SPECIAL INVITED PAPER

The ecological side of an ethnobotanical coin: Legacies in historically managed trees[1]

Nanci J. Ross[2,4], M. Henry H. Stevens[3], Andrew W. Rupiper[2], Ian Harkreader[2], and Laura A. Leben[2]

[2]Drake University, 2507 University Avenue, Des Moines, Iowa 50311 USA; and [3]Miami University, 700 E. High Street, Oxford, Ohio 45056 USA

- *Premise of the study:* A growing body of literature now documents how ancient human management of the landscape echoes through to extant environments in eastern North America. Plant domestication is a major theme in the study of human–nature interactions. Long-term ecological impacts of human selection may last for centuries after management ends, yet little work has focused on legacies in the evolution of historically used trees. Ecological data will be valuable in teasing apart myriad variables that confound questions of land-use legacies. We discuss the potential for legacies of ancient human selection and present a preliminary case study for the approach of integrating ecological and historical data for *Diospyros virginiana*, the American persimmon.
- *Methods:* Herbarium samples of *D. virginiana* (28 male and 40 female) from across the species range provided specimen localities for edaphic analysis. Soil and environmental data were analyzed using nonparametric ordination, Wilcoxon summed rank test, and permutational MANOVA.
- *Key results:* Edaphic data demonstrated substantial variation among sites, but revealed no significant differences between sexes. Permutational MANOVA showed no difference in environmental preferences for the tested variables between male and female trees ($R^2 < 0.01$, $P = 0.8$).
- *Conclusions:* Extending our understanding of landscape history to the long-term impacts of artificial selection at the species or population level would be valuable in both theoretical and applied botanical research. Multidisciplinary approaches integrating ecological data will be essential for investigation of the evolutionary implications of historical human selection in economic species and the potential for adaptive flexibility in reproductive systems of long-lived perennials.

  Key words: American persimmon; artificial selection; *Diospyros virginiana*; domestication; Ebenaceae; land use legacies; leaky dioecy; unconscious selection.

Effects of recent historical land use (~50–150 yr ago) on natural systems have received significant attention, especially in diverse tropical forests where slash-and-burn agriculture, logging, and monoculture industrial agriculture have created huge areas of secondary forests (Chazdon, 2014). The last few decades, however, have also seen a growing body of literature on the investigation of ancient human management of landscape as it echoes through to extant environments. In Europe, impacts of ancient Roman agriculture can still be seen in plant species richness and composition patterns (Dupouey et al., 2002; Vanwalleghem et al., 2004; Dambrine et al., 2007; Plue et al., 2008). In Mesoamerica, ancient Mayan silviculture still echoes in the species composition and distributions of the modern forest (Campbell et al., 2006a; Ross, 2011; Ross and Rangel,

2011). Amazonian Dark Earth (ADE), patches of rich, anthropogenic soils scattered throughout the Amazon forests of South America (Schmidt et al., 2014), as well as the growing evidence of extensive, pre-Colombian urbanism that has impacts on modern biodiversity (Heckenberger et al., 2007, 2008) have ended the belief in a pre-Colombian pristine tropical forest in Amazonia (but see Barlow et al., 2012).

In eastern North America, an extensive history of past land use has provided a rich body of research questions on long-term land-use legacies. The colonization by Europeans approximately 500 yr ago and the concomitant Native American population crash provides us with an opportunity to consider legacies of both historical (i.e., post-European) and ancient (i.e., pre-European) management strategies in the extant landscape (Table 1). European agriculture generally consisted of clearing plots in nearly continuously forested landscapes of colonial America (Williams, 1989), resulting in highly fragmented systems that are still echoing in the modern landscape (Hall et al., 2002; Dyer, 2010; Hermy and Verheyen, 2007). Contrary to previous beliefs, landscape management by pre-European Native American cultures was also both extensive and intensive, leaving a lasting trace in modern species diversity, composition, and distributions, soil characteristics, and broader landscape patterns (Delcourt, 1987; Delcourt and Delcourt, 1997; Foster and Aber, 2004; Abrams and Nowacki, 2008; McEwan et al., 2011; Cook-Patton et al., 2014).

Table 1. Studies documenting ecological impacts on biodiversity and/or species composition, distribution, edaphic composition, and plant density and/or productivity of historical and ancient human land-use legacies in eastern North America. References are separated into the four analyzed variable categories. In North America, historical land-use coincides with post-European settlement, and ancient is pre-Columbian Native American land-use.

Analyzed variables	Historical	Ancient
Biodiversity/Species composition	Bromley, 1935; Bellemare et al., 2002; Hall et al., 2002; Vellend, 2004; Flinn et al., 2005; Flinn and Vellend, 2005; Flinn, 2007; Flinn and Marks, 2007; D'Amato et al., 2009; Dyer, 2010; Rhemtulla et al., 2009; Kuhman et al., 2011; Steen-Adams et al., 2011; Brudvig et al., 2013	Bromley, 1935; Delcourt and Delcourt, 1997; Foster and Aber, 2004
Distribution	Bromley, 1935; McEwan et al., 2011; Buchanan and Hart, 2012	Bromley, 1935; Keener and Kuhns, 1997; Murphy, 2001; Abrams and Nowacki, 2008; McEwan et al., 2011
Edaphic composition	Latty et al., 2004; Flinn et al., 2005; Fraterrigo et al., 2005; Flinn and Marks, 2007; Brudvig et al., 2013	Matlack, 2009; Cook-Patton et al., 2014
Plant density/Productivity	Fraterrigo et al., 2006; D'Amato et al., 2009	

While there is extensive ethnographic literature on the legacy of ancient land use, identification of a definitive signal in the modern landscape generally requires a multidisciplinary approach integrating ecological as well as ethnographic and historical data (Lepofsky et al., 2003; Lepofsky and Lertzman, 2008). As an example, using solely ethnographic texts, MacDougall (2003) attempted to answer the question of whether ancient Native Americans acted as long-distance dispersal agents for postglacial advances of dispersal-limited forest herbs. He found that biased reports due to observer familiarity and/or intentionality, nonrandom site selection, vague locality descriptions, and unreliable species identification made it impossible to assess his research question based on ethnographic reports alone. The author concluded that integration of archaeobotanical and ecological data was essential.

Such integration of ethnobotanical data with climatic, edaphic, and community ecological data has been extensively used for investigation of the role of anthropogenic fire in the development of eastern North American forests (e.g., Delcourt and Delcourt, 1997; Stewart, 2002; Fowler and Konopik, 2007; Abrams and Nowacki, 2008). Delcourt and Delcourt (1997) argued that Native Americans used fire to increase landscape heterogeneity, thus increasing both local and regional diversity. Abrams and Nowacki (2008) integrated historical records of multiple landscape management practices including forest thinning, selective removal of competing species, and periodic burnings, with fire ecology data to suggest that the predominance of fire-tolerant mast species (oaks) in the precontact forest of eastern North America was promoted by Native Americans as an important food source. McEwan et al. (2011), however, argued that the evidence of pre-European fire is mixed. They expanded the analysis to include climate change, compositional change due to the near extinction of American chestnut (*Castanea dentata*), and increasing browse from growing deer populations. The authors concluded that the compositional shift from oak-to-maple dominance in the eastern forest is the result of multiple, interacting ecological factors including, but not limited to, the cessation of anthropogenic fire regimes. The controversy over the significance of fire and its use as an ancient land management tool highlights the complexity of teasing apart the multiple potentially confounding variables affecting the search for a signal of ancient land use in the modern landscape.

Ecological data has primarily been used to address questions of community and ecosystem level impacts of pre-Colombian cultures. This integrated, multidisciplinary approach, however, may also be valuable in evaluating the potential long-term legacy of utilization and landscape management on the evolution of individual tree species. Here we consider the conceptual basis for long-term legacies of ancient selection in perennial species. We then provide a case study assessing whether ecological variables contribute to sex segregation as a preliminary step toward identifying such a legacy in a historically managed native fruit tree species in Eastern North America.

HISTORICAL SELECTION AND LONG-TERM EVOLUTIONARY CHANGE

Plant domestication has been a major theme in investigations of long-term legacies of human–nature interactions. Plant domestication is the process by which human influence and selection leads to population genetic changes in chosen species over generations (Hancock, 2004). As such, it is clearly an evolutionary process. Domestication, thus, provides us with an opportunity to investigate long-term evolutionary change in species using diverse data from historical records and accounts, archaeology, ethnobotany, botany, and molecular biology (e.g., Casas et al., 1997; Zohary and Hopf, 2000; Doebley et al., 2006; Emshwiller, 2006; Miller and Schaal, 2006; Brown et al., 2009; Purugganan and Fuller, 2009; Provance et al., 2013). The main evolutionary force in domestication is artificial selection, but this force is influenced by both cultural and ecological factors. A holistic multidisciplinary approach to plant domestication creates a research approach to study domestication as an evolutionary biocultural continuum (Lins Neto et al., 2014).

While plant domestication in pre-Colombian Eastern North America has received significant attention (e.g., Heiser, 1951; Fritz, 1990; Smith, 2006; Blackman et al., 2011), the focus has primarily been on cultivated annual species. These species exhibit morphological and genetic changes (i.e., domestication syndromes) that are difficult to distinguish in species in incipient stages of domestication (Lins Neto et al., 2014) as well as in woody perennials (Miller and Gross, 2011). Native American peoples used a wide variety of woody, perennial species. This utilization was often outside of direct management by cultivation, but rather through various land management techniques (Hammett, 2000). Cultural management practices of perennial species show both direct and indirect effects of artificial selection (Heiser, 1988; Zohary, 2004; MacFadyen and Bohan, 2010; McKey et al., 2010). However, while the influence of artificial selection via land management on the evolution of perennial tree species has been identified in modern populations, it is unclear whether we can identify a signature of artificial

selection in the biology of historically managed species. "Historically managed species" refers to species that were at one time culturally important, but have since faded from use, thus altering or releasing the selective pressure on that species.

Conscious and unconscious selection—Darwin (1859) initially divided artificial selection into conscious or unconscious selection. In conscious selection, growers intentionally and directly select breeding individuals for subsequent generations. This intensive selection results in clear domestication syndromes and reduced genetic variation found in most annual crops. Weedy, annual species (e.g., corn, rice, wheat) form the basis of nearly every agricultural foodway (Heiser, 1990). With their comparatively short generation times, artificial selection has led to drastic changes in the anatomy, morphology, and reproduction of domesticated annuals (Gepts, 2004). Along with these changes is a corresponding change in overall genetic diversity. Annual crops retain, on average, only around 60% of the diversity found in their wild relatives (Miller and Gross, 2011). For perennial, woody species, however, the effects of artificial selection are often less apparent. A recent review of domestication of perennial fruit crops (Miller and Gross, 2011) found that cultivated, perennial fruit species maintain an average of 94.8% of the genetic diversity of wild populations.

Darwin originally described unconscious selection as the result of humans preserving useful varieties and destroying others; thus, unintentionally altering the gene pool of breeding individuals. In spite of the observed genetic changes, some researchers have argued that unconscious selection could be responsible for the majority of evolutionary change in seed-propagated annuals (Heiser, 1988; Gepts, 2004; Zohary, 2004). Zohary (2004) later expanded the concept of unconscious selection to describe changes resulting indirectly from environmental differences experienced by transplanting desired individuals into managed, anthropogenic landscapes. Use of cuttings and transplants is common for cultivation of woody perennial species. Such vegetative propagation allows farmers to sidestep the difficulties of the long juvenile period and/or dioecious reproductive strategies that are major complications in use and management of most perennial fruit crops (McKey et al., 2010).

Studies of contemporary management of perennial species in traditional agriculture, however, suggest yet a further expansion of the delineation of unconscious selection. Researchers describe a variety of in situ landscape-scale management techniques employed by traditional farmers that can also lead to evolutionary changes (Casas et al., 1997, 2007; Abrams and Nowacki, 2008; Blancas et al., 2010; Parra et al., 2010). Ethnobotanical studies in Mesoamerica have documented extensive in situ management of perennial tree species by local people (Alcorn, 1990; Rico-Gray et al., 1991; Casas et al., 1997; Gómez-Pompa and Kaus, 1999; Gómez-Pompa et al., 2003; Toledo et al., 2003; Lins Neto et al., 2014). Casas et al. (2007) surveyed traditional agricultural techniques of indigenous cultures in Mexico, one of the global centers of plant domestication. In addition to field agriculture, the authors described four common landscape management techniques: (1) "systematic gathering" by which selective means of harvesting, rotation of harvesting locations, or other methods may result in artificial selective forces on populations; (2) "let standing" by which selected individuals are allowed to remain when land is cleared for use; (3) "encourage growing" as part of management practices designed to increase the density of selected species; and

(4) "protection" by farmers to eliminate competitors, predators, or other threats to the success of focal plants.

Many of the landscape management techniques described by Casas et al. (2007) have been historically and currently documented as practices around the world from Amazonia (e.g., Balée and Gely, 1989; Miller and Nair, 2006) to the Tibetan Himalaya (e.g., Salick et al., 2007). Similarly, at the time of European contact in the southeastern United States, historical writings and archaeological reconstructions described a mosaic of different management types across the landscape created by Native Americans for management of, among other species, native tree crops (Hammett, 2000). While few of these practices fall into the category of cultivation per se, all represent continuous selective influence on focal populations through management of the landscape. The question then is how to identify effects of incipient artificial selection in genetically diverse perennial tree populations.

Clement (1999) described incipient domesticates as exhibiting phenotypic variation within the range of wild populations. Analyses by Casas et al. (2007) of managed populations showed significant morphological and physiological changes, yet failed to discern significant differences in genetic diversity between wild and managed populations (Casas et al., 2007). Rather than genetic approaches, several studies have identified significant variation in reproductive traits and/or breeding systems of perennial species as indications of evolutionary change due to selection (Otero-Arnaiz et al., 2003; Lins Neto et al., 2014).

Variability in breeding systems: "Leaky" dioecy—Humans have been selecting, modifying, managing, and otherwise influencing plant evolution in myriad species throughout history. These practices can have profound effects on the reproductive biology of species, both intentionally and not. Miller and Gross (2011) provided an excellent review of the effects of domestication on the reproductive systems of cultivated woody perennials, so we do not repeat it here; however, while perennial tree crops use a variety of reproductive systems, dioecy—plants produce unisexual flowers with male and female flowers on separate individuals—provides an interesting condition to consider the potential long-term impact of both conscious and unconscious selection.

Dioecy is a rare condition, existing in only about 6% of all angiosperms (Renner and Ricklefs, 1995). Theoretically, dioecy evolved to maximize outcrossing (i.e., avoid inbreeding depression) and/or to reallocate resources in heterogeneous environments (Miller and Venable, 2000). Some authors, however, argue that the evidence for dioecy leading to an evolutionary advantage in dealing with the stochasticity inherent in natural selection is lacking (Allem, 2003). Plant breeding systems are often less clear than commonly believed. For example, nominally dioecious species may occasionally diverge from strict dioecy to produce perfect (i.e., hermaphroditic) flowers or even reproduce asexually through apomixis under certain environmental conditions. Such inconsistency results from breeding systems controlled both by genetics and by environmental variability (Richards, 1996). Therefore, many plants use more than one breeding strategy with their evolutionary potential depending on a balance between outcrossing, selfing, and/or apomixis (Allem, 2003; Richards, 1996). The threshold point for this balance is determined by both biotic and abiotic selection.

This variability in breeding systems further increases the complexity in identifying potential signals of human selection

in the reproductive biology of historically managed species and supports the integration of ecological and ethnobotanical approaches. In managed dioecious species, artificial selection is, in part, a result of farmer preferences for solely fruiting individuals. This commonly results in two adaptive solutions: (1) "leaky" dioecy, where individuals diverge from strict dioecy to produce branches bearing opposite sex (i.e., monoecy) or even perfect (i.e., hermaphroditic) flowers; or (2) parthenocarpy where female flowers skip pollination entirely and produce primarily seedless fruits (Zohary, 2004). This shift away from strict dioecy is thus caused by artificially induced nonrandom mating system ratios that are relatively independent of environmental controls.

Alternatively, leaky dioecy may correlate with environmental heterogeneity that causes biased sex ratios (Delph, 1999). Male and female distributions and life-history trade-offs often vary across resource gradients (Barrett and Hough, 2013). Differential resource costs for male and female reproduction can lead to divergence from a 1 : 1 sex ratio especially in fleshy-fruited species, which often have higher maternal costs for females than the pollen-producing males (Sinclair et al., 2012). Species that occupy a broad range of environmental conditions may experience greater influence on sex ratios leading to spatial segregation of the sexes (SSS) (Bierzychudek and Eckhart, 1988; Sinclair et al., 2012). Such segregation may be the result of niche partitioning (Cox, 1981) or simply the result of differential mortality between the sexes (Bierzychudek and Eckhart, 1988).

A first step, then, in investigating long-term impacts of ancient human management in the biology of historically managed species is to begin isolating and testing edaphic and environmental variables. Such an approach brings us to our case study where we describe a preliminary study within a larger investigation of long-term legacies of ancient selection by Native Americans. Specifically, we describe statistical approaches to analyzing and interpreting edaphic data and how that data can function as a springboard for future studies.

CASE STUDY: *DIOSPYROS VIRGINIANA* L. (EBENACEAE), THE AMERICAN PERSIMMON, A HISTORICALLY USED PERENNIAL FRUIT TREE

History and management—The Greek word "diospyros" roughly translates as "fruit of the gods", and the use and popularity of several species worldwide attest to this name. Persimmon fruits have been cultivated for millennia and hold an important place in the diet and medicine chests of many cultures. *Diospyros* is a relatively large (~500 species) pantropical genus in the ebony family (Ebenaceae) with a small, but significant, number of temperate species including the Asian persimmon, *D. kaki*, and the Native American persimmon, *D. virginiana* (Mabberley, 1997). Phylogenetic analysis of plastid DNA indicates a close evolutionary relationship between *D. kaki* and *D. virginiana* (Yonemori et al., 1998; Duangjai et al., 2009). The Asian persimmon has been argued to be the most popular fruit in the world and is the subject of extensive agro-evolutionary research. Yet, in the United States, persimmon is nearly a forgotten fruit. The American persimmon (*D. virginiana*) has a broad distribution throughout the United States from Connecticut south to Florida and west to the eastern edge of Nebraska (USDA-NRCS, 2014). Archaeological and historical records provide evidence of the extensive use and management of American persimmons by Native Americans historically, yet

the species is now viewed as a rare, weedy, wild fruit tree that is known primarily by hobbyists and wild harvesters.

When Europeans arrived in North America, they discovered the sweet, custard-like fruits of American persimmon described in the earliest published book by an English colonist as "lushious sweet" (Harriot, 1590). Other writers of the time also noted the fruits and their potential value including Jamestown's Captain John Smith (Briand, 2005). Historically, *D. virginiana* was a common component of the Native American diet throughout the southeastern Unites States (Briand, 2005; Fogelson, 2004) and is regularly found in archaeobotanical remains (e.g., Wilson, 1977; Gremillion, 1984, 1998; Fritz, 2000; Hammett, 2000). Archaeobotanical data from three Late Archaic period (300–800 BC) sites in the Lower Mississippi Valley found persimmon to be the most ubiquitous fruit crop used (Fritz, 2008). Paleobotanical analysis of refuse pits from two Mississippian (900–1700 AD) sites identified *D. virginiana* in 78–80% of pits (Scarry, 1993). The Mississippian culture gave rise to the largest, most complex civilization in what is now the United States prior to European settlement. Even after the Native American population crash brought about by introduced European diseases (e.g., smallpox), early writers describe remnant forests rich in fruit and nut trees around abandoned settlements (Hammett, 2000). Ethnobotanical research of indigenous, postcontact use found that American persimmon was used by the Cherokee, Rappahannock, Creek, Comanche, and Seminole tribes among others (Moerman, 1998; Fogelson, 2004).

Evidence of cultivation of persimmon is difficult to discern using historical records. An early explorer identified American persimmon in what he believed to be remnants of ancient Native American orchards in the Atlantic Seaboard region (Bartram, 1773). A previous writer in that same area, however, described not cultivated orchards, but rather managed landscapes where useful trees were allowed to grow wild in fields "…as large and as vigorous as if they were cultivated and irrigated in gardens." (Anonymous, 1557, p. 87). This writer, a chronicler with the de Soto expedition (1539–1542), could have mistaken the unfamiliar tree crop management practices of Native Americans for uncultivated "wild" sproutings. Regardless, either practice suggests management that may result in unconscious selection in desired species after consistent and extensive management over time. The archaeobotanical record described argues for such long-term management.

Despite the long history of persimmon use, as European settlers expanded throughout its range and indigenous populations decreased, widespread management of American persimmon was essentially abandoned, and these selective pressures on the tree were released. One might imagine that the centuries since the extensive use and selection of American persimmon trees may have obscured any signal of human influence that may have existed; however, perhaps not. Discontinuities in the reproduction system of American persimmon may be the result of just such an echo.

Divergence from dioecy in persimmon—Reproductively, American persimmon is highly variable. Although *Diospyros virginiana* is classified as strictly dioecious, individuals that are monoecious, gynomonoecious (bearing both female and hermaphroditic flowers), and andromonoecious (bearing both male and hermaphroditic flowers) have all been reported (Spongberg, 1979). Even more interesting, some individuals are reported to switch from strict dioecy one year, to producing some perfect or opposite sex flowers in other years (Hague, 1911; Spongberg,

1979). Finally, both seedless and seeded parthenocarpic fruits have been reported (Goodell, 1982). Leaky dioecy and parthenocarpy were the characteristics appearing to arise frequently from artificial selection (Zohary, 2004); however, divergence from dioecy may also result from environmental pressures.

Diospyros virginiana is reported to be tolerant of a wide range of habitats and soil types (Spongberg, 1979; Goodell, 1982). Nixon et al. (1977) reported that persimmon was significantly associated with acidic alluvial flats in East Texas, while Baskin and Baskin (2000) identified it as a drought-tolerant pioneer species in calcareous, shallow limestone glade communities in the Ozarks and Midwest of the United States. Gilliam et al. (1993) reported persimmon to be strongly correlated with coarse, sandy soils in lowland communities. Wall and Darwin (1999) listed persimmon as a facultative species, equally likely to be found in wetland or nonwetland areas. While American persimmon may, in fact, exploit various habitats, microsite edaphic differences have been found to relate to phenotypic variations within populations of many species (Owuor et al., 1999) including species of *Diospyros*. In a study of 11 *Diospyros* species on the island of Mauritius, leaky dioecy was found in one species living on very poor soils in fragmented populations (Venkatasamy et al., 2007).

American persimmon was reportedly more common historically than it is today (Skallerup, 1953). Its tolerance of various habitats and its reputation as a weedy pioneer species argues that this lower frequency is not due to limiting niche requirements. Skallerup (1953) suggested that its current rarity is due to dispersal limitation instead. The large fruit of American persimmon preclude birds as dispersers, but deer, coyote, raccoon, fox, and other mammals are known to eat them (Murphy, 2001). Heilbuth et al. (2001) modeled that limited dispersal requires dioecious species to be substantially more fit than their cosexual competitors in order remain selectively competitive. *Diospryos virginiana* is rare when considered across its range (Iverson et al., 2004); however, in localized areas it can be a dominant species (e.g., Nixon et al., 1977; Wall and Darwin, 1999; Baskin and Baskin, 2000). Because the plants can sprout suckers from the root, the genetic diversity of such populations is not known, but it is reasonable to assume that subsets of individuals in each community are, in fact, clones. As discussed, dioecy is proposed to enforce outcrossing and avoid inbreeding depression, which would seem selectively advantageous in species like American persimmon with patchy, clumped distributions. Dioecy is also correlated with fleshy fruits, which may increase long-distance dispersal (Muenchow, 1987; Sakai et al., 1995). Dispersal of persimmon has been recorded for opossum (Murphy, 2001), raccoon (Cypher and Cypher, 1999), white-tailed deer (Skallerup, 1953), and coyote (Cypher and Cypher, 1999; Roehm and Moran, 2013), supporting this thesis.

Divergence from dioecy in persimmon begs further investigation. We hypothesize that leaky dioecy in *D. virginiana* is a result of historical selection by Native Americans for trees with copious fruit production. If true, we would expect to see increased proportions of leaky dioecy in the breeding systems of populations on or near ancient Native American population centers. Alternatively, we might expect to see higher proportions of leaky dioecy in disjunct populations known to be of anthropogenic origins outside the normal range of *D. virginiana*. The complexity created by the long time since widespread human management of American persimmons, however, requires that multiple potentially confounding environmental variables that could influence sex expression must be addressed before we can directly assess our main research question. Our particular goal here is to evaluate whether sex expression is related to local climate and soil characteristics. The null hypothesis is that distributions of male and female trees will have no significant relation to distinct suites of edaphic characters. Such a relationship would suggest evidence for environmental selection for sex in American persimmon (H_1).

MATERIALS AND METHODS

Due to the surculose (i.e., producing suckers) growth of American persimmon and the sometimes patchy distribution of American persimmon thickets (Goodell, 1982), individual specimens from across the range of the species were required to investigate possible differential sex distribution in relation to edaphic variability (Fig. 1). These specimen localities were then overlain on the USGS reported distribution of *D. virginiana* (http://esp.cr.usgs.gov/data/little/) (R language and programming environment v. 3.1.1, R Development Core Team, 2014).

The extensive distribution and relative rarity of American persimmon makes fresh collection during flowering time difficult, however. For this reason, air-dried herbarium specimens were used to provide samples from across the range (Appendix 1). Herbarium samples were collected from the Missouri Botanical Garden herbarium (MO), Turrell Herbarium at Miami University (MU), and Drake University herbarium (DU). The specimens ranged in age from <5 to >100 yr old. Only reproductive specimens were used to determine whether they were male or female. Location coordinates for most specimens were recorded when the sample was initially collected in the field. Older specimens, however, often only had a description of the collection location. Therefore, those specimens were geolocated using the closest proximity to described localities. The use of herbarium specimens unfortunately precluded our ability to check for incidences of gyno- or andromonoecy in the individual plants.

Coordinates for individual specimens were used to collect data on edaphic characters at each specific locality. Data on pH, available cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), annual precipitation (in inches), drainage class, elevation, and slope were collected for each locality. Soil chemistry data (pH and available cations) for 20-cm-deep soil cores were retrieved from the database of the Kellogg Soil Survey Laboratory (KSSL) at the National Cooperative Soil Survey (2014; http://ncsslabdatamart.sc.egov.usda.gov). Environmental data were derived from the Web Soil Survey reports (Soil Survey Staff, 2014) in conjunction with known soil series documents. The KSSL database is a database published online by the National Resource Conservation Service of the USDA. It is a collection of soils data (minimum delineation is 1 acre [4046 m^2]) collected by state soil scientists in all 50 states. The listed characters were chosen because they were the only soil characteristics consistently included in the soil survey reports for every locality. The pH was measured using a 1 : 1 soil to water ratio. Cation content is reported in cmol/kg of soil material. Elevation and slope were identified using the program ArcMap version 10.2 (ESRI, 2014).

Differences between sexes were assessed in an exploratory data analysis. The nonrandom sampling of specimens (those from available herbaria) prevented us from drawing strong inferences regarding the true nature of the environment associated with persimmon sex. Putative differences we observed could be due to either actual interactions of sex expression, fitness, and the environment or to the nonrandom collection procedures and storage by the herbaria. Nonetheless, approached as data exploration and hypothesis generation (Tukey, 1980), our approaches would allow us to reveal meaningful differences among the groups of specimens, which happen to have different sexes, and to propose promising hypotheses. We used a wide variety of approaches, detailed in Appendix S1 (see Supplemental Data with the online version of this article). We selected analyses to present here that are widely used and easily interpreted. The supplemental information shows that other approaches would lead to similar conclusions.

Differences between sexes in each of the environmental variables were assessed visually with box and whisker plots and the Wilcoxon summed rank test using the R programming language and environment (v. 3.1.1, R Development Core Team, 2014). Individuals respond to complex multivariate aspects of the environment, and therefore we used nonmetric multidimensional scaling (NMDS) to visualize differences among specimens and sexes. Prior to analysis of Bray–Curtis distances, environmental variables were standardized to between 0 and 1 by dividing each by its own maximum, helping to weight the variables relatively equally. We quantified these multivariate distances within and between sexes

using permutational MANOVA in the vegan package in R (v. 2.0-10, Oksanen et al., 2013). Like parametric MANOVA, permutational MANOVA assesses whether multivariate differences among samples are associated with the groups to which those samples belong (McArdle and Anderson, 2001).

RESULTS

Figure 1 shows the current U. S. Geological Survey (USGS) distribution map, as well as our specimen localities. The herbarium specimens successfully represent the natural distribution of *D. virginiana* and provide adequate sampling for broad-scale edaphic analyses.

Environmental heterogeneity—Data collected from the NRCS database and Web Soil surveys demonstrated substantial variation among sites from which specimens were collected, with orders of magnitude variation within most of the environmental variables (Fig. 2). Nonetheless, these revealed no significant differences between sexes as assessed visually in box and whisker plots or Wilcoxon summed rank tests (all $P >$ 0.10). Further, permutational MANOVA showed no difference in environmental preferences for the tested variables between male and female trees ($R^2 < 0.01$, $P = 0.8$; Fig. 3). Additional analyses, including logistic regression and classification trees confirmed these approaches (see Appendix S1).
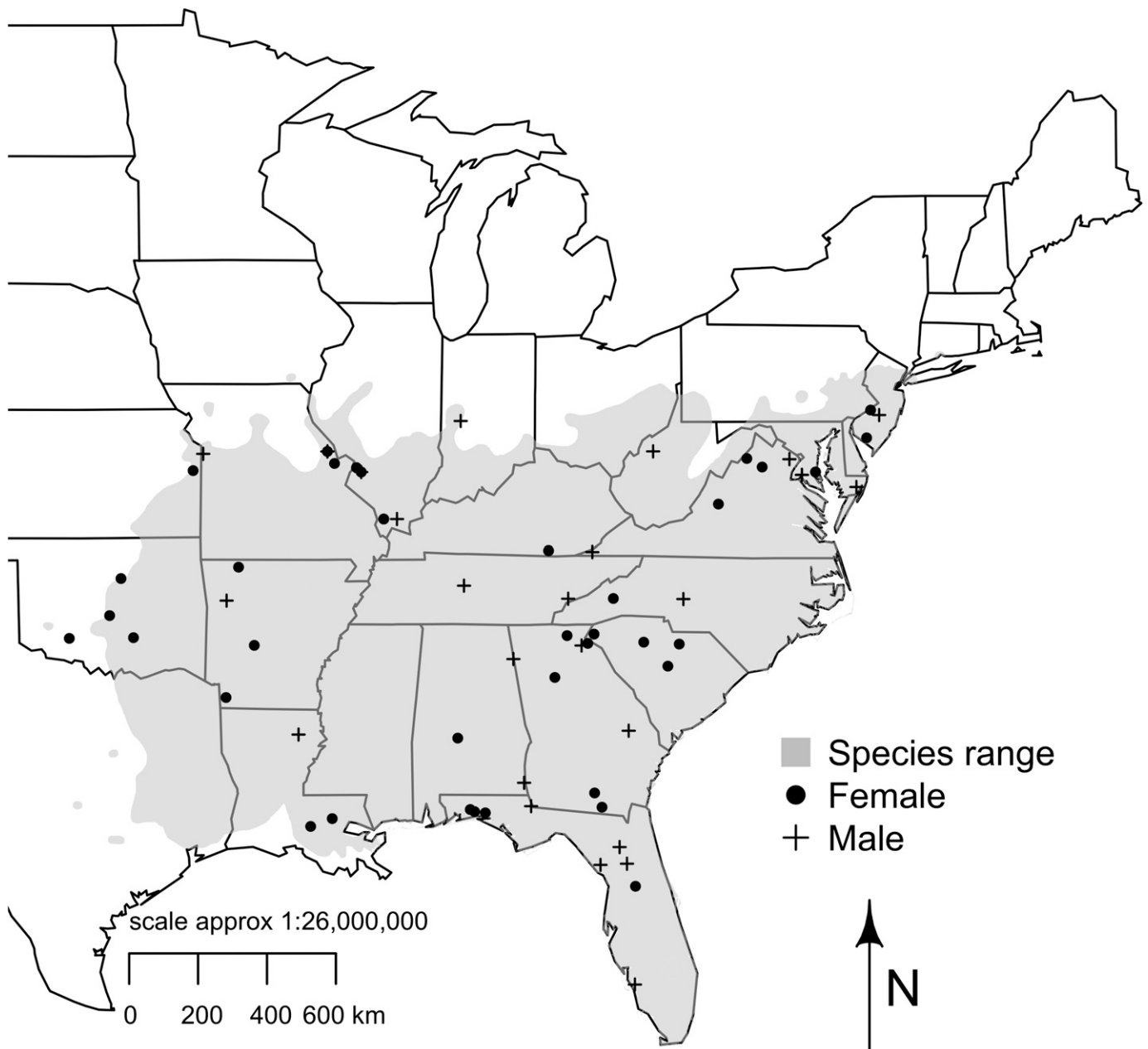

Fig. 1. Distribution map of *Diospyros virginiana* including the locations of herbarium specimens used for soil analyses and the U.S. Geological Survey reported range of *D. virginiana* (http://esp.cr.usgs.gov/data/little/) (in gray). Map was drawn with R v. 3.1.1 (R Development Core Team, 2014). (See Appendix S1 for more detail).
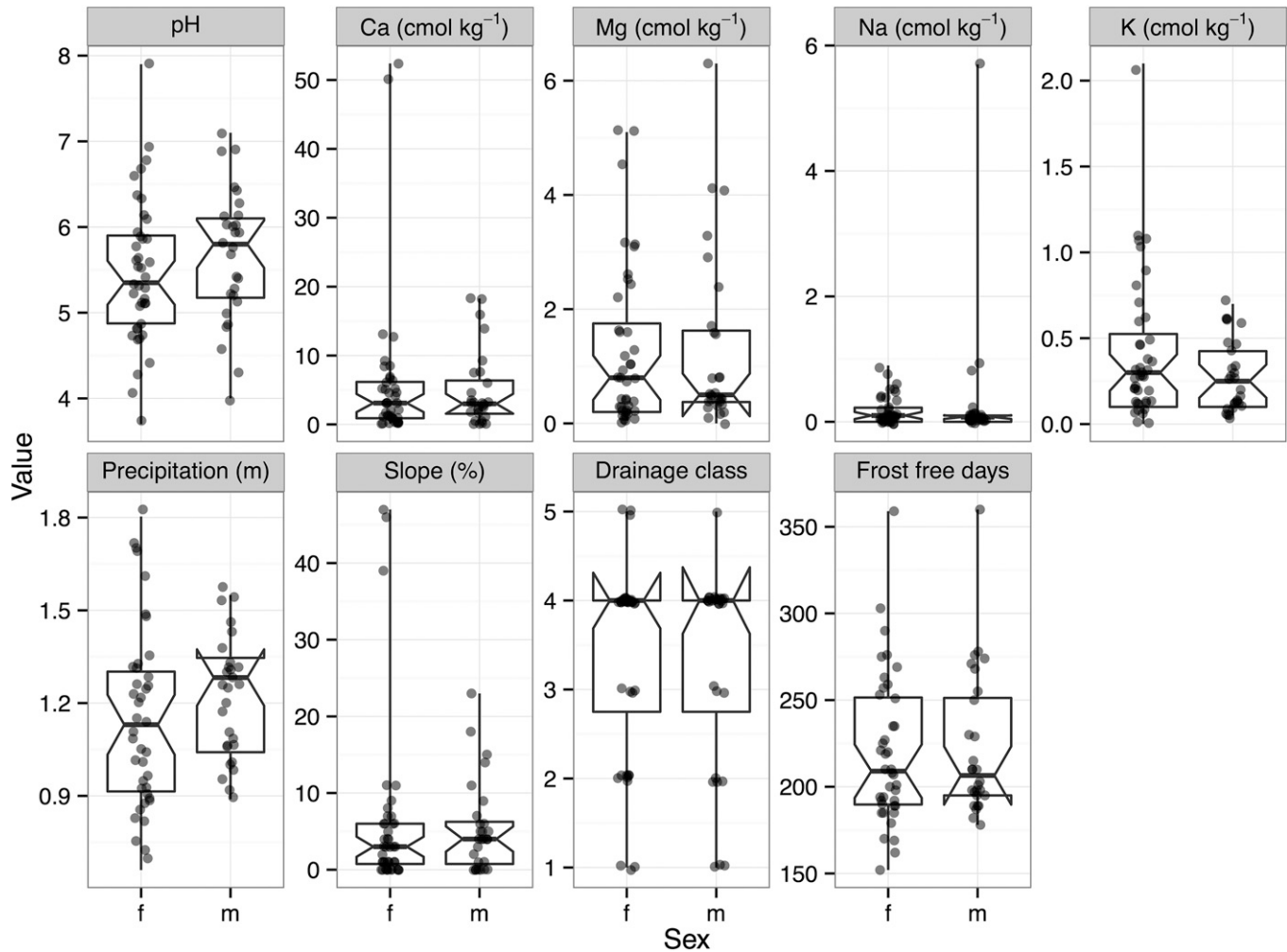
Fig. 2.   Box and whisker plots show that sites from which the specimens of *Diospyros virginiana* were collected spanned a broad range of environments. Boxes represent the quartiles (0.25, 0.50, 0.75), and whiskers extend to the most extreme points. Observations were superimposed for clarity (investigated: $N = 40$ females, 28 males). Notches indicate that the medians (0.5 quartiles) of the two groups (sex) do not differ. No variable differed between sexes (all Wilcoxon summed rank tests $P > 0.10$).

DISCUSSION

In contrast to the argument that influences of environmental variation on sex distribution differences are often especially strong in fleshy-fruited species (Sinclair et al., 2012), our analyses provided no evidence that the climate or the edaphic environment causes segregation of sexes in American persimmon. Although we used a wide variety of approaches (see Appendix S1), no compelling evidence arose to suggest segregation due to environmental factors. This finding supports the null hypothesis of no relationship between suites of measured edaphic characters and sex distributions in *D. virginiana*. Lack of evidence for such a relationship suggests that we can move forward in our investigation of potential long-term impacts of historical management on the evolution of reproductive traits in American persimmon without the noise of a strong environmental driver.

Our results suggest that if such ecological segregation does occur, then it is occurring at a finer scale than we sampled. If an environmental characteristic does facilitate segregation, it is likely related to soils, rather than climate, which varies only at larger scales. A preliminary assessment of this "fine-scale hypothesis" could derive from intensive sampling of soils in one or more local extant populations of persimmon. If fine-scale variation in edaphic characteristics correlates with sex ratio, this correlation would provide some evidence for the fine-scale hypothesis. A rigorous test of this hypothesis could use a common garden experiment calibrated to field conditions. If no environmental segregation of sexes actually occurs in natural populations, as suggested by our results, then one can move forward to investigate the role of historical management in contributing to the persistence of leaky dioecy in American persimmon.

Future studies might benefit from attention to soil features related to the variables for which we found a suggestion of correlation with sex, including potassium and calcium (see Appendix S1). Annual precipitation appeared to show a hint of a correlation with sex as well, which may reflect climate, but may also reflect water-holding capacity of the soil, or even a variation in spatial geography that is correlated with precipitation. A signature of nonrandom sex ratios in natural populations would provide an opportunity to investigate hypotheses of niche
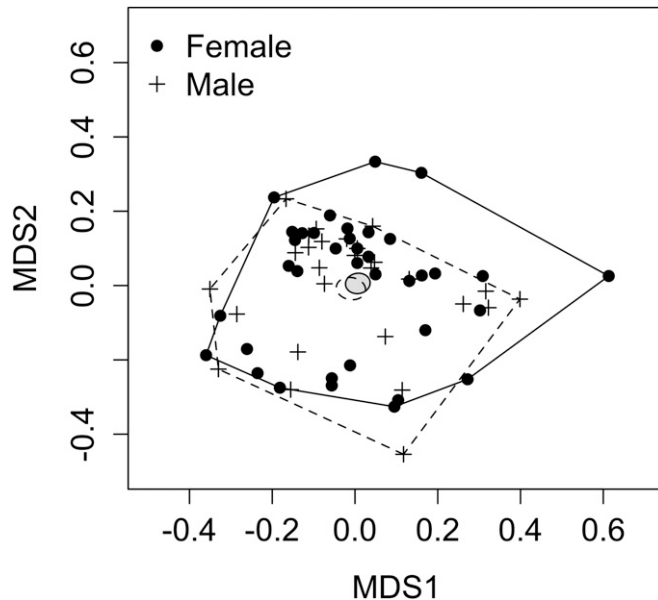
Fig. 3. Nonmetric multidimensional scaling plot of the first two NMDS axes of the environmental data show that the environments do not differ between sexes for *Diospyros virginiana*. Ovals in the center illustrate one standard error around the centroids for males (dashed) and females (solid). The outer polygons are convex hulls for each group (male = dashed, female = solid). Permutational MANOVA showed no significant differences by sex.

partitioning (Cox, 1981) vs. differential mortality (Bierzychudek and Eckhart, 1988) and whether these relate to the occurrence of leaky dioecy.

There are several explanations why we may see 1 : 1 sex ratios in American persimmon in spite of countervailing pressures. As mentioned previously, often the higher reproductive costs of fruit production lead to females facing resource limitations that could result in a divergence from 1 : 1 sex ratios, yet this is far from universal (Barrett and Hough, 2013). In dioecious species, males and females fundamentally play different roles in populations potentially resulting in differential selection on the sexes. Differential life-history trade-offs between males and females in terms of reproductive costs and currencies may actually result in an equal burden on the growth and survival of both sexes. For example, Cornelissen and Stirling (2005) found that males often face significantly higher rates of herbivory. As herbivory pressures increase it could differentially impact male fitness thus reducing or eliminating any male bias in the sex ratio.

The tendency for American persimmon to produce suckers or root sprouts from damaged roots suggests another possibility. Clonal propagation has been used for millennia as a means of preserving and promoting selected phenotypes (McKey et al., 2010). Precontact Native Americans could have promoted the surculose growth of selected individuals such as fruit-producing males over struggling to find and relocate multiple bisexual individuals. Such selection could, however, have quickly led to inbreeding depression, which is significantly more profound in trees than other crops (Petit and Hampe, 2006). Once such management stopped, the limited diversity and lower fitness within local populations would result in their rapid replacement by more fit, naturally outcrossed wild individuals, resulting in a rapid return to a 1 : 1 sex ratio.

Finally, the long time since extensive management of *D. virginiana* may simply make it difficult to uncover a signal of pre-contact management. A signature of historical planting or promotion of fruiting persimmon may have been obscured by subsequent recruitment of a more even sex ratio in the intervening centuries since European settlement began. While all events in an ecosystem leave an imprint on that system, not all events leave a strong enough record to be "heard" through the noise of centuries.

Of course, the lack of significant results may simply be due to limitations of the methods used in our pilot study. The sample size in this preliminary study (40 females and 28 males) was small for a species with such a large range. The small sample size relates to the limitations of using herbarium specimens. Our requirement for reproductive specimens with a relatively specific collection locality greatly reduced the number of useable specimens. The relative rarity of American persimmon resulted in surprisingly few reproductive specimens to be found in herbaria. Of the specimens with reproductive features, females are overrepresented (N. J. Ross, personal observation), perhaps because the bright orange fruits are more noticeable and memorable for collectors. Second, the use of government soil and environmental data meant that any microsite edaphic variables were essentially missed. Future work should include soil and climate data collection at the site of the measured populations and individual trees. However, the goal of this study was to conduct a preliminary analysis on sex distributions across the range of *D. virginiana* to provide a starting point for addressing a complex and challenging question.

Although leaky dioecy is often thought to evolve as a result of male-skewed sex ratios, it is important to remember that it may also evolve in the absence of this skew. For example, Humeau et al. (1999) studied the endemic dioecious *Dombeya* (Sterculiaceae) species on La Reunion Island. The small surface area (2500 km²) and large elevational gradient (sea level to 3069 m) on the island created large environmental gradients across small scales. The authors found that the *Dombeya* species in lower, drier, more disturbed populations all exhibited leaky dioecy. Despite the prevalence of leaky dioecy, no species exhibited differences in male to female abundance (although this lack does not preclude the possibility that there was a skewed sex ratio in the past).

How long do the impacts of [ancient] humans on the landscape persist? It is now accepted that an understanding of landscape history is important in understanding current ecological patterns and processes (Szabó, 2010). Extending this understanding to the long-term impacts of artificial selection at the species or population level would be valuable in both theoretical and applied botanical research. Arising out of Carl Sauer's (1925) work, the historical ecology approach defined in the anthropological literature views the interaction of humans and environments holistically (Balée, 2006). The landscape is a place "upon which past events have been inscribed, sometimes subtly, on the land" (Balée, 2006, p. 77). Smith's (2012) cultural niche construction theory brings this anthropological approach together with a biological perspective for the initial domestication of plants. The basic precepts of the biological niche construction theory (Odling-Smee et al., 2003) state that organisms do not only adapt to their environments, but they, in turn, alter their environments to their own needs. Initial plant domestication, then, was part of the alteration of the environment by humans to create "opportunities for human societies to expand and enrich their overall integrated resource management

strategies" (Smith, 2012, p. 197). *Diospyros virginiana* may provide a fascinating and potentially valuable case study on such evolutionary implications of ancient human selection.

Once a commonly eaten native fruit, American persimmon is still eagerly sought by wild harvesters and naturalists throughout its range. It is reported to have few natural enemies (Goodell, 1982). In addition to being already adapted to grow in a variety of soil types, it has potential as a valuable native fruit crop. This project establishes a baseline for future investigation of the distribution of reproductive diversity in American persimmon. Beyond enhancing our theoretical understanding of historical ecology and ecosystem dynamics, this research can be used to inform conservation (Foster et al., 2003) and agricultural landscape management (Flinn and Vellend, 2005) and guide us in identification of useful native species for future crop development (Goodell, 1982). Regardless of whether a connection to ancient cultivation and selection can be established, the system provides an example to investigate evolutionary lability of dioecious reproductive systems and the potential for adaptive flexibility in reproductive systems of long-lived perennial species.

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Appendix 1. *Diospyros virginiana* herbarium specimens included in edaphic analyses. Collection numbers are the unique identification numbers given to specimens at the various institutions from which the sample was taken. *Herbaria abbreviations:* MO, Missouri Botanical Garden; MU, Miami University Herbarium; DU, Drake University herbarium.

ID no.	Collection no.	State	Collection year	Sex	Herbarium
27	50587	Alabama	1964	female	MO
54	46193	Alabama	1972	male	MO
56	52924	Alabama	1974	male	MO
12	6343	Arkansas	1914	female	MO
4	23122	Arkansas	1923	female	MO
18	13467	Arkansas	1936	female	MO
63	C0647	Arkansas	1989	male	MO
73	54445	D.C.	1995	male	MU
14	2033	Florida	1895	female	MO
58	35188	Florida	1929	male	MO
65	1014	Florida	1930	male	MO
41	s.n. 1940	Florida	1940	male	MO
43	51557	Florida	1981	male	MO
22	9176	Florida	1997	female	MO
40	9136	Florida	1997	female	MO
13	9473	Florida	1998	female	MO
51	8472	Florida	1999	male	MO
53	s.n.	Georgia	1897	male	MO
10	2588	Georgia	1967	female	MO
28	6751-D	Georgia	1970	female	MO
19	16942	Georgia	1975	female	MO
9	18245	Georgia	1976	female	MO
61	1702	Georgia	1976	male	MO
44	1744	Georgia	1982	male	MO
29	s.n.	Illinois	1875	female	MO
30	s.n.	Illinois	1875	female	MO
59	15207	Illinois	1919	male	MO
55	15185	Illinois	1960	male	MO
32	46	Illinois	1979	female	MO
35	5566	Illinois	1990	female	MO
39	122	Illinois	1991	female	MO
3	12	Illinois	2012	female	MO
60	6679	Indiana	1941	male	MO
20	758 a	Kansas	1897	female	MO
78	54481	Kentucky	1985	male	MU
24	9511	Kentucky	2001	female	MO
36	17144	Louisiana	1961	female	MO
34	6	Louisiana	1973	female	MO
70	54429	Louisiana	1983	female	MU
62	133533	Louisiana	1993	male	MO
5	16720	Maryland	1956	female	MO
6	17266	Maryland	1957	female	MO
42	10264	Maryland	1981	male	MO
46	14260	Maryland	1984	male	MO
50	14131	Maryland	1984	male	MO
64	7041	Missouri	1913	male	MO
17	12-38	Missouri	2012	female	DU

APPENDIX 1. Continued.

ID no.	Collection no.	State	Collection year	Sex	Herbarium
48	12-39	Missouri	2012	male	DU
49	12-28	Missouri	2012	male	DU
33	998	New Jersey	1927	female	MO
57	4411	New Jersey	1933	male	MO
1	792	New Jersey	1993	female	MO
66	10261	N. Carolina	1911	male	MO
11	1416	N. Carolina	1920	female	MO
72	54440	Ohio	1938	female	MU
71	54439	Ohio	1953	female	MU
76	54472	Ohio	1980	male	MU
75	54461	Ohio	1989	female	MU
74	54459	Ohio	2002	female	MU
69	36116	Ohio	2010	female	MU
26	S0138	Oklahoma	1905	female	MO
23	265	Oklahoma	1939	female	MO
25	778	Oklahoma	1944	female	MO
7	74424	S. Carolina	1974	female	MO
21	1984	S. Carolina	1976	female	MO
15	39	S. Carolina	1994	female	MO
2	1017	S. Carolina	2010	female	MO
77	54475	Tennessee	1895	female	MU
52	s.n.	Tennessee	1929	male	MO
47	1/16/2	Tennessee	1932	male	MO
45	34787	Tennessee	1969	male	MO
16	43888	Texas	1993	female	MO
38	s.n.	Virginia	1892	female	MO
8	648	Virginia	1924	female	MO
67	18343	Virginia	1941	male	MO
37	24091	Virginia	1945	female	MO
31	2090	Virginia	1967	female	MO
68	W4063	West Virginia	1955	male	MO

Note: D.C. = District of Columbia.