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Genetic diversity and differentiation of yellowwood [*Cladrastis kentukea* (Dum.Cours.) Rudd] growing in the wild and in planted populations outside the natural range

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Abstract Yellowwood (*Cladrastis kentukea*) grows in small, widely scattered populations in the wild, but is also a popular ornamental tree that thrives when planted in urban areas outside its natural range. Since the small native populations of yellowwood in several states are considered at risk of extirpation, the cultivated population could serve as an ex situ repository of yellowwood genetic diversity that could be used to restore lost local populations of the species. The potential value of cultivated yellowwood for conservation depends on the genetic diversity among cultivated trees compared to natural populations. Using nuclear microsatellite markers, we genotyped 180 yellowwoods from natural populations in Indiana, Missouri, Arkansas, and Kentucky, along with 61 trees from urban parks and landscapes in Indiana, Ohio, and Missouri. We found that, even when statistics were adjusted based on population size, the urban "population" had higher genetic diversity than any of the wild populations sampled, indicating that commercially-grown yellowwood is most likely a mixture of genotypes from isolated wild populations. We observed strong genetic differentiation among wild populations, and evidence for inbreeding in at least one of the wild populations.

Keywords Ex situ conservation · Urban forest · Landscape genetics · Fabaceae

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Introduction

Many native tree species that perform well in cultivation, and therefore make up a large portion of the tree canopy in urban and suburban areas, are also abundant in the wild. Red maple (*Acer rubrum*) and eastern redbud (*Cercis canadensis*) are examples in eastern North America. Some native trees, however, that are rare or have very small distributions in the wild can become abundant and widespread in cultivation if they have outstanding ornamental characteristics, such as showy flowers, fast growth, tolerance of urban conditions, and resistance to pests and diseases. In the eastern United States, examples include northern catalpa (*Catalpa speciosa*), Osage-orange (*Maclura pomifera*), and yellowwood; ginkgo (*Gingko biloba*), European horse chestnut (*Aesculus hippocastanum*), and radiata pine (*Pinus radiata*) are notable international examples. In cases where wild populations of a tree are threatened with extirpation, the large number of trees in cultivation could serve as a reservoir of genetic diversity for reintroduction or genetic replenishment of wild populations.

The importance of conservation as a goal for horticultural plant collections has expanded as wild plants have become threatened in shrinking natural habitats (Havens et al. 2006). Botanical gardens and arboreta are primary sites for ex situ conservation of trees, but for species that are widely cultivated, the enormous number of trees growing in parks and along streets could serve as a diffuse ex situ conservation population. Cultivated specimens growing successfully outside their natural range could provide genetic material for assisted migration, which may become necessary for some trees affected by climate change (Dumroese et al. 2015). Ideal plant conservation collections should take into account allelic capture, baseline genetic variation, and reintroduction potential (Cibrian-Jaramillo et al. 2013). In several taxa, including cycads (Griffith et al. 2015), *Coffea* species (Andrianasolo et al. 2013), and *Zelkova carpinifolia* (Christe et al. 2014), living ex situ collections captured a large portion of the genetic diversity in natural populations; in some Madagascan *Coffea*, genetic diversity was higher in some collections than in the wild, and *Z. carpinifolia* collections included some chloroplast haplotypes not sampled in the wild. In a second *Zelkova* species (Christe et al. 2014), ex situ collections failed to capture diversity because they were all sourced to a single locality, illustrating the potential problems of using garden plants as conservation resources.

The unique value of yellowwood as an ornamental tree was recognized shortly after its description by botanists in the late 18th century (Peattie 1950). Yellowwood is a small-to-medium-sized deciduous tree with smooth gray bark, pinnately compound leaves, panicles of showy white flowers, and a symmetrical flat-topped crown when it is grown in the open. In the wild, yellowwood is typically found in sheltered locations along cliffs, bluffs, and hillsides, on well-drained sites with rich, moist, alkaline soil (Hill 2007). It is rare throughout its native range, and typically occurs in small, widely scattered groves. Yellowwood's range encompasses a small portion of the southern Appalachian Mountains in North Carolina and Tennessee; it is completely absent from the Allegheny Mountains and plateau. It occurs west of the Appalachians on the Interior Low Plateaus and Highland Rim regions in Tennessee and Kentucky, where it is probably most abundant. Examining FIA (Forest Inventory and Analysis; http://www.fia.fs.fed.us/tools-data/) data from states within yellowwood's native range, yellowwood was recorded at one site each in Kentucky, Arkansas and North Carolina and four sites in Tennessee. A second population center is found along the southern edge of the Ozark plateau and throughout the Ouachita Mountains in Arkansas and Missouri, and a few small disjunct populations occur in the hills of

southern Illinois and Indiana, and others are found in Oklahoma, Mississippi, and Alabama (Leopold et al. 1998; Hill 2007). Yellowwood's population throughout its range appears to be stable, although some marginal populations may be in decline. Threats include destruction of stands by clearcutting, flooding of ravine habitat by reservoirs, poor recruitment, and loss of adult trees to fungal diseases.

In general, wild forest tree populations maintain high levels of genetic diversity, even when large population declines have taken place; this is the case with slippery elm (*Ulmus rubra*) (Brunet et al. 2016) and butternut (*Juglans cinerea*) (Ross-Davis et al. 2008). These species are wind-pollinated: although yellowwood's reproductive biology is not well-known, its large, showy flowers suggest pollination by insects, which could make a difference in gene flow among spatially isolated populations. Insect pollination does not necessarily lead to reduced gene flow versus wind-pollinated species (see Nason et al. 1998; Kamm et al. 2009), but it can if insect pollinators have short dispersal distance or if tree populations are widely scattered (Burczyk et al. 2004). Furthermore, leguminous trees like yellowwood may self-pollinate more frequently than other taxa (Ward et al. 2005; Rodger and Johnson 2013), which could allow isolated populations to remain viable, but could also lead to genetic drift and inbreeding (Hamrick and Godt 1996). If yellowwood has managed to maintain gene flow among populations so that populations are poorly differentiated, yellowwood could be considered one large genetic population for conservation purposes. If, on the other hand, populations in different regions are strongly differentiated, conservation efforts should focus on preserving regional genetic resources.

To investigate the population genetics of wild yellowwood and assess the suitability of cultivated yellowwood for ex situ conservation, we asked the following questions: (1) What levels of genetic diversity and differentiation exist within and among wild yellowwood populations? (2) Is there any evidence for deleterious genetic effects of isolation in yellowwood populations? (3) How does the genetic diversity of a sample of urban yellowwood trees compare to samples from wild populations?

Materials and methods

Plant material

Yellowwood leaves were collected from Yellowwood State Forest in Indiana, Tom Dorman State Nature Preserve in Kentucky, and Henning Conservation Area in Missouri in 2011, and private land near the Remmel Dam near Jones Mills, AR in summer 2012 (Fig. 1). The Arkansas and Kentucky sites were located on bluffs above the Ouachita and Kentucky rivers, respectively. For the Indiana samples, detailed data including GPS coordinates, size (DBH), and condition were recorded. Samples of cultivated yellowwood from sites in Ohio, Indiana, and Missouri were collected in summer 2013. DNA was extracted from leaves using a CTAB buffer and a phenol–chloroform extraction based on the method of Doyle and Doyle (1987) and quantified using a Nanodrop ND8000 (Thermo Scientific).

Microsatellite marker development and genotyping

DNA from two yellowwood trees on Purdue University's main campus in West Lafayette, IN was pooled to use for microsatellite marker development. Cornell University's

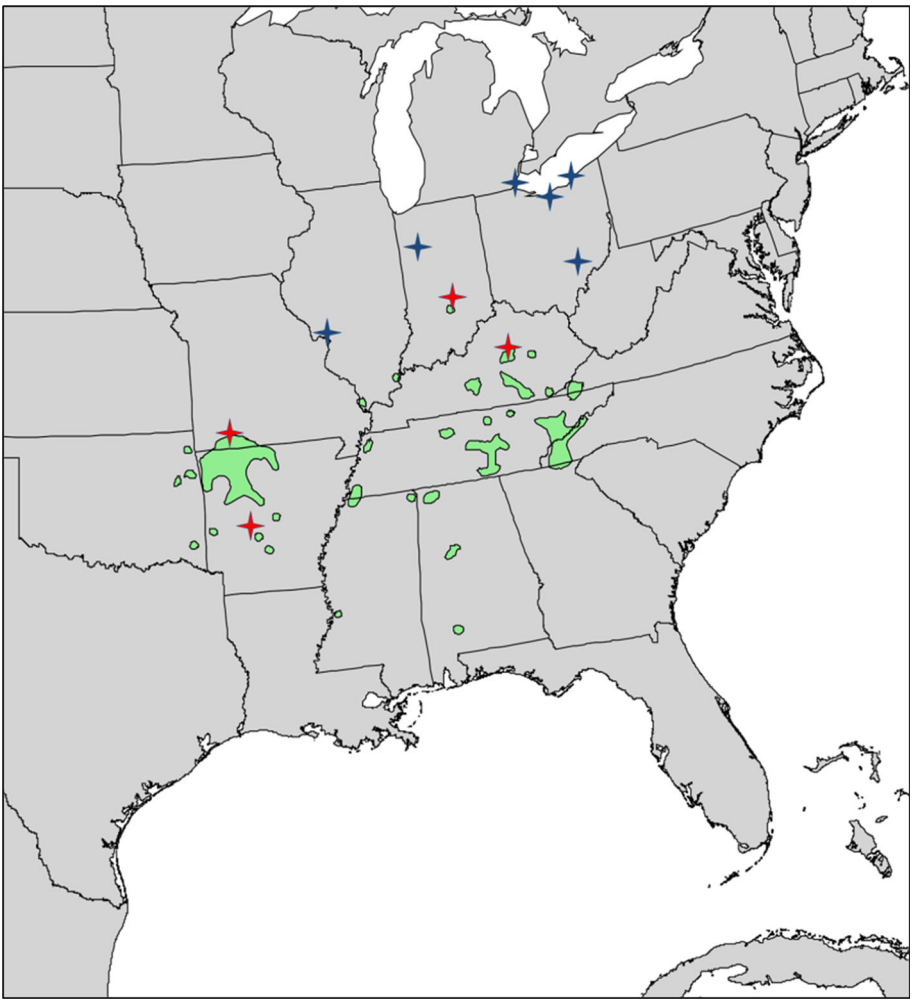

Fig. 1 Map of yellowwood range showing locations of samples from wild stands (*red stars*) and cultivated trees (*blue stars*). (Color figure online)

Evolutionary Genetics Core Facility used the pooled sample to construct a library enriched for nuclear microsatellites and sequenced the library using the Roche 454 platform. The core facility also assembled the reads into ~20,000 contigs with an average length of 300 bp. Contigs were manually examined to identify suitable microsatellite repeat sequences with adequate flanking regions. Primer design was accomplished using the Primer3 web tool (Koressaar and Remm 2007). Markers were amplified using M13-tagged primers and fluorescent dyes (NED and HEX) in a three-primer reaction (Schuelke 2000). Eleven loci amplified consistently, were polymorphic, and were used for genotyping (Table 1). However, after removing loci with large amounts of missing data and low levels of polymorphism, only six loci were used for the final analysis. The full sequences of these six loci are stored at GenBank with consecutive accession numbers KY230374-KY230379. PCR products were genotyped using an Applied Biosystems 3730xl platform at the Purdue

Table 1 Microsatellite loci used for genotyping

Name	Motif	Primers	Length (bp)	N_A	H_O	H_E
321	GA	F: GTTGTCACCCTTGGCTCAAC R: GGAGGAGGCCTTGTTTCTTC	321	21	0.403	0.739
796	GTT	F: AGAAACTGCCCAAGGGATG R: AACGATGTATTTCTATCAAGAGCTG	162	16	0.601	0.69
1000	GTT	F: CCACATGCTGTACCCAACAA R: GTTGTGTCGTGTGGCAGTTT	268	9	0.322	0.407
1192	GTT	F: TGATTAGCACAATTAGTCCTTTGG R: CCCGAGGATACCCTTACCAT	351	12	0.487	0.577
4257	GT	F: CAGCATTTTCATCTCTCGGTCT R: CATTTCTGCAAGCGTTTATTGA	245	12	0.465	0.619
6018	GAA	F: CTTCCGGAATGAGGAAATCA R: CGGAACCCGTGTGTCTTC	363	21	0.377	0.473

N_A number of observed alleles, H_O observed heterozygosity across 6 samples, H_E expected heterozygosity

Genomics Core Facility and Genemapper v3.7 (Applied Biosystems) was used to manually score microsatellite alleles.

Data analysis

Allelic richness was calculated with rarefaction to adjust for sample size using HPRar (Kalinowski 2005). Other genotype summary statistics (F_{ST}, inbreeding coefficient, Nei's unbiased genetic distance) were calculated using GenAlEx 6.5 (Peakall and Smouse 2012). Structure (Hubisz et al. 2009) was used as an alternative assessment of population differentiation. Structure's simulation settings were as follows: K set from 1 to 12; 15 iterations; 1,000,000 MCMC reps with 500,000 discarded as burn-in; admixture model true; and default settings for other parameters. Structure simulations were conducted separately for the entire dataset, for the Indiana trees alone, and for the complete dataset with Indiana trees removed. The most likely value of K was determined by using the web program Structure Harvester (Earl and vonHoldt 2012), which calculates delta K using the method of Evanno et al. (2005).

Results

Genetic diversity

Allelic richness, after adjusting for sample size, varied widely across sampled yellowwood populations (Table 2), from 3.17 (Missouri) to 6.17 (Kentucky) and 9.97 for the sample of urban trees. Private alleles were observed in every population, but Kentucky again had the greatest richness of private alleles among wild samples (0.97). Private allelic richness for the sample of cultivated trees was 3.71.

Observed heterozygosity was lower than expected heterozygosity in every population sampled, including cultivated trees (Table 2), ranging from 0.26 (Missouri) to 0.51 (Indiana) in natural stands and 0.56 for cultivated trees. The fixation index (F) was highest for

Table 2 Allelic richness and heterozygosity in sampled yellowwood populations

Sample	n	N_A	$N_{A(private)}$	H_O (SE)	H_E (SE)	F
AR	24	5.67	0.94	0.382 (0 076)	0.544 (0.103)	0.251
IN	106	5.46	0.7	0.511 (0.059)	0.639 (0.064)	0.185
KY	26	6.17	0.97	0 494 (0 053)	0 570 (0 069)	0.106
MO	24	3.17	0.57	0.257 (0.072)	0.424 (0.123)	0.312
URB	61	9.97	3.71	0.568 (0.083)	0.743 (0.074)	0.243

Rarefaction was used to adjust N_A for sample size n number of individuals in sample; N_A allelic richness, $N_{A(private)}$ private allelic richness, H_O average observed heterozygosity across 6 loci with standard error, H_E expected heterozygosity across 6 loci, F fixation index

the Missouri wild population (0.43) and lowest for the Kentucky population (0.11). F_{IS}, the inbreeding coefficient, ranged from 0.15 in Kentucky to 0.41 in Missouri.

Heterozygosity was calculated for trees in different size classes in Indiana to test for generational differences in heterozygosity. Of the 106 trees sampled in Indiana, 43 had diameters less than 4 inches (8.5 cm) and were counted as seedlings. Seedlings had a slightly lower heterozygosity across loci (0.492) than adult trees >4 inches diameter (0.524), although at two loci the observed heterozygosity for seedlings was slightly higher. Some very large, presumably old trees were also sampled, with diameters up to 29 inches (71 cm). Trees greater than 12 inches diameter (n = 25), which is fairly large for yellowwood, actually had slightly lower heterozygosity (0.480) than younger trees (0.521).

Population differentiation

F_{ST} among wild populations ranged from 0.114 between Indiana and Kentucky to 0.234 between Kentucky and Missouri (Table 3). When Structure was used to assess population differentiation, delta K supported K = 2 when IN, KY, AR, MO and cultivated trees were analyzed together, with one cluster containing MO trees and the second containing all others. When Indiana trees were analyzed on their own, K = 3 was supported, and when the dataset without the large Indiana sample was analyzed, K = 6 was supported, with AR, KY, MO separated along with three clusters from the urban sample. Within Indiana, three genetic clusters were identified among the six individual stands sampled (Fig. 2). Although each genetic cluster was represented in most stands, the prevalence of a given genetic

Table 3 Pairwise F_{ST} (below diagonal) and Nei's unbiased genetic distance (above diagonal) values for six samples of yellowwood

	IN	AR	KY	MO	URB
IN	0	0.795	0.482	0.694	0.5
AR	0.162	0	0.805	0.574	1.371
KY	0.114	0.183	0	0.896	0.53
MO	0.186	0.194	0.234	0	1.276
URB	0.084	0.174	0.104	0.211	0

All values were significantly different from zero ($p < 0.01$) based on 999 permutations of the data

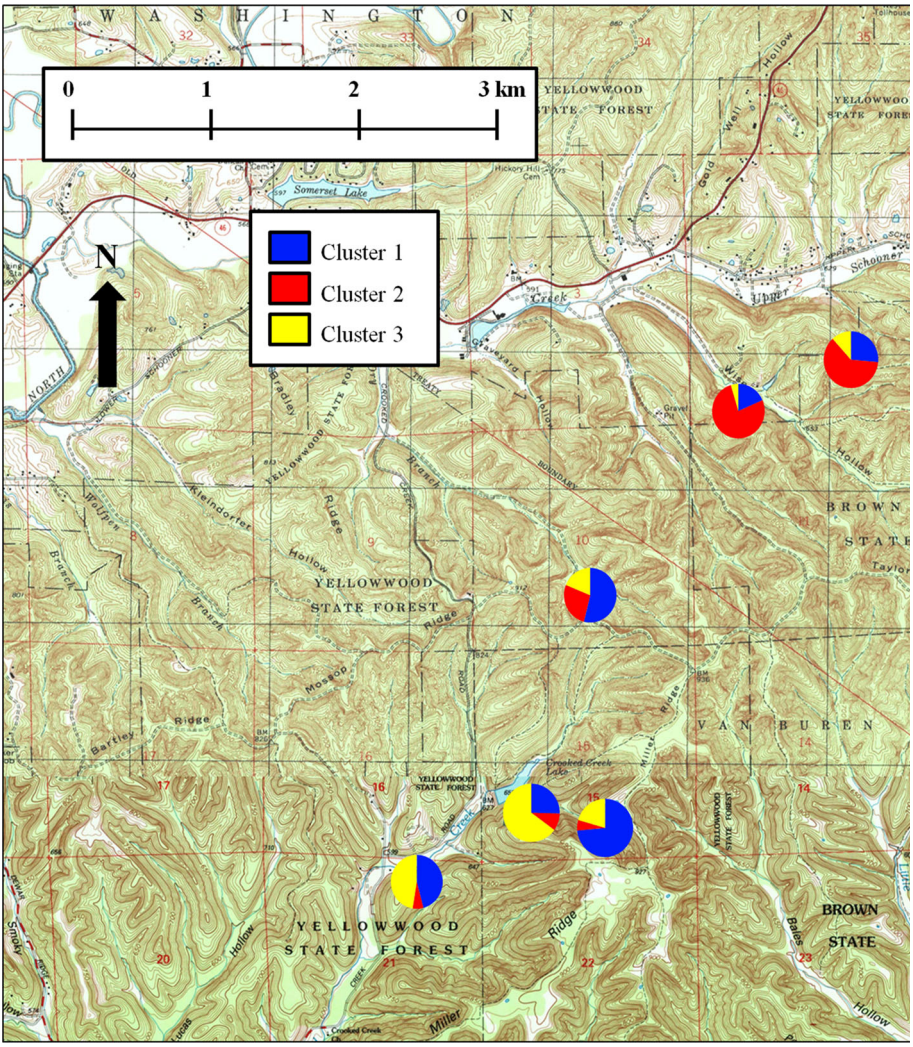

Fig. 2 Genetic clusters identified by STRUCTURE among Indiana yellowwoods and their distribution among six sampled stands

cluster varied with the geographical proximity. When F_{ST} was calculated among the six Indiana stands, stands that were farthest apart had F_{ST} values ranging from 0.037 to 0.074. An AMOVA of the data in GenAlEx indicated that 5% of the genetic variation in Indiana yellowwood is among stands rather than among and within individuals.

Discussion

Genetic diversity and gene flow are important for the long-term viability of populations and species because they provide a basis for populations to adapt to changing environmental conditions. Because they have long lives and can disperse pollen and seeds a great

distance, forest trees may endure population declines, habitat disruption, and poor recruitment without suffering serious declines in genetic diversity, heterozygosity, or gene flow. Red elm (Brunet et al. 2016), butternut, and American chestnut (*Castanea dentata*) have all suffered enormous population declines due to introduced pathogens, but maintain relatively high genetic diversity. In one study, American chestnut retained an average of 17.7 alleles per microsatellite locus and an average heterozygosity of 0.8 (Kubisiak and Roberds 2005), while butternut maintained a heterozygosity of 0.75 at seven microsatellite loci (Ross-Davis et al. 2008). Much of the eastern North American forest outside of rugged mountain terrain was logged and converted to agriculture c. 1800–1900 C.E., and in most areas the regenerated forest is highly fragmented, but there is little or no genetic signature of this demographic history in even the most heavily and systematically exploited species, such as eastern white pine (*Pinus strobus*) (Marquardt et al. 2007) and black walnut (*Juglans nigra*) (Victory et al. 2006). Flowering dogwood (*Cornus florida*), an understory tree, also has maintained high genetic diversity and low differentiation in the face of habitat loss and an introduced disease, dogwood anthracnose (Hadziabdic et al. 2010).

Based on our results, yellowwood is an exception to this rule. The yellowwood populations we sampled showed signficant differentiation, and particularly in the Missouri population, depressed heterozygosity. Compared to other forest trees, yellowwood's allelic richness was low. Differentiation between populations was moderate based on F_{ST} values and Nei's unbiased genetic distance, and much stronger than differentiation observed in other hardwood species. The reason for this strong differentiation may be related to yellowwood's reproductive biology and ecology. Yellowwood is insect-pollinated, and some insect-pollinated species in eastern North America, such as black locust (*Robinia pseudoacacia*) (Chang et al. 1998) and flowering dogwood (*Cornus florida*) (Hadziabdic et al. 2010) show low levels of population differentiation and high genetic diversity. Black locust, despite the similarities of its flowers to yellowwood, is a fairly common canopy tree. Yellowwood, although it occasionally reaches a codominant position, tends to exist as an understory tree in well-developed stands. Hamrick and Godt (1996) noted that stature, and its effects on seed and pollen dispersal, could explain some of the low genetic differentiation found in trees. Few North American understory trees have been subjected to population genetics studies. Most widely-distributed insect-pollinated understory trees exhibit high genetic diversity and gene flow, such as serviceberry (Campbell et al. 1999) and flowering dogwood, for which an AMOVA showed less than 3% of genetic variance occurred among populations (Hadziabdic et al. 2010). In contrast, in umbrella magnolia (*Magnolia tripetala*), a mid-sized tree with insect-pollinated flowers and a patchy distribution very similar to and nearly overlapping yellowwood's, strong differentiation ($F_{ST} > 0.4$) exists between some populations, and genetic diversity is not uniformly high (Gilkison 2013). Genetic differentiation among populations was also strong (Nei's $D > 0.7$) in a study of *Magnolia sieboldii* ssp. *japonica*, a rare small tree found in Japan (Kikuchi and Isagi 2002). *Sorbus torminalis,* a rare, small-stature, insect-pollinated tree, displays differentiation over short distances ($F_{ST} = 0.12–0.42$) near its northern range limit in Denmark (Rasmussen and Kollmann 2008). It seems likely that a combination of reproductive biology and highly specific site requirements for establishment are the main causes for limited gene flow in wild yellowwood. Yellowwood's rarity and patchy distribution is somewhat puzzling, considering that it grows with ease in cities and suburbs far outside its native range, and the calcareous soils it prefers are widely distributed in eastern North America. At the seedling stage, however, yellowwood may require specific conditions for establishment that are only rarely present on the landscape. It is also possible that yellowwood's seeds do not disperse widely enough to allow established stands to colonize

potentially suitable habitat nearby. Little is known about seed dispersal in yellowwood, but seeds are borne in papery, persistent pods and appear to be wind-dispersed.

Our largest sample of wild trees came from Indiana, and this sample included trees from six distinct stands within the Yellowwood State Forest, scattered along an 8-mile stretch of a ridge feature. F_{ST} among these stands indicate weak differentiation, comparable to values (0.03–0.05) observed for scattered stands of butternut, separated by up to 30 miles, in different parts of Great Smoky Mountains National Park (Parks et al. 2014). The AMOVA of this data indicates that some gene flow does occur between these separate stands. Our samples from other states were too small to test for this kind of small-scale genetic structure, but given the rarity of yellowwood throughout its range it is possible that many yellowwood stands rarely exchange genetic material even among their nearest neighbors.

If yellowwood's range in the wild is restricted by its inability to establish as a seedling outside of narrow environmental parameters, it is possible that its scattered distribution and lack of gene flow among populations is partly the result of forest clearing during settlement of the eastern and Midwestern United States. Clear-cutting, conversion to agriculture, and subsequent soil erosion may have destroyed many yellowwood stands that have not been re-colonized, but it seems doubtful that the strong genetic differentiation we observed in surviving stands is a result of this history. Yellowwood is a long-lived organism. It is possible that some of the oldest trees alive today were young trees during settlement, and it is unlikely that the genetic differences we observed could have accumulated in the few generations since then. It is possible that yellowwood self-pollinates; this would help isolated populations reproduce and survive, but accelerate the process of genetic differentiation. If selfing is widespread in yellowwood stands, we would expect to see a loss of heterozygosity in younger trees, compared to older trees. While it seems likely that declines in heterozygosity over time are occurring in more isolated populations, like the one we sampled in Missouri, there is no evidence that younger trees in Indiana are the offspring of extensive selfing or that their heterozygosity is lower than that of their parents.

The sample of cultivated yellowwood trees from urban areas that we analyzed had higher genetic diversity than any of the natural populations sampled. It appears to meet the genetic conservation criterion of allelic capture (Cibrian-Jaramillo et al. 2013), and exceeds the baseline genetic diversity of the wild yellowwood populations we sampled. If cultivated yellowwood was derived from just a few individual trees, it would be expected to have low genetic diversity and would be a poor germplasm bank for ex situ conservation. This does not appear to be the case. Instead, it seems likely that cultivated yellowwood is derived from a number of independent collections, and that its unusually high alleleic richness is the result of mixing genetic material from long-separated wild populations. The large number of private alleles in the urban samples indicates that we did not sample any source populations for the urban stock, although the Kentucky and Indiana samples were more similar to the urban trees It is likely that some of the parent trees used to produce cultivated yellowwood planting stock were from the core of yellowwood's range west of the Appalachians in Kentucky and, particularly, in Tennessee. Therefore, the high allelic richness we observed in urban trees might be due partly to sampling from wild populations that have maintained greater genetic connectivity, and higher allelic richness, than the peripheral populations we sampled.

There appears to be some genetic structure within the urban yellowwood population. The software STRUCTURE identified three genetic clusters; two were a mixture of trees from different states (one mostly Indiana and Missouri, the other mostly Ohio) and the third was only found in Missouri. This differentiation may be the result of different nurseries using different seed sources and supplying different regions, or it may be related

to when a tree was planted. Municipalities routinely purchase their trees from a few large nurseries who, in turn, purchase tree seeds from a handful of seed brokers. Most wholesalers of tree seeds do not ask for or keep records of where their seeds originated, but at least one vendor does list its source for yellowwood as "Tennessee." It is possible that at least one of the genetic clusters of urban trees reflects a Tennessee source population. The Indiana sample consisted mostly of older trees growing around the Purdue university campus, which all belonged to one genetic cluster, while the two recently planted trees belonged to a different cluster that was dominated by urban trees growing in Ohio. Heterozygosity was uniformly high (0.51–0.58) in the three urban clusters relative to the wild samples, but the inbreeding coefficients were slightly higher than the least-inbred wild populations.

What is the potential conservation value of the enormous population of yellowwood growing in urban areas? As the field of ecological restoration has developed, practitioners have championed the use of local seed from remnant populations to re-plant restoration sites. This is done to ensure that the plants are well-adapted to local conditions, and to ensure that local genetic diversity of the restored species is preserved in restoration plantings (e.g. Krauss et al. 2013). Using this criterion, the urban trees we sampled would not be suitable for restoration projects in any of the states where we sampled wild trees: their original source is unknown, and they were clearly distinct genetically from all the wild populations we sampled. There are practical considerations for yellowwood, however, that could give the urban trees some value as an impromptu ex situ conservation collection. First, restoration using local seed is only possible if a local seed source is available. In areas at the edge of yellowwood's range, there may be no local seed source. Second, even if the local source is in an adjacent county, it may be genetically distinct, as we observed in the Indiana stands. Essentially, it is possible that a yellowwood would be as genetically similar to a random tree from an urban planting as it is to a tree from a separate stand in a neighboring county. Third, local adaptation may not be crucial to yellowwood's success, as it seems to be limited not by climate (it can naturalize in areas far north of its natural range) but by a combination of highly specific site requirements for seedling establishment. Unlike species with more extensive ranges and less specific site requirements, like white oak or black walnut, the sites where yellowwood grows naturally in Arkansas are very similar (in terms of soils and microclimate) to the sites where it is found in Tennessee or Indiana. A lack of local adaption might not impede yellowwood restoration as much as it would many other species. Fourth, some natural stands, like the one we sampled in Missouri, may be so isolated and inbred that they would not produce high-quality seed for restoration projects.

Conclusion

Using six neutral nuclear markers, we observed variable heterozygosity and inbreeding coefficients in wild yellowwood populations in four states, and relatively strong differentiation among stands in different states. Among stands a few kilometers apart in Indiana there was evidence of genetic differentiation. Based on these results, we conclude that gene flow between yellowwood populations is limited, even on small spatial scales. It is likely that self-pollination and inbreeding occur in isolated yellowwood stands. The cultivated population of yellowwood, based on samples from three states, contains greater genetic diversity than natural stands of yellowwood. We conclude that the cultivated population of

yellowwood could serve as a valuable reservoir of genetic diversity for yellowwood conservation in cases where using locally-sourced seed for restoration is not possible, or in areas where the genetic diversity of natural populations has been reduced by inbreeding.

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