaflet blades mostly 8–15 cm long, distinctly larger than adjacent laterals; well-developed lateral buds mostly 30–90% as wide as leaf scars; samaras mostly 30–50 × 4–6 mm, with L/W 7–12, strictly linear-lanceolate

5. Fresh twigs, rachises and leaflets largely glabrous, except along midrib on lower leaflet surfaces; leaflets mostly 3.5–4.5 cm wide, often weakly serrate [especially those of vigorous sprouts]; samaras mostly 4–5 mm wide
.... **F. pennsylvanica** Marsh. var. **subintegerrima** (Vahl) Fern.
[= *F. lanceolata* Borkh., *F. viridis* Bosc]

5. Fresh twigs, rachises and lower leaflets surfaces densely pubescent; leaflets mostly 4–5 cm wide, usually subentire; samaras mostly 5–6 mm wide .... **F. pennsylvanica** Marsh var. **pennsylvanica**

2. Leaflets mostly 9–15 × 3.5–7 cm [L × W 40–90 cm^2], usually truncate to rounded at base, the petiolules (3)4–14(20) mm [distal pair]; pubescence usually dense on fresh twigs, petioles and rachises [but sparse to absent in some plants], often also on lower leaflet surfaces; female flowers with calyx (1)2.5–5(7) mm long; anthers with slender terminal cusp 0.5–1 mm long; samaras (32)42–65(74) × (5.5)6–11(12) mm, wing decurrent to 50–100% below apex of body, apex usually emarginate [with notch often about 1 mm deep], the mature bodies usually 18–30 × 2.5–4.5 mm, plump [with less distinctive ridges and channels], pale to dark reddish-brown .... **F. profunda** (Bush) Bush [= *F. michauxii* Britt., *F. tomentosa* Michx.]

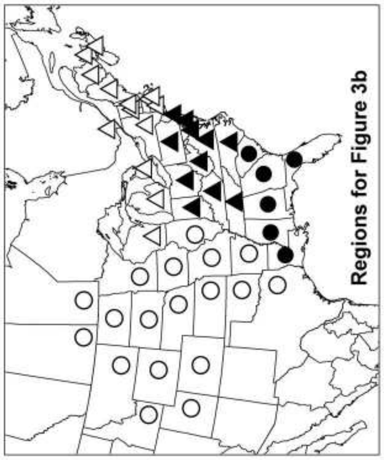
1. Lower leaflet surfaces whitish to pale green, with dense minute [1–10 microns wide] waxy papillae and connecting ridges [especially mature leaves in sun] or [juvenile or shaded leaves] uniformly fine-textured bluish-silvery appearance [with pale yellowish-brown glands somewhat obscured by wax], at least between veinlets, the ultimate veinlets usually somewhat obscured; upper surfaces usually plain green to [especially in herbaria] olive or bluish; leaves turning golden yellow, pinkish, reddish or purplish in fall, their hairs usually whitish; leaflets truncate to rounded at base [versus gradually cuneate], with distinct largely unwinged petiolules 3–13 mm long, abruptly acuminate at apex, entire to crenulate; bud scars sometimes with deep notch [>20%]; terminal buds dark reddish-brown to blackish, mostly obtuse-deltoid [but apiculate], about as wide as twig; twigs often with flaking waxy surface [especially 2nd year]; wing of samara decurrent to ca. 10–30% below apex of body and often abruptly narrowed, usually blunt to emarginate at apex, the mature bodies [enclosing seeds] with L/W ca. 4–5.5, indistinctly ridged .... **Americana group**

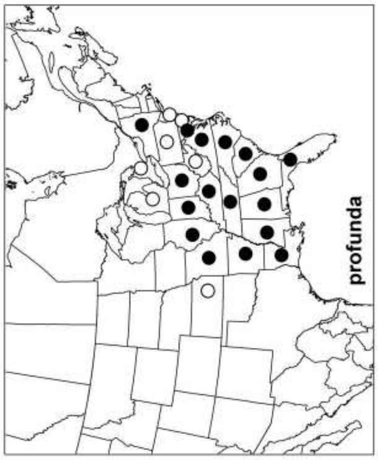
6. Petiole bases deeply notched (mostly 30–50%) around buds, the margins often sharp; rachises and fresh twigs usually glabrous; lower leaflet surfaces usually with hairs restricted to midrib and proximal parts of primary veins, sometimes more widespread to dense; upper leaflet surfaces usually plain yellowish-green; mature samaras mostly 25–38 × 3–5.5 mm [or 38–45 × 5.5–7 mm in some more northern populations], the bodies mostly 6–11 × 1.5–2.5 mm .... **F. americana** L. (sensu stricto)

6. Petiole bases slightly notched [mostly 0–20%] around buds, the margins usually blunt; rachises and fresh twigs densely pubescent to almost glabrous, lower leaflet surfaces usually with hairs dense along veins, often widespread over lower leaflet surfaces, sometimes thin to glabrous; upper leaflet surfaces usually somewhat bluish-green; mature samaras mostly 30–50 × 5–7 mm, the bodies mostly 10–15 × 2–4 mm.

7. Rachises and fresh twigs largely glabrous, sometimes irregularly or thinly hairy especially in distal parts of rachis; leaflets subentire to crenulate or weakly serrulate [as in *F. americana*] .... **F. biltmoreana** Beadle var. **subcoriacea** (Sarg.) J.J.N. Campb. [= *F. smallii* Britt.]

7. Rachises and fresh twigs usually covered with dense minute hairs, sometimes moderately dense to sparse but still uniform; leaflets usually subentire .... **F. biltmoreana** Beadle var. **biltmoreana** [= *F. americana* L. var. *biltmoreana* (Beadle) J. Wright ex Fern.]


Regions for Figure 3b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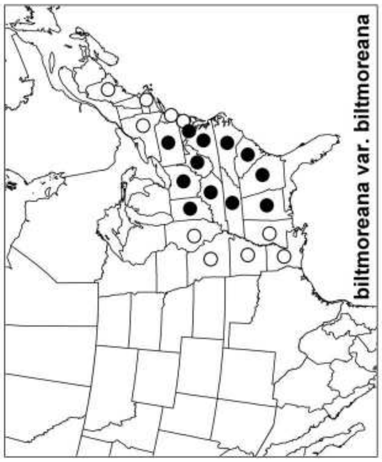
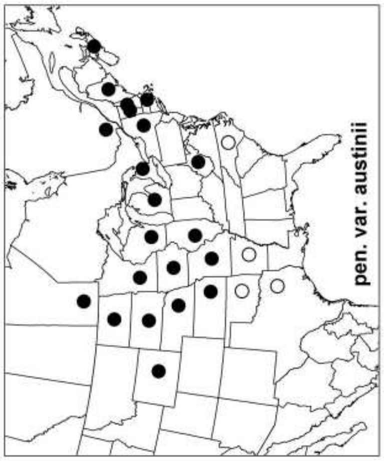
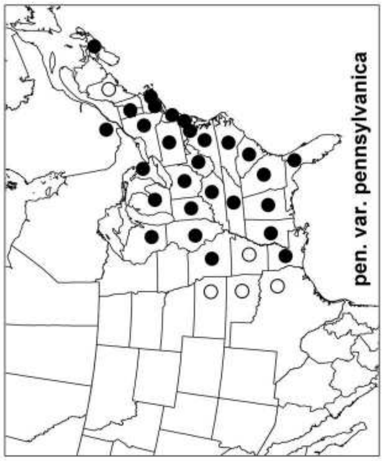
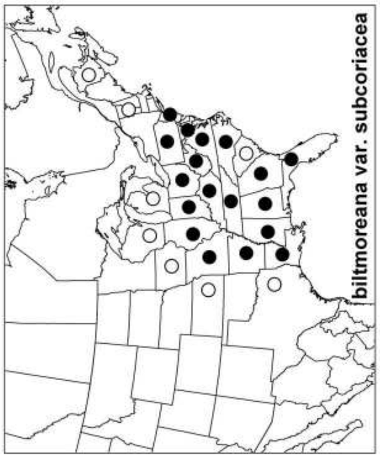
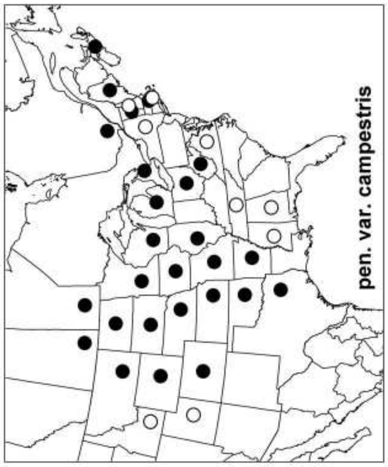

biltmoreana var. biltmoreana


pen. var. austinii


pen. var. pennsylvanica


biltmoreana var. subcoriacea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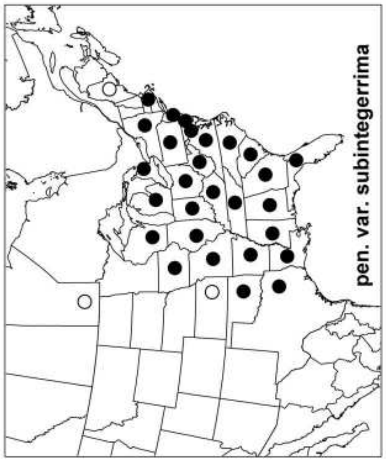
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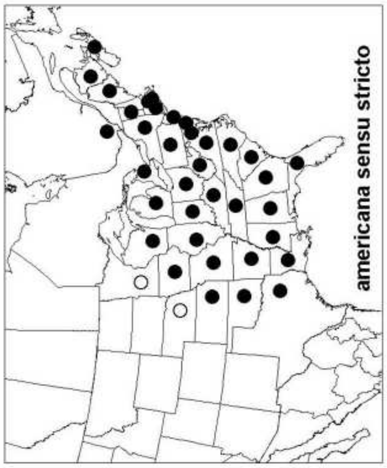
americana sensu stricto

Figure 1 [previous page]. Distribution maps of *Fraxinus* taxa that are the focus of this study. These are provisional maps based largely on collections seen at APSC, AUA, FLAS, GA, MISS, MU, MUHW, NA, NY, TENN, US, VPI and WVU, plus records compiled by Nesom (2010a, c, f). Solid dots indicate that the taxon is reliably recorded with many typical collections. Open dots indicate that the taxon is uncommon (generally less than 10 counties), or that plants are not generally typical (perhaps intergrading with another taxon in some cases). Maps for *F. pennsylvanica* are divided into the four provisional variants (see key): var. *campestris* (informally "NW smooth"); var. *austinii* ("NW hairy"); var. *subintegerrima* ("SE smooth") and var. *pennsylvanica* ("SE hairy").

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Distribution maps

Figure 1 presents maps of each suggested taxon's native occurrence in states and Canadian provinces. In the green ash complex, the two northwestern variants have ranges that are distinct from the two southeastern variants, extending further into the Great Plains and largely absent from southeastern states. See Discussion for notes on new records of *F. profunda*. In the white ash complex, there are only a few minor additions to Nesom (2010f). There are *smallii*-like or *biltmoreana*-like collections as far north as Maine and New Brunswick, but these might just be unusual collections of *Fraxinus americana* without a distinct notch in the petiole base and bud scar.

Polyploidy

Table 1 lists the 24 collections from central Kentucky for estimation of ploidy using flow cytometry. Estimations were all diploid (2n = 46)—for the 14 collections determined as *Fraxinus americana*—or hexaploid (2n = 138)—for the 10 collections determined as *F. smallii* (5) or *F. biltmoreana* (5). Three collections of *F. americana* were initially misidentified as *F. smallii*, generally due to more obscure notching of petiole bases on vigorous shoots. No fruiting material was located during the survey. The proportion of *F. americana* collections tended to increase from south to north (M to K to B under "region"); P = 0.06 with chi-square test in 2 × 3 contingency table. The lack of tetraploids in central Kentucky is consistent with sampling by R. Olsen and A. Whittemore (pers. comm.) in mid-Atlantic states. [A much more extensive survey using flow cytometry is currently underway, and there will be efforts to correlate stomatal sizes with these results, as well as direct chromosome counts.]

Principal Components Analysis [PCA]

Despite the rudimentary nature of morphometric data used here, with only 15 characters (interdependent in some cases) and only 160 thinly sampled collections, there is a surprising degree of separation in the analysis between the provisional taxa (Figure 2). The first and second axes are sufficient to display all readily interpretable patterns in the data, accounting for 27% and 23% of the total sum-of-squares variance; in a trial with six axes the sequential percentages were 23, 20, 13, 10, 10 and 9. The first axis emphasizes dimensions of leaf size and, to a lesser extent, samara size; characters with opposing trends are leaf serration and narrow leaf shape (Table 2a). The second axis emphasizes samara size and pubescence, together with notched petioles and waxy-papillose leaf

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Table 1 [next page]. Determinations of ploidy with flow cytometry from collections of white ashes from central Kentucky. See text for key to identifications. Collections marked with asterisks (*) were initially identified as *F. smallii* (= *F. biltmoreana* var. *subcoriacea*) but closer examination, after results of flow cytometry, confirmed that they are better placed in *F. americana*. Collections that were from unusually vigorous sprouts are indicated under "sprout"; these were mostly along roads with occasional cutting. Under region: M = Mississippian Plateaus; K = Knobs and transitions; B = Bluegrass.

Table 1 [caption on previous page].

Identification	Ploidy	Number	Sprout	County	Region	Latitude	Longitude
americana	diploid	1b-i		Metcalfe	M	37.09239	-85.69832
americana	diploid	2c-i		Green	M	37.22714	-85.51380
americana	diploid	3a-i		Taylor	K	37.36717	-85.30770
americana	diploid	3b-i		Taylor	K	37.43954	-85.28601
_americana_*	diploid	3b-ii		Taylor	K	37.44007	-85.28705
americana	diploid	4a-i		Marion	K	37.44836	-85.29196
americana	diploid	4c-i		Marion	K	37.57442	-85.15495
americana	diploid	5b-i	+	Boyle	B	37.68918	-84.92180
_americana_*	diploid	6a-i	+	Mercer	B	37.70434	-84.89778
americana	diploid	6a-ii		Mercer	B	37.70429	-84.89856
americana	diploid	6b-i		Mercer	B	37.81660	-84.73783
americana	diploid	7a-i	+	Jessamine	B	37.86182	-84.70140
_americana_*	diploid	7b-ii		Jessamine	B	37.86760	-84.69138
americana	diploid	7b-iii		Jessamine	B	37.86731	-84.69108
smallii	hexaploid	1a-i		Metcalfe	M	37.09832	-85.70263
smallii	hexaploid	2a-i	+	Green	M	37.17769	-85.52903
smallii	hexaploid	2b-ii	+	Green	M	37.19907	-85.54435
smallii	hexaploid	4b-i		Marion	K	37.47062	-85.29901
smallii	hexaploid	5a-ii	+	Boyle	B	37.63821	-84.95070
biltmoreana	hexaploid	1a-ii		Metcalfe	M	37.09838	-85.70234
biltmoreana	hexaploid	2a-ii	+	Green	M	37.17739	-85.52907
biltmoreana	hexaploid	2b-i	+	Green	M	37.19743	-85.54287
biltmoreana	hexaploid	5a-i	+	Boyle	B	37.63811	-84.95082
biltmoreana	hexaploid	7b-i		Jessamine	B	37.86761	-84.69174

surface at the other extreme. Figure 2 reverses the algorithm-generated order for the second axis in order to align the diagram with ecological concepts developed elsewhere (Campbell 2011). It also compresses the scale of the second axis relative to the first, in order to provide a more convenient visual display on the page; no information is lost.

The first axis clearly segregates *Fraxinus profunda* (to right) from *F. pennsylvanica* (to left), as well as collections of *F. americana* and *F. biltmoreana* with larger leaves and fruits (to right). The second axis emphasizes segregation of *F. americana* and *F. biltmoreana* (above) from *F. pennsylvanica* and *F. profunda* (below). However, variants within *F. pennsylvanica* have little or no segregation; a more focused analysis restricted to this species is presented below. Moreover, there is only weak segregation of more pubescent plants within *F. biltmoreana* from the relatively glabrous plants here named var. *subcoriacea*. The latter are concentrated in a zone along the second axis between (above) more pubescent plants (*F. biltmoreana* sensu stricto), with much overlap, and (below) *F. americana,* with almost no overlap.

The separation of Pennsylvanica and Americana groups is imperfect, and exceptional collections deserve closer inspection. In the upper right section of Figure 2, there is an outlying collection of *Fraxinus pennsylvanica* (as var. *pennsylvanica*) surrounded by *F. americana*: *Norton 323a* from Kansas. With current taxonomic concepts, its identification is appropriate but the collection has unusually large leaves with long petiolules. Moreover, a few other collections of *pennsylvanica* have relatively large leaves and long petiolules, leading to positions in the upper right half of the diagram (above the dashed line); these are from Kansas, Tennessee, Alabama and perhaps Mississippi (*Bryson 7447* at MISS but without samaras). Further sampling and deeper analysis is needed to determine if such collections represent a distinct segregate or perhaps originate from hybridization. In the lower left half of the diagram dominated by *F. pennsylvanica,* there are several collections of *F. biltmoreana* (including var. *subcoriacea*). These have relatively small leaves, narrow leaflets or short petiolules compared to most *F. biltmoreana.* However, they all have distinctive white waxy papillose lower leaf surfaces and there is no reason to doubt identifications. The third axis provided no further useful separation of Pennsylvanica and Americana groups.

Within *Fraxinus americana* (sensu stricto), there is a wide spread of collections from left to right in the upper half of Figure 2, and some indication of two distinct clusters (upper central and upper right). Further sampling would allow a more definitive analysis, but this clustering does appear to be driven largely by the frequency distribution of samara sizes, which displays a degree of bimodality (Campbell 2017). The outlying collection at upper left is *Small s.n.* (8 Aug 1892) from Virginia which has extremely small samaras ca. 14 × 2.5 mm; such plants have been named var. *microcarpa* Gray. In contrast, the outlying collection at upper right (*Smith et al. 3895* from Kentucky) has unusually large terminal leaflets (10–15 cm long), not larger samaras.

Within the Pennsylvanica group, there is general separation of *Fraxinus profunda* from *F. pennsylvanica* but some outliers deserve comment. Two collections with uncertain identification, lying between typical collections of these two taxa, have been reexamined and assigned to *F. profunda* with more confidence but they appear transitional to *F. pennsylvanica*. Both of these collections are from the northeastern range-margins of *F. profunda*: *Rose et al. 8469* from Pennsylvania (with unusually sparse pubescence); and *Long 6056* from New Jersey (with unusually short samaras). Another transitional collection (*Ruth 459* from Tennessee) is retained within *F. pennsylvanica* due to its short petiolules and largely glabrous leaves, but its fruits are exceptionally large and somewhat *profunda*-like (50 × 5.5 mm). A more convincing collection from east Tennessee (*Kearney 832*) is provisionally identified as *F. profunda*, although it has somewhat serrate leaflet margins. Thus, even when fruits are present distinction of *F. profunda* can be somewhat subjective in such cases, especially if collections are poor.

In the analysis restricted to *F. pennsylvanica*, the first axis generally separates the southeastern variants from northwestern variants (Figure 3a). However, there is still a zone of overlap at the center, along about 20% of the whole sampled axis, especially among more pubescent plants (lower in the diagram). Outliers at the upper and lower right have already been noted in the analysis of all combined collections above (*Norton 323a* and *Long 6056*, respectively). As in the combined analysis, the second axis tends to segregate plants with larger samaras and more pubescence (Table 3). The provisional taxonomic separation of more pubescent plants is partly supported with this axis. But there is a broad zone of overlap concentrated along about 40% of the whole sampled axis, and a few collections are greatly misplaced, including some of the anomalous collections noted above.

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Table 2a. Morphometric characters used in the analysis. *Note that WHI is the only character with diagnostic value by itself for distinguishing the Americana versus Pennsylvanica groups.

CHARACTERS	**DESCRIPTION**
EMA	**Samara emarginate: 0.5 = slight notch (< 1 mm); 1 = clear notch (ca. 1 mm)**
FRP	**Samara length × width**
FRR	**Samara length / width**
FRW	**Samara width in mm**
FRL	**Samara length in mm**
WHI*	**Whitish waxy papillose reticulum on lower leaf surface: 0 = absent; 1 = present**
SER	**Leaflet serration: 0 = < 0.25 mm deep; 0.5 = up to 0.5 mm; 1 = up to 0.75+ mm**
LLP	**Terminal leaflet blade length × width**
LLR	**Terminal leaflet blade length / width**
LLW	**Terminal leaflet blade width in cm**
LLL	**Terminal leaflet blade length (down to proximal inflexion of blade) in cm**
LEF	**Total leaf length in cm**
PLL	**Petiolule length of distal lateral leaflets (up to proximal inflexion of blade) in mm**
PET	**Petiole base distinctly notched around most of bud: 0 = absent; 1 = present**
PUB	**Pubescence dense on rachis and stem: 0 = absent or sparse; 1 = present**

Table 2b. Loadings and weightings of characters in the PCA for all collections. Loadings are correlations of scores with the axis; weightings are coefficients in the linear relationship.

Variables	**First Axis Loading**	**First Axis Weighting**	**Variables**	**Second Axis Loading**	**Second Axis Weighting**
LLP	**0.913**	**0.226**	**PET**	**− 0.695**	**− 0.225**
LLW	**0.881**	**0.228**	**WHI**	**− 0.574**	**− 0.202**
LLL	**0.769**	**0.179**	**SER**	**− 0.144**	**− 0.024**
LEF	**0.748**	**0.185**	**PLL**	**− 0.125**	**− 0.087**
PLL	**0.735**	**0.197**	**FRR**	**− 0.040**	**− 0.012**
FRP	**0.399**	**0.049**	**LLW**	**− 0.022**	**− 0.065**
WHI	**0.397**	**0.140**	**LEF**	**0.109**	**− 0.016**
FRL	**0.368**	**0.045**	**LLP**	**0.132**	**− 0.020**
FRW	**0.347**	**0.042**	**LLL**	**0.278**	**0.034**
EMA	**0.242**	**0.031**	**LLR**	**0.339**	**0.120**
PET	**0.187**	**0.094**	**EMA**	**0.478**	**0.130**
PUB	**0.019**	**− 0.037**	**PUB**	**0.640**	**0.195**
FRR	**− 0.008**	**0.001**	**FRW**	**0.719**	**0.197**
LLR	**− 0.242**	**− 0.085**	**FRL**	**0.747**	**0.204**
SER	**− 0.302**	**− 0.068**	**FRP**	**0.819**	**0.224**

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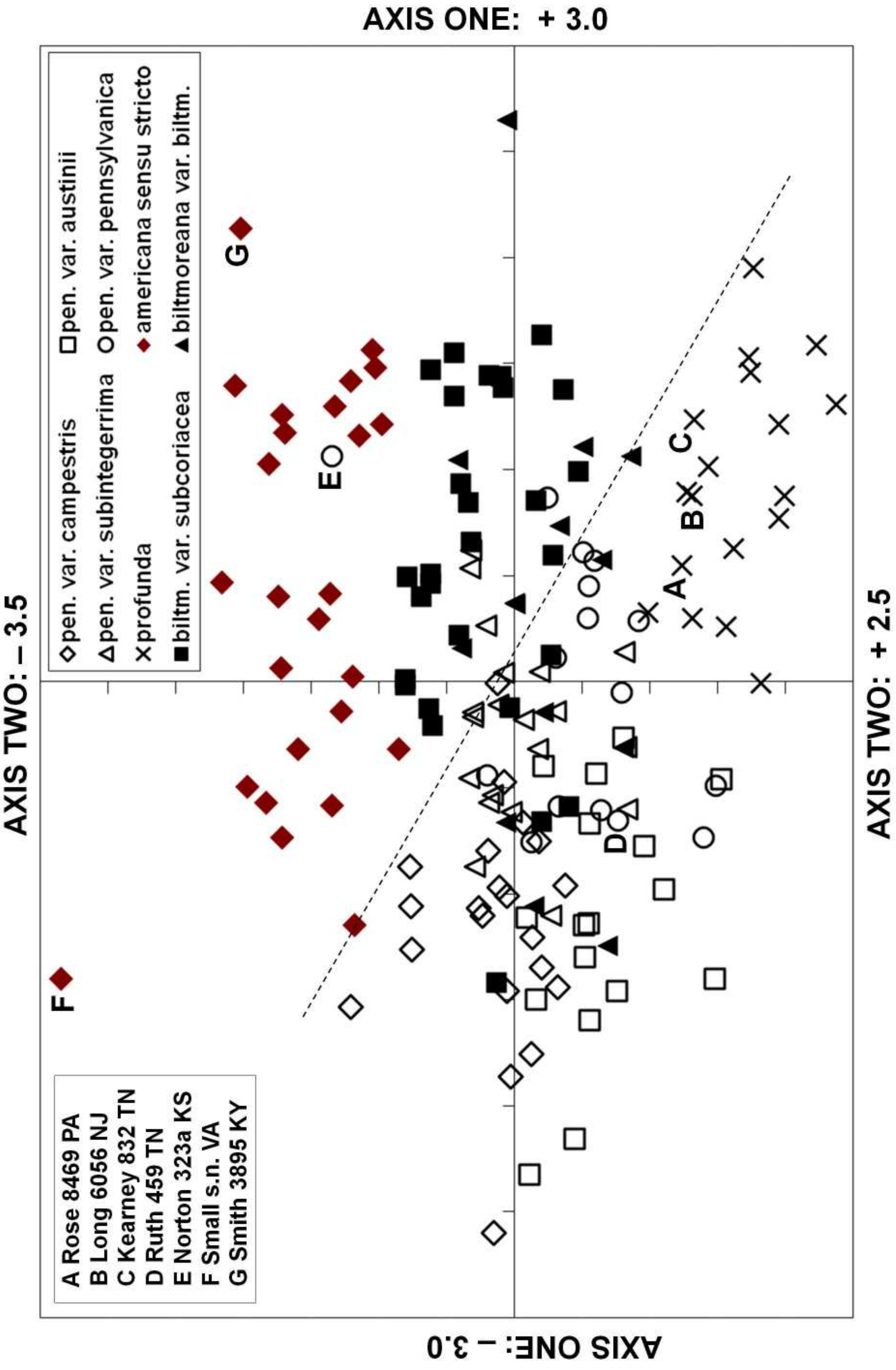
Figure 2 [next page]. Principal components analysis of morphometric data from 160 collections at US. See text for outline of taxa. See Tables 1 and 2 for list of characters, loadings and weights in the analysis. First axis is horizontal; second is vertical. Tick marks are units of 0.5 in the analysis. Dashed line maximizes separation of white ashes (solid) versus greens (open/crosses): 5 greens are mixed with whites, 12 whites with greens. Listed-and-marked collections are noted in the text.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□pen. var. austinii
△pen. var. subintegerrima
○pen. var. pennsylvanica
×profunda
◆americana sensu stricto
■biltm. var. subcoriacea
▲biltmoreana var. biltm.
A Rose 8469 PA
B Long 6056 NJ
C Kearney 832 TN
D Ruth 459 TN
E Norton 323a KS
F Small s.n. VA
G Smith 3895 KY
A
B
C
D
E
F
G

Table 3. Ranked loadings of characters (plus weightings) in the PCA for *F. pennsylvanica*. Loadings are correlations of scores with the axis; weightings are coefficients in the linear relationship. PET and WHI are excluded since those characters are uniform in this species.

Variables	First Axis Loading	First Axis Weighting	Variables	Second Axis Loading	Second Axis Weighting
LLP	**0.920**	**0.195**	**FRR**	**− 0.392**	**− 0.178**
LLL	**0.856**	**0.182**	**PLL**	**− 0.162**	**− 0.078**
LLW	**0.774**	**0.164**	**LLW**	**0.107**	**0.037**
LEF	**0.730**	**0.156**	**SER**	**0.141**	**0.066**
PLL	**0.619**	**0.128**	**LLR**	**0.171**	**0.073**
FRR	**0.544**	**0.110**	**LLP**	**0.202**	**0.076**
FRL	**0.398**	**0.089**	**LEF**	**0.248**	**0.099**
LLR	**0.122**	**0.027**	**LLL**	**0.254**	**0.100**
PUB	**0.006**	**0.006**	**PUB**	**0.432**	**0.188**
FRP	**0.050**	**0.020**	**EMA**	**0.472**	**0.206**
EMA	**0.001**	**0.005**	**FRL**	**0.531**	**0.226**
FRW	**− 0.270**	**− 0.047**	**FRW**	**0.908**	**0.399**
SER	**− 0.374**	**− 0.077**	**FRP**	**0.921**	**0.401**

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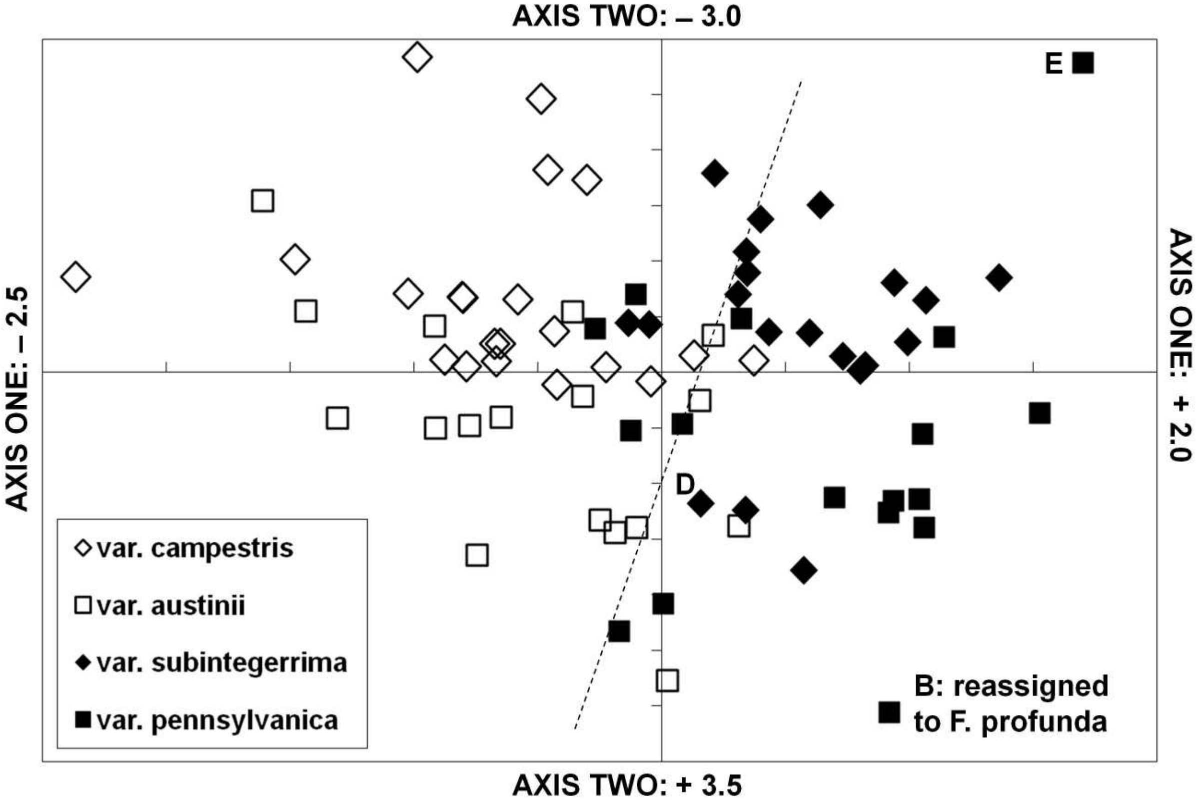
Figure 3a [next page upper]. PCA restricted to 74 collections of *Fraxinus pennsylvanica* scored at US. See text for outline of the four groups overlaid here, and for notes on outliers. Collections marked B, D and E are identified in Figure 2; B was reassigned to *F. profunda* after this analysis. Dashed line maximizes separation of SE variants (vars. *subintegerrima* and *pennsylvanica*) versus NW (vars. *campestris* and *austinii*): 5 NW plants mix in with the SE group; 7 SE plants mix in with the NW.

Figure 3b [next page lower]. As in Figure 3a but with geographic region overlaid instead of suggested taxa. See Figure 1 for definition of each region and further details. Dashed line maximizes separation of collections from S or E regions versus N or W: 16 N/W plants mix in with the S/E group, 5 S/E plants mix in with the N/W group.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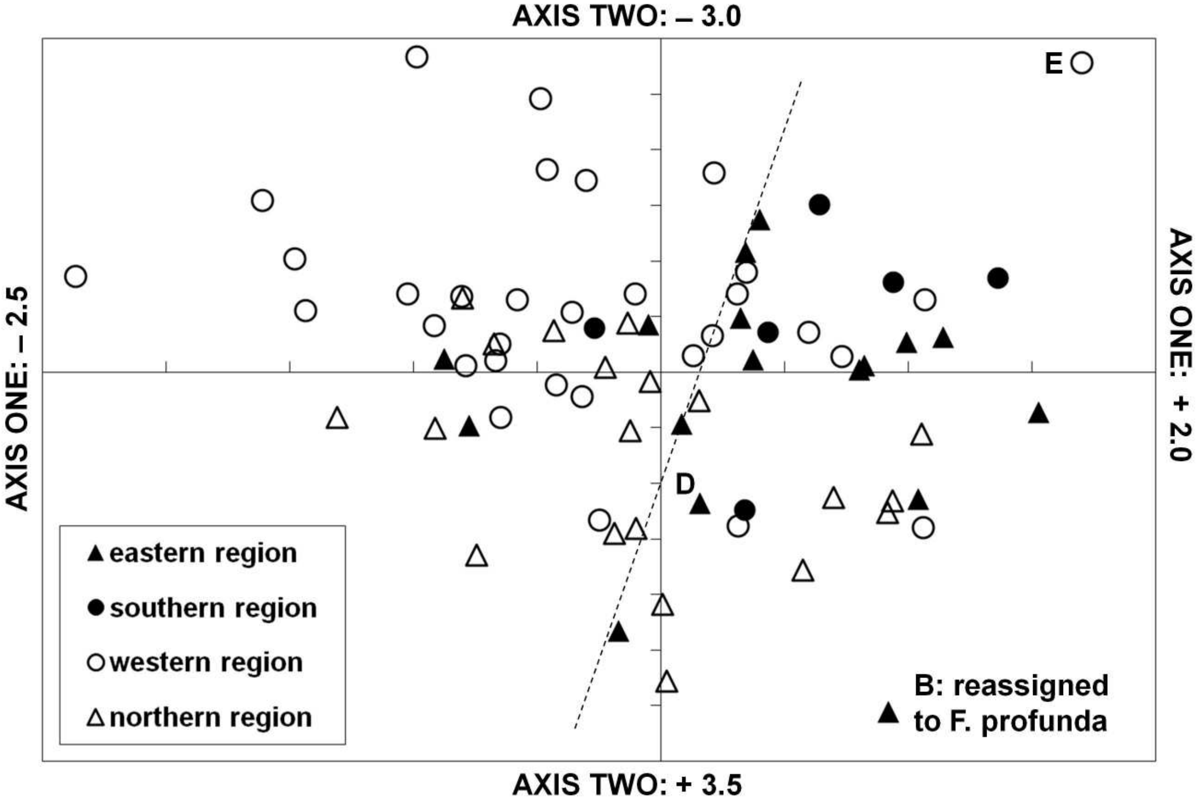
AXIS TWO: + 3.5
AXIS ONE: – 2.5
AXIS ONE: + 2.0
E
D
◇ var. campestris
□ var. austinii
◆ var. subintegerrima
■ var. pennsylvanica
B: reassigned
■ to F. profunda


AXIS TWO: – 3.0
AXIS TWO: + 3.5
AXIS ONE: – 2.5
AXIS ONE: + 2.0
E
D
▲ eastern region
● southern region
○ western region
△ northern region
B: reassigned
▲ to F. profunda

DISCUSSION

Distinction of Americana group versus Pennsylvanica group

Nesom's (2010a-f, 2014) work has supported a relatively divisive treatment of sect. *Melioides*, leading to about 15 species in North America, mostly assigned to the Americana group and the Pennsylvanica group. However, he noted potential difficulties in initial phylogenetic analysis due to uncertain identifications, and due to possible hybrid origin of some taxa. Distinction of the two major groups can be challenging among collections without samaras; about 1-10% of such collections remained uncertain in assignment after initial inspection by this author. The most useful key characters that separate the two groups include color and texture of lower leaflet surfaces (with waxy papillae and ridges in Americana group), decurrence of samara wings below seed summits (less so in Americana group), and elongated shape of seed-containing bodies (less so in Americana group). As detailed above (in notes before keys), several additional characters have been suggested by other authors, at least with reference to *Fraxinus pennsylvanica* versus *F. americana* (e.g., Miller 1955, Taylor 1972). Taylor reported that even if leaflet waxiness and petiole-notching are omitted from analysis, consistent multivariate discrimination of these two species is possible based on other characters. And in the field, habitat is often a useful guide to identification, or perhaps a bias—the Americana group generally occurs on drier ground, except perhaps for *F. pauciflora* (Tables 5 and 6).

As reviewed in the Introduction, there is little published evidence of natural hybridization between these two groups. After examination of over 2000 herbarium collections for this study, less than 20 were noted as possible hybrids. These puzzling collections mostly lack fruits, and definitive identification has not yet been possible; the few with samaras need to be studied further (e.g., from Calumet Co., Wisconsin, and Coos Co., New Hampshire, at MU). It can be sometimes be difficult to classify non-fruiting collections based on petiole bases, bud scars, or the presence versus absence of whitish waxy-papillose covering on lower leaflet surfaces (especially leaves from seedlings or shade). Some extra waxy covering can still rarely appear in *Fraxinus pennsylvanica*, as in a fruiting collection from North Dakota (*Lunell*, 16 Jul 1905 at NA), but without the papillose condition of *F. americana*.

The existence of *Fraxinus pauciflora* as a distinct species in the Americana group has been recently confirmed (Nesom 2010d), and estimates of its chromosome number are underway (A. Whittemore, pers. comm.). But provisional identifications for some collections of *pauciflora*-like plants have suggested to Miller (1955) and this author that there may be an intergrading complex between *F. caroliniana* (a highly variable taxon) and other species. There is a need for deeper investigation of such collections at US, NCU, VPI, GA, FLAS and elsewhere, including those named by Fernald (1937, 1938) as *F. caroliniana* var. *pubescens* (*Fernald 15140* from Virginia at NY) or *F. pennsylvanica* var. *austinii* (*Fernald & Long 11110* from Virginia at VPI); a possible *caroliniana-pennsylvanica* hybrid (*Fox 1738* from North Carolina at FLAS); and a possible *caroliniana-profunda* hybrid (*Price*, 9 May 1949, from North Carolina at NA).

Although several authors, as reviewed by Nesom (2014), have hypothesized that supposed polyploids in some eastern species of *Fraxinus* (*profunda, smallii, biltmoreana*) originated from hybrids, these taxa display no characters to indicate that crosses between two existing parental species are more likely than autopolyploidy from a more homogeneous ancestral pool. In *F. biltmoreana* (including *F. smallii*), the lack of notches in petiole bases has suggested hybrid origin from an *americana*-like ancestor with notches and a *pennsylvanica*-like ancestor without notches. But since petiole-notching is unique to *F. americana* (sensu stricto) within sect. *Melioides*, this character is probably derived from an un-notched ancestral pool within the Americana group. Moreover, those ancestors were presumably closer to the Pennsylvanica group than their modern derivatives. Such closeness would accord with other claimed similarities to the Pennsylvanica group in some collections of *F. biltmoreana*, including "lateral buds rounded, usually reniform" (Miller 1955), paler brown versus darker to blackish buds in general (Yatskievytch 2013), samaras with "bodies that tend to be dark orange at maturity" (Nesom 2010f), and flavonoid profiles (Black-Schaefer & Beckmann 1989). Yet these characters are not clear or consistent enough to be diagnostic. Flavonoids may be useful but much more sampling is needed; an earlier study provided different results (Fitzgerald & Reines 1969).

Extent of polyploidy and status of associated segregates

Within the Pennsylvanica group, almost all reported chromosome counts are 2n = 46 (Saxe and Abbe 1932; Taylor 1945; Wright 1957; S. Taylor 1972; Mukherjee & Ware 1979; Löve & Löve 1982; Hickman 1993; Nesom 2010c-d). The only report of a tetraploid (2n = 92) is for *Fraxinus coriacea* (Taylor 1945). The only report of a hexaploid (2n = 138) is for *F. profunda* (Wright 1957). These sparse data do not provide strong support for a general polyploid condition in any taxon, but it is often assumed that *F. profunda* is hexaploid and derived from diploid *F. pennsylvanica* through autopolyploidy, or from a cross with tetraploid *F. americana* (Miller 1955; Wright 1965). More reliable counts are clearly needed. Within the Americana group, there are more reports of polyploidy, but there is again much uncertainty in the geographic extent and degree of correlation with morphology. And although there have been several independent reports of diploid status (2n = 46) for *F. americana*, even these are mostly from old or obscure literature without cited collections (Sax & Abbe 1932; H. Taylor 1945; S. Taylor 1972; Mukherjee & Ware 1979; Weng & Zhang 1992); only the latter two references are provided for the *F. americana* complex in the Index to Plant Chromosome Numbers (Goldblatt & Johnson 2015).

Virtually no cited collections have been clearly associated with estimations of tetraploid or hexaploid status. The only verifiable report may be Santamour's (1962) linkage of hexaploid status with "*biltmoreana*" morphology in the following collections: "One specimen each of two lots (Nos. 152 from Owen County, Indiana, and 471 from Marshall County, West Virginia)." And even in this case it is not known if collections were provided to a public herbarium. Taylor (1945) had reported 2n = 46 for "*F. biltmoreana*" from "SCS Nurs., Shiprock, N. Mex., col. Farmington, N. Mex.," with accession number 2147-39 at the Blandy Experimental Farm, University of Virginia. But Miller (1955) reported that an immature collection of this same tree at BH (Bailey Hortorium) was definitely not *Fraxinus biltmoreana* and probably in the *F. pennsylvanica* complex.

Tetraploids of *Fraxinus americana* sensu lato were not indicated in this study using flow-cytometry with material from central Kentucky (Table 1), which included plants referable to *F. smallii* in the sense of Nesom (2010f). Moreover, C. Carlson, R. Olsen, and A. Whittemore (National Arboretum, pers. comm.) have been unable to find tetraploids in mid-Atlantic states using flow-cytometry, even with several plants that have the morphology of Nesom's *F. smallii*. There are two general explanations for this discordance with the research summarized by Nesom (2010f): either (1) recent flow-cytometry at the National Arboretum is flawed as an indicator of ploidy, although direct counts of chromosomes have been made in a few cases for verification (A. Whittemore, pers. comm.); or (2) some of the earlier cytological results are unreliable and led to an overestimated range for tetraploids.

It is unlikely that recent usage of flow-cytometry at the National Arboretum has provided erroneous indications of ploidy in *Fraxinus*, but further checking and refinement of methods may be needed. R. Olsen and A. Whittemore (pers. comm.) will address these issues in a broader report of data from across the range of *F. americana* sensu lato. It is notable that their laboratory has produced good evidence of diploids and tetraploids in *Ulmus americana* (Whittemore & Olsen 2011). Nevertheless, any attempt to estimate ploidy from nuclear DNA content must consider the potential challenges and biases in methods used (Bennett & Leitch 2005, Doležel & Bartoš 2005). Moreover, there is general evidence among angiosperms that DNA content does not increase in linear proportion to ploidy, suggesting that "loss of DNA following polyploid dormation, or genome downsizing, may be a widespread phenomenon of considerable biological significance" (Leitch & Bennett 2004). And at much smaller, physiological scales, one can expect considerable variation in DNA content within one organism. For example, Zhong et al. (1995) found that DNA content of cambial cells in *F. americana* varies by ca. 10–20% through the seasons.

The second general explanation for discordance with Nesom's summary (2010f) is more likely—that earlier estimates of ploidy were partly erroneous. Wright (1944a-b, 1957) claimed to have made direct counts of chromosomes, but he provided few details of methods or results and no figures of chromosomes. He appears to have relied largely on measurements of stomatal guard-cells as an indicator of ploidy. It would be desirable to have a more robust statistical distinction of supposed diploids, tetraploids and hexaploids using stomatal data (Table 4). Nesom (2010f) also noted general concerns about the accuracy of such distinctions.

Santamour's (1962) paper also lacked sufficient detail for robust conclusions, since he appeared to rely largely on Wright's initial assessments of ploidy in the plantation at Morris Arboretum, and perhaps subsequently on guard-cells: "In the spring of 1959 root tips were collected from these trees, pre-fixed in a saturated solution of paradichloro-benzene, killed in 3:1 alcohol-acetic, and prepared for examination by standard aceto-carmine squash techniiques. However, exact chromosome counts by this technique proved to be difficult, and some stumps did not produce new roots. Therefore, it was decided to make counts on only one tree of each progeny and to rely on stomatal guard cell measurements for verification of the degree of ploidy." Curiously, Santamour provided guard-cell measurements only from supposed diploids and tetraploids, without any from supposed hexaploids (Table 4).

Schaefer and Miksche (1977) did provide convincing indication of the three ploidies, using "photometrically determined nuclear DNA content from root tip cells of germinating embryos". Morevoer, they did check chromosome numbers using direct counts, and provided one figure of stained chromosomes that appears to show examples of the three ploidies. But, curiously, they found that only a minority (3/12) of the plants estimated to be more or less hexaploid had DNA content close to expected: 9.70±0.10 pg (0.4% less than expected). The remainder (9/12) had 8.11±0.08 pg, which was 16% less than expected. They invoked aneuploidy, suggesting that "some loss of chromosomal material is responsible for the lower average." In addition, one "putative pentaploid or possible aneuploid" had measured DNA content of 7.08 pg. Leser (1978), in an unpublished thesis, and Clausen et al. (1981), without new cytological data, extended the work of Schafer and Miksche (1977), and deeper assessment of their work is still needed. Armstrong and Funk (1980; Armstrong 1982) made photometric determinations of DNA content in bark cells, reporting putative diploids, tetraploids, and hexaploids as well as possible triploids and pentaploids, but they made no direct counts of chromosomes.

Black and Beckmann (1983) used photometric methods to indicate diploids, tetraploids, and hexaploids "within immediate proximity of one another" in Granville Co., North Carolina; they also reported a possible pentaploid. But, again, the degree of checking with direct counts remains dubious. Black and Beckman (1983) stated [in Methods] "Because of great difficulty in working with chromosomes of this species and in order to sample a large number of individuals, cytophotometry was used both to determine ploidy and to obtain information about the variability of nuclear DNA ... [but in Results] Chromosome counts of ten leaf cell nuclei of each tree confirmed the ploidy, within a maximum interpretation error of 2% for diploids, 7% for tetraploids, and 12% for hexaploids." However, they did not provide details of methods for making the counts, and they did not explain the meaning of "maximum interpretation error".

Taylor (1972) reported an intensive effort to count chromosomes in eastern ashes but it was restricted to *Fraxinus pennsylvanica* and *F. americana* in one southern Michigan county. Her results indicated that 2n = 46 for all 112 meiotic counts and all 76 mitotic counts. But, again, she noted difficulties in several cases, noting that "Preparations which contain a high proportion of pollen mother cells in early meiosis I stages may also contain a few tapetal cells undergoing free nuclear division and having chromosomes which appear to be paired. Because of the many, apparently paired, chromosomes of these cells, they can easily be mistaken for meiotic cells by one unfamiliar with ash tree cytology, and chromosome counts indicating high ploidal levels can resuIt" (p. 28); and "Mitotic chromosomes from root tips were much smaller and more difficult to count. Pretreatment with paradichlorobenzene did not change their appearance significantly. At 1000× magnification it was rarely possible to distinguish more than 40 or 42 chromosomes. No count exceeding 46 chromosomes was made" (p. 29).

Given these uncertainties about earlier reports of polyploids, the current results from flow-cytometry are tentatively accepted here: that *Fraxinus smallii*, as circumscribed morphologically by Nesom (2010f), is indeed largely hexaploid rather than tetraploid (Table 1; and unpublished data). Unfortunately, there has not yet been enough accumulation of chromosome counts—or even estimates—to allow a definitive association of tetraploid or hexaploid status with the type collection of *F. smallii* by J.K. Small, which comes from the Piedmont of Georgia. Indeed, there are no published estimates of ploidy from the *Fraxinus americana* (sensu lato) complex anywhere in Georgia, where all three of the morphological taxa treated by Nesom are locally frequent.

This author's general impression across east-central states is that typical pubescent *Fraxinus biltmoreana* is often mixed with less pubescent plants in populations, especially among seedlings and saplings. Thus, it is proposed here that these less pubescent plants can be grouped with *F. smallii* as a less pubescent variant of hexaploid *F. biltmoreana*. Lack of pubescence on stems and rachises remains the only consistent character used to distinguish *F. smallii* from *F. biltmoreana*. Although Nesom (2010f) also used samara size in his key, the data from over 50 fruiting collections examined so far by this author show no significant difference in dimensions (Campbell 2017). And there are no significant differences in foliar dimensions among data analyzed above (Figure 2).

It is notable that in the most definitive cytological study of *Fraxinus americana* and allies so far, by Schafer and Miksche (1977), tetraploids were reported only from southeastern Texas (3 of 3 from that state), southern Louisiana (3 of 3) and central Mississippi (1 of 6). However, Clausen et al. (1981) reported that samaras from these same trees were similar in size to diploids and smaller than hexaploids, unlike the plants defined as *F. smallii* by Nesom (2010f). Moreover, they found that seedlings from these trees had much faster growth rates and longer growing seasons than seedlings of *F. americana* sensu lato from elsewhere across its range, when planted together in southern regions—but much lower growth and survival in northern plantations (especially Wisconsin). Thus, it remains likely that a distinct tetraploid variant does exist on the Gulf Coastal Plain. There is also some evidence that tetraploids exist on or near the Piedmont from central Georgia to central North Carolina (Black & Beckmann 1983; unpublished data of J. Campbell and A. Whittemore). It will be important to investigate the status of such plants in more detail, including consideration of the potential for "genome downsizing" (Leitch and Bennett 2004).

Table 4. Reported mean stomatal guard-cell length in *Fraxinus* sect. *Melioides*. Standard errors are based on substantial samples (see sources for details), but: "In the red ash especially it is usual to find a variation of 50 per cent in guard cell length within the same microscope field."*

Species epithet	**Length (microns)**	**Sources**
"*americana*"	15-30 [in his key]	Wright 1944b
	12-18	Taylor 1972
2x (14 progenies)	18.8±0.2	Wright 1944b
2x (11 parents)	18.2±0.3	Wright 1944b
2x (8 progenies)	16.8±1.1	Santamour 1962
4x (2 progenies)	21.6±0.3	Wright 1944b
4x (2 parents)	22.5±1.1	Wright 1944b
4x (9 progenies)	22.6±0.9	Santamour 1962
6x (4 progenies)	24.2±0.6	Wright 1944b
6x (4 parents)	23.7±0.4	Wright 1944b
"*biltmoreana*"	15-30 [in his key]	Wright 1944b
pennsylvanica	15-24	Wright 1944b*; Miller 1955
	16-28	Taylor 1972
profunda (*tomentosa*)	22-34	Wright 1944b; Miller 1955

Table 5 [next page]. Some typical characteristics of taxa in the Pennsylvanica group, based on the literature (Miller 1955, Correll and Johnson 1970, Clausen et al. 1981, Burns and Honkala 1990, Hickman 1993, Nesom 2010a-f, etc.) plus direct study of herbarium collections (especially *campestris, pennsylvanica, profunda*).

See Nesom (2010a-f) for full names with authors. The names of taxa are provisional in some cases; those with asterisks (*) are treated in this paper as weakly segregated varieties of *Fraxinus pennsylvanica*: *campestris*, *austinii*, *subintegerrima* and *pennsylvanica*. Note that: *velutina* should include *papillosa* according to Nesom (2010f); *profunda* includes *tomentosa* and *michauxii*; *caroliniana* has often been defined to include *cubensis* and *pauciflora* (Nesom 2010d, Ward 2011).

Shading indicates higher character values.

Under "Pubescence": S/s = stem; R/r = rachis; B/b = blade lower surface. Upper case indicates wide extent; lower case indicates limited or variable extent; parentheses indicate largely restricted to veins.

Under "Leaflet Width": S/s indicates taxa with more distinct margin serration; upper case indicates especially distinct.

Table 5 [see previous page for caption].

CHARACTERS	*coriacea*	*velutina*	*berlandi-eriana*	*campestris* + austinii**	*pennsyl.* + subint.**	*profunda*	*carolin-ana*	*cubensis*
Range	SW desert	SW canyons	ne Mex. Tex. Okl.	c-ne US Canada	e US	e-se US	se US coast	s Fla. Cuba
Habitat:	riparian	riparian	subhydric	subhydric	subhydric	hydric	hydric	hydric
Max Height: m	5-10	9-14	10-18	12-18	15-35	15-40	3-15	3-14
Pubescence: stem rachis blade	b	S R B	(b)	s r b	s r b	s r b	(b)	(b)
Leaf Length: cm	6-15	10-25	10-15?	15-25	20-30	20-45	12-40	12-23
Leaflets: number	3-5	3-7	3-5(7)	5-7	5-9(11)	5-9	5-9	3-7
Petiolule Length: mm	5-10(13)	0-2	2-6(12)	1-3	2-4	5-12(20)	2-3(12)	4-10
Leaflet Length: cm	3-10	2-7.5	5-10	6-12	8-14	9-15(25)	4-12	5-12
Leaflet Width: cm	2-5.5 s	1-3? s	2-4 S	2-4 s	3-5	3.5-7.5	2-3	2-5
Samara Length: mm	18-32	10-35	25-35	30-40	35-45	42-65	35-46	25-54
Samara Width: mm	4-6	3-6?	3-6?	3-8	4-6	6-11	10-23	6-10
Seed-body L: mm F = flattened	9-16?	8-15?	10-18? F	12-20	15-22	18-30 f	20-35 F	15-25 F
Seed-body Width: mm	2-4?	2-4?	2-3?	1-2?	1.5-2.5	2-4	2-3	1-2

Systematic variation among eastern taxa of the Pennsylvanica group

Table 5 summarizes visible characteristics of the eight taxa recognized here within this group. These eight taxa are largely separated by range, except that within southeastern states there is also some local segregation along the gradient of increasing wetness: *Fraxinus pennsylvanica* to *F. profunda* to *F. caroliniana.* There are general increases in leaflet size and samara size from the arid west to the humid southeast. Maximum size, leaf dimensions, and samara dimensions tend to be highest in *F. profunda*, which also has the highest reported chromosome number (Wright 1957). These taxa have all been considered species by at least one previous author, as reviewed by Nesom (2010a-f). But some authors, especially Miller (1955), have reduced them to as few as two or three species based on claimed evidence of intergradation. With little quantitative support for taxonomic concepts, uncertainty remains in some cases, such as *F. pennsylvanica* var. *campestris* (Britt.) F.C. Gates and var. *austinii* Fern. Those two taxa are separated in the key above, based largely on pubescence (which is denser in var. *austinii*). They could be reasonably combined with each other, but together they remain relatively distinct from var. *pennsylvanica* plus var. *subintegerrima.*

***Fraxinus pennsylvanica* vars. *campestris* and *austinii*.**

Despite Small's (1933) acceptance of *Fraxinus campestris* as a species, Fernald (1950) did not mention it at all. However, Fernald appears to have included some trees that are at least transitional to it under *F. pennsylvanica* var. *austinii*, which has relatively pubescent leaves and broad short samaras: "Banks of streams, Que., to Sask. and Mont., s. to N.S., N.E., Va., O., Ill. and Ia. Passing into... Var. *subintegerrima...*" Under *pennsylvanica*, Gleason (1952) stated: "Plants with subsessile lateral leaflets; sometimes pubescent as in our first variety [var. *pennsylvanica*]; sometimes glabrous like the second [var. *subintegerrima*] occur frequently to the w. of our range [ne US and adjacent Canada] and have been observed as far e. as Mich. and Ont. They have been described as *F. campestris* Britt., but probably scarcely deserve segregation."

Meuli (1936, Meuli & Shirley 1937, Rudolf 1953) showed that more northwestern plants of *Fraxinus pennsylvanica* in the Great Plains, matching var. *campestris*, tend to be more drought-resistant. Similar results were later reported by Abrams et al. (1990). Provenance trials led to one particular male tree of this type becaming widely propagated across North America as "Marshall's Seedless". According to Santamour and McArdle (1983) and others, this cultivar came from Utah, at or beyond the western range-margin of *F. pennsylvanica*. They cited: "Cole Nurs. Co., Painesville, Ohio, Fall 1955 Trade List, p. 7—handsome, shapely tree with extremely dark green glossy foliage, entirely free of seed. Porter-Walton Co., Salt Lake City, Utah, Garden Book No. 46 (1946), p. 56, offered male green ash propagated from 'non-seed-bearing trees.' Some of this material was purchased by Marshall Nurs., Arlington, Nebraska, who, in several undated listings offered 'Seedless Ash.' Cole Nurs. Co. purchased plants from Marshall, and were the first to use the cultivar name. Because of its origin as 'trees' in Utah, this cultivar name may actually apply to several genotypes." Cultivars from western states, especially Marshall's Seedless, have been widely planted in developed areas of eastern states within the past 50 years but tend to grow poorly in southeastern states (Santamour & McArdle 1983; Gilman & Watson 1993). A "very similar" cultivar from Alberta, Canada, is "Patmore" (U.S. Plant Patent PP04,684 in 1981); see <missouribotanicalgarden.org> for details.

We need more thorough surveys in herbaria to map distributions of these *Fraxinus* taxa. It is usually easy to distinguish typical plants of var. *campestris* such as Marshall's Seedless from southeastern variants of *F. pennsylvanica,* but identification can be difficult, especially for collections of seedlings. Seedlings and sprouts of var. *subintegerrima* often appear to have more serration on leaf blades than mature trees, leading to confusion with var. *campestris*. Pubescence is not a useful character; both northwestern and southeastern plants include relatively glabrous and pubescent variants, as detailed in the key above. Several collections do suggest transitions between var.

campestris and var. *subintegerrima* (e.g., *England 1402* from Marengo Co., Alabama, at AMAL). The cultivar “Bergeson” (US Plant Patent USPP4904 P in 1982) from Minnesota may match var. *campestris* but it appears transitional to var. *subintegerrima* in its relatively long petiolules, weaker leaflet serration, and longer growing season. An atypical collection of *F. pennsylvanica* from Virginia that has been referred to var. *austinii* appears transitional to *F. caroliniana* (*Fernald and Long 11110* at GH, VPI). A possible collection of var. *austinii* is known as far south as Madison Co., North Carolina (*Bozeman et al. 45185* at MUR, ?NCU), but it has relatively large terminal leaflets.

The apparent similarities of *Fraxinus pennsylvanica* var. *campestris* and var. *austinii* to *F. berlandieriana* and *F. velutina* deserve deeper investigation (Table 5). Var. *campestris* appears generally intermediate in overall dimensions of leaves and samaras between southeastern variants of *F. pennsylvanica* (including var. *subintegerrima*) and *F. berlandieriana* or *F. velutina*. Moreover, its leaves are more clearly serrate than those of typical *F. pennsylvanica*, a character shared with all western members of the Pennsylvanica group—*F. berlandieriana*, *F. velutina* and *F. coriacea*. Var. *campestris* may be closest to *F. velutina*, given its short petiolules, its tendency to dense pubescence (when grading into var. *austinii*), and its generally somewhat abrupt expansion of samara wings above the middle (unlike some *F. berlanderiana*, most *F. profunda* and *F. caroliniana*); see Nesom (2010c-d) for details. If such similarities prove stronger than those with southeastern variants of *F. pennsylvanica* then species status might even become considered for *campestris*.

Western extent of native range in *Fraxinus pennsylvanica*.

The western boundary of this species extends from eastern Alberta through eastern Colorado to southern Texas (Little 1971; Gucker 2005; Kartesz 2015). Kartesz has indicated that trees in western Washington, western Oregon, Idaho, Utah, Arizona and New Mexico are all adventive. No other species in sect. *Melioides* are mapped as native or adventive along the western boundary of *Fraxinus pennsylvanica*, other than slightly overlapping species further to the west and south (*latifolia*, *velutina*, *berlandieriana*). But given that “Marshall’s Seedless” comes from a nursery in Utah, as noted above, is it possible that *F. pennsylvanica* var. *campestris* is native to that region? Some herbarium collections of *F. pennsylvanica* from Utah and New Mexico could be interpreted as native without further information, for example: *L.C. Higgins 10349* (NY), 27 Jul 1977, Utah, “Cache National Forest, Bear River Range, Logan Canyon about six miles east of Logan along Logan River”; and *B.F. Jacobs 10* (NPS: BAND), 30 Aug 2007, New Mexico, Sandoval, Frijoles Canyon at mouth, White Rock Canyon, 35.75313 - 106.25516. The latter is at the eastern edge of the range of *F. velutina*, and intergradation with that closely related species was indicated by Miller (1955); see also Table 5 and text above. However, Nesom (2010c) did not recognize any intergradation or overlapping range in New Mexico.

Under “green ash” Kuhns (2015) stated: “A tough, durable, large tree used extensively in Utah in landscapes since pioneer times. Also good for windbreaks. Lilac borers can be an especially severe problem that is difficult to treat effectively, though they may be most likely to affect stressed trees. I have seen many old green ashes that show no signs of borers, while a nearby young, vigorous, 30' tall tree might be riddled with borers. Seedless cultivars are available, but they can set large amounts of seed under stressful conditions.” Yet he added (M. Kuhns, pers. comm.): “I have rarely seen green ash growing in the wild, and when I have it was in situations where it was likely volunteering from seed from domestic trees [including the *Higgins 10349* location].” Native status in Utah may be unlikely, but it still cannot be ruled out.

Variation of pubescence within *Fraxinus pennsylvanica*.

Several previous authors have distinguished relatively glabrous versus pubescent plants with different varietal names, as followed here. But this highly variable character alone is a weak basis for defining these taxa. Many collections have intermediate degrees of pubescence, and occasional densely pubescent sprouts have been found attached to much less pubescent plants referable to var. *campestris* (e.g. *J. Campbell 2016.08.06A & B* from Vermont, and *2016.08.33A & B* from Maine, both at NY). Moreover, Taylor (1972) found that among 3-year old seedlings from glabrous mothers 20–35% were largely pubescent; and among those from pubescent mothers 40-45% were largely glabrous. However, using multivariate analysis Taylor did partially discriminate more pubescent plants (mostly referable to var. *pennsylvanica*) from less pubescent (mostly var. *subintegerrima*).

Nevertheless, some formal recognition of pubescent versus glabrous variants may be useful, pending deeper analysis of *Fraxinus pennsylvanica*. Samaras do tend to be larger in more pubescent plants, especially among the northwestern variants: *austinii* versus *campestris* (Campbell 2017). The proportion of pubescent plants appears to increase from west to east, based on overall mapping (Figure 1) and local observation. For example, in New England pubescent trees (here named var. *austinii*) are much more common than glabrous (var. *campestris*), as indicated by local floras (e.g., Haines 2011; Gilman 2015) and personal observation. But largely glabrous plants predominate in the Great Plains (e.g., Gates 1938; Correll & Johnson 1970; Lesica 2012; Yatskievytch 2013).

***Fraxinus profunda*.**

This remains a poorly understood taxon that is highly variable and, without samaras, it is often indistinguishable from *Fraxinus pennsylvanica*. Even when samaras are present, a few collections appear intermediate between these two species, with samaras that are smaller than typical *F. profunda* or that have less decurrent wings, and with leaflets that are generally smaller or less pubescent (e.g., *Long 6056* from New Jersey at US, *McAvoy 5959* and *Naczi et al. 10463* from Delaware at APSC). Some of these intermediates have been named *F. michauxii* Britt., which Britton (1908) reported "from southern New York to North Carolina, but probably has a much wider range". There are indeed similar collections from Georgia (*Duncan 23167* at MISS) and Alabama (*Hudsen 223*, and *Jackson 138* at AUA). If these intermediate collections are included within *F. profunda*, this species appears to have some multimodality of samara sizes (Campbell 2017); deeper analysis is needed.

Although typical *Fraxinus profunda* tends to have larger samaras and more decurrent wings, compared to *F. pennsylvanica*, these differences need further assessment. There is probably some overlap in size (Campbell 2017), as just noted for "*F. michauxii*". Within what is now generally accepted as *F. pennsylvanica*, trees described as *F. darlingtonii* Britt. were reported to have "long-linear" samaras about 50–75 × 4 mm (Britton 1908), but otherwise appeared close to typical *F. pennsylvanica*, which has samaras mostly 30–50 × 4-7 mm. Yet samaras longer than 50 mm may indeed be rare within *F. pennsylvanica*, including *F. darlingtonii*. At US, only one of the 74 collections of *F. pennsylvanica* had typical samara length >50 cm (51 cm in *Morton et al. 11754* from Ontario). Elsewhere, this author has seen very few collections of any eastern *Fraxinus* with samaras 60–75 mm long, as Britton (1908) reported for both *F. darlingtonii* and *F. profunda*. In *F. pennsylvanica*, the largest seen is 64 × 5 mm from Santa Rosa Co., Florida (FLAS, "var. *darlingtonii*"). Jeffrey Carstens (U.S.D.A., Ames, Iowa; pers. comm.) has measured mean lengths >50 cm in this species at only one locality (51–60 mm near Corning in sw. New York), with just 4 trees out of 210 trees sampled across the range; his maximum for an individual samara was 67 mm.

Further verification is needed for reports of samaras over 65 mm long as typical in *Fraxinus profunda*, which seem to have been repeated in several treatments (e.g., Fernald 1950; Miller 1955, Gleason & Cronquist 1991; Yatskievytch 2013). From results of this paper (Figure 4) and other studies focussed on this species (McCormack et al. 1995; Nesom 2010a; Reznicek & Voss 2012), a more accurate statement of samara size in this species (including *F. michauxii*) would be (32)42–65(74) × (5.5)6–11(12) mm, where parentheses include the outlying 1–5% of observations. The largest samaras seen so far by this author are as follows:

Shiflet, 2 Aug 1975 (VPI), Louisiana—mean 59 × 5.5 mm; maximum 65 × 6 mm;
Athey 2968 (MUR), Kentucky—mean 60 × 8.5 mm; maximum 67 × 9 mm;
Schneck, 8 May 1901 (NY), Illinois—mean 65 × 8.5 mm; maximum 72 × 10 mm;
Snyder 1262-6 (NY), New Jersey—with only two samaras, 66 × 8.5 and 74 × 9 mm.
Deam 11987 (NY), Indiana—mean 68 × 8 mm; maximum 73 × 8.5 mm;
Fox 4879 (NA), North Carolina—with few samaras but maximum of 73 × 10 mm;

Although typical *Fraxinus profunda* tends to have larger leaf dimensions (especially petiolules), compared to *F. pennsylvanica*, there is again much overlap. For example, an extraordinary fruiting collection from northern Florida clearly matches *F. pennsylvanica* var. *subintegerrima* except that its leaflets are 18–22 × 6–8 cm, within the upper range of sizes for *F. profunda* (*Godfrey 54998* at NA).

Fraxinus profunda typically has densely pubescent young twigs and lower leaf surfaces (e.g., Yatskievytch 2013), but there are several collections with relatively large *profunda*-like samaras that are partly glabrous (e.g., *Rose et al. 8469* from Pennsylvania at US; *Vincent 7501 & 7509* from Ohio at NA and MU; *Mellinger 16 Jun 1958* and *Duncan 23167* from Georgia at MISS; *Keener 3649* and *Bryson 23140* from Mississippi at AMAL; and several from Florida at FLAS). Some of these plants have been referred to *F. profunda* var. *ashei* Palmer, which was described as a largely glabrous expression of the species, distributed across its range (Palmer 1932). But Nesom (2010e) found that the type of var. *ashei* at NCU belongs with *F. pauciflora*.

Other reported characteristics of *Fraxinus profunda* include: pubescence of lower leaflets surfaces "conspicuously banded [1-5 mm wide], the edges of the bands usually irregular, the hairs longish and notably tangled" (Godfrey 1988), more scales on lower leaflet surfaces, slightly deeper notching of petiole bases and bud scars, more elongated bark ridges (Nesom 2010a, and his citations), and more swollen trunk bases, at least in deep swamps (hence "pumpkin" ash). These features deserve more quantitative investigation.

The distribution of *Fraxinus profunda* remains somewhat uncertain due to the limited numbers of clearly identified collections with mature samaras attached, due to occasional plants that appear intermediate, and due to possible plantings. Nesom (2010a) provided an updated map of county records. A few extensions are added here (Figure 1), including the largely glabrous collections noted above. Some relevant details are as follows.

Kansas. There is a convincing collection with samaras at US: *Scott Oct 1911*.

New York. Clarification of most records from the state is needed. As noted above, there are apparently native trees in Bronx Co. that have been called *F. michauxii* (Nesom 2010a). Kartesz (2015) has mapped a few other counties in southeastern New York with "adventive or introduced" status. There are also reports of possibly native trees from Tompkins Co., New York, "at the head of Cayuga Lake in Ithaca" (Miller 1955), but botanists in that state have generally considered these trees to be derived from plantings. According to R. Wesley (pers. comm.): "There are several herbarium specimens from the local area, [but] they were in people's yards or public parks and always near a road" (also http://newyork.plantatlas.usf. edu). This author recently collected vegetative material of possible *F. profunda* in Greene Co.

Tennessee. Two notable collections from eastern Tennessee at US have been referred to *F. pennsylvanica* in the past but they have relatively large samaras and leaves, appearing at least transitional to *F. profunda*: *Ruth 459* from Knox Co. and *Kearney 832* from Cocke Co. The latter may indeed be transferable to *F. profunda* (following Figure 3), although it has slightly serrate leaflet margins. More recently, good material of *F. profunda* has been collected in Rutherford Co. of central Tennnessee by D. Estes (APSC) and J. Campbell (NY).

West Virginia. The species remains largely unverified in West Virginia. But there are some unusually large-leaved collections without samaras at WVU, initially filed under *F. pennsylvanica*, that are suggestive of *F. profunda*. And one collection from West Virginia with samaras at MUHW does appear to be *F. profunda*: *James Meadows 010*, 29 Oct 1983, "bridge at Golden Jones, 3 mi above mouth of Little Clear Cr, rich bottomland soil" [Greenbrier Co.].

Systematic variation among eastern taxa of the Americana group

Table 6 summarizes visible characteristics of the five taxa in this group, as recognized by Nesom (2014), plus two allied species that may originate from a more basal position within the phylogeny of sect. *Melioides*: *Fraxinus latifolia* and *F. papillosa* (Williams & Nesom 2010). This table also includes the Mexican species, *F. uhdei*, a semi-evergreen tree that may originate from a basal position and appears closer to the Americana group than the Pennsylvanica group. These taxa are largely separated by range or, in southeastern states, by habitat. However, their recognition in previous treatments has been inconsistent. Again, there are general increases in leaflet size and samara size from the arid west to the humid southeast, which presumably reflects ecological adaptations. Maximum size, leaf dimensions and samara dimensions tend to be highest in *smallii* and *biltmoreana*, which are the only taxa with reported polyploidy.

Distinction of *Fraxinus americana* (sensu stricto) versus *F. biltmoreana* (sensu lato).

Diagnostic differences to distinguish *Fraxinus biltmoreana* from *F. americana* remain poorly understood. Even the characteristic notched bases in petioles of *F. americana* can be difficult to assess in some cases, despite the general significance of this character (Santamour 1962). This notching often appears to be less pronounced in the middle of more vigorous shoots. Although both *F. americana* and *F. biltmoreana* appear to vary much in pubescence, the latter tends to be more pubescent on average and *F. americana* is generally considered to have glabrous leaf rachises and young twigs. Yet there are rare collections of otherwise typical *F. americana*, with fruits, that do have densely pubescent rachises and/or twigs (e.g., from sand dunes of Indiana at MU; *Chester 1609* from Montgomery Co. Tennessee at APSC; and *Olsen et al. 148* from Washington Co., Maryland at NA, a confirmed diploid). Occasional vegetative collections of this sort are known from Florida (FLAS) to New England (NY), and deserve further investigation. Although there is no current evidence of hybridization, it will be important to explore this potential process, which could lead to tetraploids (Lyrene et al 2003, Ramsey and Ramsey 2014).

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Table 6 [next page]. Some typical characteristics of taxa in the Americana group and allies (see notes in text), based on literature (Miller 1955; Correll & Johnson 1970; Clausen et al. 1981; Burns & Honkala 1990; Hickman 1993; Nesom 2010a-f; Ares 2016, etc.) plus direct study of herbarium collections, especially *Fraxinus americana* (sensu stricto), *F.* cf. *smallii* (= *F. biltmoreana* var. *subcoriacea*) and *F. biltmoreana* (sensu stricto). See Nesom (2010a-f) for full names with authors.

Shading indicates higher character values.

Under "Habitat": hx = hydroxeric; sh = subhydric; sm = submesic; sx = subxeric.

Under "Pubescence": upper case indicates wide extent; lower case, limited or variable extent; parentheses, minor extent along veins.

Note that samara and seed size may be bimodal in *F. americana*; "+" indicates that some northern populations have similar size to *F. biltmoreana* (Campbell 2017).

Table 6 [see previous page for caption].

CHARACTERS	*latifolia*	*uhdei*	*papillosa*	*albicans*	*americana*	*smallii*	*biltmore.*	*pauciflora*
Range	Wash. Ore. Cal.	central Mexico	n Mex. Ariz.	ne Mex.-Okl.	e US-Canada	se US (Gulf CP)	se US (Appal.)	Fla. Ga.
Habitat:	subhydric-submesic	submesic ?	subxeric ?	subxeric	mesic-submesic	submesic	submesic-subxeric	subhydric-submesic?
Max. Height: m	18-24	25-40	6-12	10-15	15-30	7-25?	8-35	4-15
Pubescence: stem rachis blade	s r b	s r? (b)	(b)?	(b)	r b	(r) b	S R B	(b)
Leaf Lengh: cm	12-33	15-30	8-15	13-20	20-30	20-35	20-35	15-30
Leaflets: number	5-9	7-9	5-9	5-7	5-9	7-9	7-9	3-7
Petiolule Length: mm	0-5	3-13	0-3?	5-15	3-9	3-15	3-15	5-11
Leaflet Length: cm	5-9	7-11	3-7	3-8	5.5-12	7-14	8-14	7-15!
Leaflet Width: cm	3-7	2-5	1-3	2-5	2.5-6	3-8	3-9	3.5-7
Samara Length: mm	25-50	20-40	25-30	15-26	25-38+	30-55	30-55	28-65!
Samara Width: mm	5-9	5-6	?	3-5	3-5.5+	5-8	5-8	7-12
Seed-body L: mm F = flattened	15-18	7-12	?	5-8	6-12+	8-13	8-13	16-26 F
Seed-body Width: mm	3-5?	2-3?	?	1.5-2	1.5-3	2-3.5	2-3.5	2-4

Characters of *Fraxinus biltmoreana* that are currently more difficult to assess, but which deserve further study, include leaflet color (often more deep or bluish green) and bark pattern (often with deeper or longer fissures in vertical and lateral directions); see images in Campbell (2015). In Indiana, Deam (1912, 1919) also stated that: "the leaflets of the Biltmore ash stand in a plane higher above the rachis than those of the white ash," and that leaves fall later than *F. americana*. He noted: "young trees acquiring the fissured bark character earlier than the white ash, fissures of the bark of mature trees usually deeper and the ridges correspondingly farther apart..." Deam has been almost the only botanist to publish comments on the ecology of this taxon: "In the original forest, the pioneers called the very large ash with deeply fissured bark "the old fashion" ash. It is believed that most of the trees so described were of this variety. In the hilly parts of Indiana, this variety is found in situations too dry for the species, and for this reason should be given preference in hillside planting." There are also provisional data from Kentucky and other states that indicate a concentration of *F. biltmoreana* on drier or poorer soils than typical *F. americana*, especially in Appalachian regions (Braun 1950; Campbell 2011).

Variation within *Fraxinus americana* (sensu stricto).

If the Americana Group contains just two species in east-central states—*Fraxinus americana* (sensu stricto) and *F. biltmoreana* (including *F. smallii*), there is still considerable variation in samara size, leaf pubescence, leaflet shape and other characters within each of these. However, there is no evidence to support further taxonomic segregates of *F. americana* at this time.

As detailed elsewhere, there does appears to be a bimodal tendency in the size distribution of samaras within *Fraxinus americana* (Campbell 2017), and larger samaras are locally predominant in northern regions (Miller 1955; Nesom 2010f). Also, a few collections have exceptionally small samaras, including some that have been named *F. americana* var. *microcarpa* Gray, such as the outlier in Figure 2. Collections with samaras ≤26 mm were recorded at US and NY only from south-central states (AL, GA, KY, MO, NC, SC, TN and VA). However, the increase in average size of *F. americana* samaras with latitude is gradual, based on initial analysis of collections (Campbell 2017). In contrast, there is less variation in samara size of *F. biltmoreana* (including *smallii*), a more southern species, and this has no relationship to latitude. There is much overlap in samara size between these two species, and if they are combined as traditional "white ash", the clinal relationship of samara size to latitude largely disappears (Campbell 2017).

Clausen et al. (1981) also studied variation in samaras of *Fraxinus americana* sensu lato, and showed that overall size, seed size, and percent filled seed increases from southwest to northeast. *F. albicans* (= *F. texensis*) is a closely related species, treated as a variety or subspecies by some authors, that is largely restricted to Texas (Nesom 2010b). It has generally smaller leaves, with distinctive suborbicular-obovate leaflets, and samara size only (12)15–26(35) × 3–5(6) mm. As reviewed by Nesom, intergradation with *F. americana* is not verified, but deeper genetic analysis of the overall geographic trend in samara size is clearly warranted.

Other characters probably have latitudinal trends as well. And Wright (1944a) indicated that *Fraxinus americana*, excluding *F. biltmoreana*, could be divided into northern versus southern ecotypes, based on an extensive survey. His northern plants tended to have less leaf pubescence, narrower leaflet shape, and faster seedling growth rates in Massachusetts (at Harvard Forest). However, this author could find no significant geographic trend in leaflet size or shape among herbarium collections at US.

Additional characters should be explored in more detail with larger samples. For example, it is notable that, in the sample here from US, distinctly serrate leaflets with teeth mostly 0.5–1 mm deep were recorded only in a few northern and western collections. These were from Kansas, Iowa*, Quebec and Vermont*; see also photos from Minnesota (Campbell 2015, p. 37). In addition, some of these collections have relatively large samaras (30–41 mm long) and in two cases (*) petioles appear to lack distinctive basal notches, suggesting *F. smallii*; some have been named *F. americana* var. *juglandifolia* (Lam.) K. Koch.

Variation within *Fraxinus biltmoreana* (sensu lato).

Britton (1908) described *Fraxinus smallii* as distinct from *F. biltmoreana* in its more decurrently winged samaras, suggesting a closer similarity to *F. pennsylvanica*. However, neither Nesom (2010e) nor this author have found that *smallii*-like plants have distinctly decurrent wings. Moreover, both *smallii*-like plants and typical *F. biltmoreana* can easily be confused with *F. pennsylvanica* based just on overall dimensions of samaras and leaves (Figure 2). The inclusion of *F. smallii* within *F. biltmoreana*, under the new combination var. *subcoriacea*, is a provisional suggestion that allows continued classification of relatively glabrous versus pubescent plants. Reasons for this combination are as follows.

(a) Both taxa appear to be generally hexaploid in east-central states based on recent results (Table 1; A. Whittemore & R. Olsen, pers. comm.).

(b) Although Nesom (2010f) indicated that *F. smallii* tends to have smaller samaras, there is no significant difference in samara size among collections at US and NY (Campbell 2017), and continuing data collection from other herbaria (not shown here) has confirmed this conclusion.

(c) Among non-fruiting collections, distinction of *F. smallii* has been based largely on its less pubescent rachis and stem surfaces (Nesom 2010f). However, several collections do have an intermediate degree of pubescence—often with dense hairs on lower leaf surfaces but thinly pubescent to glabrous rachises and stems. A quantitative survey of pubescence patterns is needed to determine whether this variation is continuous or not.

(d) Within the woods of southern and western Kentucky, the author has repeatedly found a general mixing of less pubescent *smallii*-like plants with more pubescent typical *F. biltmoreana*. There appears to be little ecological segregation, although *smallii*-like plants are generally rare to absent in the Bluegrass region of north-central Kentucky. Also, they may be concentrated on more mesic sites. Again, quantitative surveys are needed.

(e) In Kentucky, the proportion of *smallii*-like plants appears to be generally higher in shade versus sun, and among seedlings or sprouts versus branches of mature trees.

(f) A tree with generally pubescent stems and rachises near Perryville in Boyle County, Kentucky, was found to have largely glabrous sprouts at its base, probably caused by roadside damage. This was collected for NA (Table 1) and illustrated elsewhere (Campbell 2015, p. 78–81). Combination of relatively glabrous and pubescent sprouts was also found in a cultivated tree at the University of Kentucky (*J. Campbell 2016.09-12* at NY).

Although *Fraxinus biltmoreana* has often been ignored by the botanical and horticultural community, there is a widely distributed cultivar that does belong to this species: “Urbanite” from a wild tree in Danville, Vermilion Co., Illinois (Wandell 1988). This plant was initially identified as *F. pennsylvanica*, but Dirr (1997), Jacobson (2003) and others have pointed out the error. Some recent collections of this cultivar have confirmed its hexaploid status (*J. Campbell 2016.09-6, 7, 10, 12, 94 & 95* at NY). A more recently introduced cultivar—“Jade Patina” or “Greenville”—is also reported to be hexaploid, with origin from the John C. Pair Horticultural Center at Kansas State University, but few details have been published (Griffin and Davis 2005; Hatch 2013).

More analysis of *Fraxinus biltmoreana* is needed for better circumscription of segregates, including *smallii*-like plants and other potential variants or cultivars. Ashe (1902) described *F. catawbiensis* as distinct from *F. biltmoreana* in its smaller calices and samaras. As more intensive analysis of biological differences continues, it will be interesting to determine the degree of genetic separation between these variants. And as more distributional data are accumulated, it will be interesting to determine the degree of difference in overall range and habitat.

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