Evaluation of Pecan [*Carya illinoensis* (Wangenh.) K. Koch] Germplasm Collections and Designation of a Core Subset

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Most plant scientists are aware of the rapid loss of biological diversity in particular habitats, such as Ecuador or Brazil. We often associate the threat to biodiversity with places where the situation is extreme. Even in extreme situations, opinions vary concerning appropriate management to preserve genetic resources (Fearnside, 1989, 1993; Long, 1992; Stepney, 1990). The subject often degenerates into a debate concerning whether to take the "holistic approach" to conservation and attempt to save everything, or to take a "market-place approach" that values only what sells (Namkoong, 1992). To avoid crisis situations, we must manage our genetic resources wisely, recognizing that future generations are impacted by our decisions. To accomplish that objective, we must see through the oversimplification represented by the extreme management alternatives. Plant genetic resources can be characterized within the context of geographic origin, and reasonable strategies to maintain, develop, and use them can be devised. The National Plant Germplasm System (NPGS) provides a national framework for a portion of that work, in relation to selected crop plants and their relatives. Traditionally, the NPGS has pursued the strategy of ex situ conservation to increase access to plant material for systematic evaluation, maintenance, and dissemination to users. Repositories of the NPGS were recently requested to address suitability of the core concept as a management tool.

The core concept, as elucidated by Brown (1989), is a series of management decisions for subdividing genetic collections. The primary justifications for creating the core subset are to 1) make the diversity of the collection more quickly and completely accessible to germplasm users; 2) reduce the number of accessions needed to develop testing procedures, while maintaining confidence that the reduced sample size encompasses the total collection variability; 3) facilitate evaluation and genetic characterization of the collection; and 4) facilitate general management. We assessed the core concept to determine if it would be a useful management tool in the evaluation and management of collections in the National Clonal Germplasm Repository for Pecans and Hickories.

This repository was designated in 1984. However, the collection of pecan cultivars that forms the basis of the repository was begun in the 1930s by Louis Romberg. He actively

acquired pecan cultivars as parents for use in controlled crosses in the early pecan breeding program. Over the years, cultivars have been acquired as they were deemed useful for the breeding program.

The Pecan Crop Advisory Committee has recognized the need to increase genetic diversity in the collection, while maintaining the utility of the collection for use in the breeding program. The strategy undertaken differentiates the pecan collection into two groups: the cultivar collection and the provenance collection. The cultivar collection is composed of selected cultivars propagated by the pecan industry, historically and currently. This collection is maintained as grafted trees in several orchards at Brownwood, Texas, with some duplicates maintained in orchards near College Station, Texas. Since most pecan cultivars can be recognized on the basis of nut characteristics, nut samples are maintained as voucher specimens for individual trees in the

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Fig. 1. Distribution of native pecan trees (shaded), showing locations of origin of cultivars in the pecan cultivar collection ●, with sites of origin for many cultivars shown by □. Collection sites for the provenance collection are indicated by ★.

cultivar collection and are verified by comparison to nut samples collected from other trees of the cultivar. The provenance collection is composed of seedlings grown from seed collected from native pecan trees throughout the range of the species. Many of these seedlings are growing in orchards either in Brownwood or near College Station. Others are being temporarily maintained in a screenhouse at College Station. We cannot indefinitely maintain the entire provenance collection, as analyzed and reported on in this paper, but rather consider it a short-term tool for characterizing genetic diversity in the species. In addition to the repository's pecan collections, other *Carya* species were targeted for collection across their ranges (Grauke, 1989).

The U.S. Dept. of Agriculture (USDA)–Agricultural Research Service Pecan Breeding Program is the primary user of the collection. Requests for germplasm are predominantly for graftwood of specific pecan cultivars, rather than for multiple accessions that represent species diversity. Occasionally, we receive requests for seed of particular cultivars. Accessions of the provenance collection are not maintained for dissemination but are used in evaluation research. Requests for other *Carya* species are generally for seed and are filled only when seasonal collections from native populations have made seed available, since the hickory seedlings in the repository are juvenile.

The repository pecan collections have enough accessions (480) to merit subdivision to increase accessibility. Pecan accessions are considered as part of either the cultivar collection or provenance collection. Accessions in the cultivar collection are the primary target for evaluation in relation to the core strategy because they are maintained separately and are the only ones currently available for dissemination. Evaluation of the provenance collection is presented here to provide insight into the adequacy of the cultivar collection in representing genetic diversity of pecan.

Cultivar collection

The Colorado River of Texas has been a preeminent site for pecan cultivar development (Fig. 1). Other outstanding "nurseries" have been Jackson County, Miss., and San Saba and Brownwood, in Texas (Thompson and Grauke, 1991).

The collection contains more cultivars that originated in Texas than in any other state (Table 1), partly because the collection has always been maintained in this state. Also, Texas has produced more named pecan cultivars than any other state, according to Thompson and Young (1985). These authors provided historic passport information for all known pecan cultivars in relation to state of origin (Table 1). Since both authors worked in Texas, these numbers may reflect their greater familiarity with local cultivars.

If Thompson and Young (1985) accurately described the "universe" of pecan cultivar germplasm, then the cultivar collection may be a fairly representative sampling of that

Table 1. Frequency distribution of the pecan cultivar collection and core subset in relation to state of origin, as compared to total cultivars known from each state, based on Thompson and Young (1985).

State	Cultivar collection		Thompson and Young		Core subset	
	No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)
Alabama	5	2.2	11	1.4	1	3.8
Arizona	2	0.9	3	0.4	0	0
Arkansas	2	0.9	5	0.6	0	0
California	1	0.4	6	0.8	0	0
Connecticut	0	0	1	0.1	0	0
Florida	10	4.4	61	8.0	2	7.7
Georgia	9	3.9	50	6.5	2	7.7
Illinois	6	2.6	51	6.7	2	7.7
Indiana	2	0.9	9	1.2	1	3.8
Iowa	2	0.9	17	2.2	1	3.8
Kansas	4	1.7	10	1.3	1	3.8
Kentucky	3	1.3	15	2.0	1	3.8
Louisiana	25	11.0	88	11.5	3	11.5
Michigan	1	0.4	1	0.1	0	0
Mississippi	25	11.0	67	8.8	3	11.5
Missouri	8	3.5	20	2.6	1	3.8
New Mexico	4	1.7	7	0.9	0	0
New York	0	0	1	0.1	0	0
N. Carolina	2	0.9	5	0.6	0	0
Oklahoma	14	6.1	26	3.4	1	3.8
S. Carolina	1	0.4	6	0.8	0	0
Tennessee	0	0	1	0.1	0	0
Texas	101	44.3	300	39.3	7	26.9
Utah	0	0	1	0.1	0	0
Virginia	1	0.4	2	0.3	0	0
Total	228		764		26	

universe, as indicated by the percentage of composition of the repository by state, relative to that state's contribution to total cultivar numbers (Table 1). Collections of cultivars from Illinois, Florida, and Georgia are possibly underrepresented, according to this analysis, while cultivars originating in Texas and Oklahoma may be overrepresented. In selecting entries for the core subset, broad geographic representation was sought (Table 1).

Of the 228 accessions of the cultivar collection, 182 have been evaluated for isozymes at five loci (Tables 2 and 3). Four loci [phosphoglucose mutase (*Pgm-1*), phosphoglucose isomerase (*Pgi-2*), malate dehydrogenase (*Mdh-1*), and leucine aminopeptidase (*Lap*)] are amenable to genetic interpretation (Marquard, 1987, 1989, 1991; Marquard et al., 1995). The fifth, diaphorase (*Dia*), has been scored for the presence or absence of a single high band, but genetic interpretation is not complete (Marquard et al., 1995).

Core subset

Stratified sampling (Brown, 1989) was used to develop a core of 26 cultivars (11% of the collection) (Table 4). Cultivars were selected with reference to geographic origin (Table 1). Documented direct selections from native populations were preferred over seedlings of more dubious origin.

Entries were chosen so that genotype and allele frequencies closely resemble those found in the cultivar collection (Tables 2 and 3). The D allele of *Pgi-2* is not represented in the cultivar collection and does not occur in the core subset. Rare alleles, such as the B allele of *Pgm-1* and the D allele of *Mdh-1*, are necessarily represented in slightly greater frequency in the core than in the cultivar collection.

Efforts were made to represent a broad

spectrum of horticultural characteristics. The most heavily selected horticultural features in pecan relate to nut characteristics. Nut shape varies from the oblong nut of 'Mahan' (2.5 nut length : width ratio) to the round nut of 'Burkett' (1.23 nut length : width ratio). Nut weight ranges from 3.9 ('Patrick') to 12.9 g/nut ('Mahan'). Kernel yield varies from 43% per nut ('Devore') to 62% ('Carmichael'). Precocity varies from the very precocious 'Evers' to 'Stuart', a cultivar that is notoriously slow to bear. Patterns of nut maturity represent the entire season of pecan harvest, from the very early maturing cultivars ('Best's Early', 'Devore', 'Hirschi', and 'Evers') to the late-season types ('Sumner', 'Greenriver', and 'Western'). Cultivars vary in resistance to pecan scab [*Cladosporium caryigenum* (Ell. et Lang.) Gottwald], from the very susceptible 'John Garner' to the very resistant 'Sumner'.

The number of accessions in the core subset is extremely small and reflects the cultural constraints facing pecan orchard establishment. Propagating just two trees of 26 cultivars would require ≈0.6 ha for the life of the collection, if trees are planted on the standard 10.7×10.7-m spacing. Requests for germplasm are predominantly for graftwood of specific pecan cultivars, rather than for multiple accessions that represent diversity of the species. However, larger collections that are less representative of the genetic diversity of pecan have been sent to other countries, such as Turkmenistan, at the request of researchers. The designation of a core subset may influence the choice of cultivars recommended for such occasions.

Provenance collection

Seeds of native pecan trees have been collected from populations ranging from Illinois

Table 2. Frequency distribution of isozymes of *Pgm-1*, *Pgi-2*, *Mdh-1*, *Lap*, and *Dia* in the cultivar and provenance collections and core subset of the NCGR-BRW.

Enzyme	Genotype	Cultivar collection		Provenance collection		Core subset	
		No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)
<i>Pgm-1</i>	AA	164	90.1	736	98.8	24	92.3
	AB	5	2.8	1	0.1	0	0
	AC	12	6.6	8	1.1	1	3.8
	BC	1	0.5	0	0	1	3.8
<i>Pgi-2</i>	AA	41	22.5	122	16.4	6	23.1
	AB	56	30.8	193	25.9	8	30.8
	AC	25	13.7	115	15.4	3	11.5
	AD	0	0	19	2.5	0	0
	BB	35	19.2	85	11.4	5	19.2
	BC	24	13.2	112	15.0	3	11.5
	BD	0	0	16	2.1	0	0
	CC	1	0.5	73	9.8	1	3.8
	CD	0	0	8	1.1	0	0
	DD	0	0	3	0.4	0	0
<i>Mdh-1</i>	AA	48	26.4	301	40.7	6	23.1
	AB	80	44.0	302	40.9	12	46.2
	AC	7	3.9	27	3.7	1	3.8
	AD	0	0	0	0	0	0
	BB	42	23.1	104	14.1	5	19.2
	BC	3	1.6	5	0.7	1	3.8
	BD	2	1.1	0	0	1	3.8
	CC	0	0	0	0	0	0
	CD	0	0	0	0	0	0
	DD	0	0	0	0	0	0
<i>Lap</i>	AA	10	5.5	22	3.0	2	7.7
	AB	56	30.8	196	26.3	7	26.9
	BB	116	63.7	526	70.7	17	65.4
<i>Dia</i>	—	155	85.2	714	98.1	22	84.6
	+	27	14.8	14	1.9	4	15.4

and Missouri south to Oaxaca, Mexico, and from Alabama west to Val Verde County, Texas (Grauke, 1990; Grauke et al., 1989) (Fig. 1). Seedlings arising from these collections have been analyzed for isozymes as described above. The frequency distributions of isozymes of *Pgm-1*, *Pgi-2*, *Mdh-1*, *Lap*, and *Dia* are shown in Table 3, for comparison with the frequency distributions of the same isozymes in the cultivar collection.

Despite the much larger number of seedlings analyzed (745 vs. 182), fewer genotypes of *Pgm-1* were recovered in the provenance collection than were present in the cultivar collection. 'Curtis' was the only cultivar with the BC genotype (Table 2). Although the BC genotype was not recovered in the provenance collection, B and C alleles are present (Table 3).

The D allele of *Pgi-2* was recovered in the provenance, but was not found in the cultivar collection (Tables 2 and 3). The D allele was recovered from more than 40 trees in several western Texas populations, where it occurred with greatest frequency and diversity in collections made from Lipan Creek and the Devil's River. The D allele was also recovered, as the AD genotype, from two seedlings that originated from the same Illinois seed stock.

The rarest allele of *Mdh-1* is the D allele, found only in 'Nugget' and its progeny 'Sullivan' as the BD genotype. The allele was not recovered from any seedlings in the provenance collection (Tables 2 and 3). During evaluation of the *Mdh-1* patterns of the provenance collection, unusual patterns of variation were observed in *Mdh-2*, which has consistently been monomorphic for all cultivars in the cultivar collection. Further research is necessary to interpret the variation.

The frequency distribution of *Lap* isozymes was similar in cultivar and provenance collections (Table 3).

The majority of seedlings analyzed in the cultivar and provenance collections lack the high band of diaphorase (Table 2).

In situ conservation

The above analysis demonstrates the value of the provenance collection in characterizing genetic variation within pecan. We are faced by the challenge of limited resources to maintain genetic resource collections. Complementary methods of conservation include the establishment of in situ reserves, where multidisciplinary research could be focused on broader ecological and historical questions. Potential sites for such an effort are on the Devil's River in Val Verde County, Texas, and along the Mississippi River in central Illinois. We have shown those two areas to be the location of the D allele of *Pgi-2*. Texas Parks and Wildlife maintains the Devil's River State Natural Area and is cooperating in the evaluation of pecan populations within their boundaries. The proximity of this Natural Area to Baker's Cave, a significant archaeological site for the study of early humans in Texas (Hester, 1981), adds to its value. Hall (1995) has suggested that Texas native pecan groves were key resources around which territories were established by archaic populations. The native pecan collection site in Illinois from which the D allele of *Pgi-2* was recovered is near Eldred. This is near the Koster Site, where intensive archaeological research has been conducted concerning pecan use by archaic man 8000 year B.P. (Asch and Asch, 1979).

Table 3. Allele frequencies in the pecan cultivar and provenance collections and core subset of the NCGR-BRW.

Locus	Cultivar collection	Provenance collection	Core subset
<i>Pgm-1</i>			
(N)	182	745	26
A	0.948	0.994	0.942
B	0.017	0.001	0.019
C	0.035	0.005	0.038
<i>Pgi-2</i>			
(N)	182	746	26
A	0.448	0.383	0.442
B	0.412	0.329	0.404
C	0.140	0.255	0.154
D	0	0.033	0
<i>Mdh-1</i>			
(N)	182	739	26
A	0.503	0.630	0.481
B	0.464	0.348	0.462
C	0.028	0.022	0.038
D	0.005	0	0.019
<i>Lap</i>			
(N)	182	744	26
A	0.209	0.161	0.212
B	0.791	0.839	0.788

Bettis et al. (1990) noted that "the co-occurrence of early Holocene *Carya* pollen peaks with the arrival of the Dalton artifact complex in the Upper Mississippi Valley suggest that humans may have played a role in the early dispersal of pecan." More detailed genetic evaluation of native pecan populations at archaeological sites may be a useful tool for the continued study of the movements of archaic people, as well as for the characterization of diversity in this important native species.

Pecan production patterns

The primary threat to pecan germplasm diversity is the elimination of native pecan populations. Pecan is a widely distributed North American species (Fig. 1). It occurs on bottomland sites adjacent to rivers and streams, making it vulnerable to elimination as land is cleared for other agricultural uses and to inundation as rivers are dammed. Reliable national inventories of native pecan acreage are not available. One indication of the status of the resource can be obtained from records of the USDA Crop Reporting Service (e.g., USDA, 1936), which has reported the production of native and seedling pecans sold annually since the first decades of this century. There are several problems associated with the interpretation of these records. In addition to questions of accuracy (records are based on accumulator surveys), the records may be subject to economic bias. In years of heavy crops, low prices may reduce the incentive for complete harvest. Records from years of low production may be more accurate, as the higher prices offered encouraged marketing. Furthermore, many "wild" trees occur in areas inaccessible to harvest in any year. Crop Reporting Service records of "native" pecan production include production from seedling trees in states where natives do not occur. To better reflect true native production, the records of "native" production from Georgia, Florida, North Caro-

Table 4. Entries in the pecan core subset, with state of origin and isozyme genotypes of *Pgm-1*, *Pgi-2*, *Mdh-1*, *Dia*, and *Lap*.

Cultivar	State of origin	Isozyme				
		<i>Pgm-1</i>	<i>Pgi-2</i>	<i>Mdh-1</i>	<i>Dia</i> high band	<i>Lap</i>
Kernodle	Alabama	AA	AB	BB	—	AB
Curtis	Florida	BC	AB	AB	—	AA
Moore	Florida	AA	AB	AB	—	BB
Brooks	Georgia	AA	AC	AB	—	AB
Sumner	Georgia	AA	AA	AB	—	AB
Best's Early	Illinois	AA	AC	AB	—	AB
Hodge	Illinois	AA	AA	AB	—	BB
Posey	Indiana	AA	AB	AB	—	AB
Devore	Iowa	AA	BB	AA	—	BB
Johnson (KS)	Kansas	AA	BC	AA	—	AB
Greenriver	Kentucky	AA	CC	AA	—	BB
CS-14	Louisiana	AA	BB	BC	+	BB
Frotscher	Louisiana	AA	BB	AA	+	BB
Van Deman	Louisiana	AA	AA	AB	—	BB
Alley	Mississippi	AC	BC	AB	—	BB
Mahan	Mississippi	AA	AB	BB	+	AB
Stuart	Mississippi	AA	BB	AC	+	BB
Hirschi	Missouri	AA	BB	AB	—	BB
Patrick	Oklahoma	AA	AB	AA	—	BB
Burkett	Texas	AA	BC	AB	—	BB
Carmichael	Texas	AA	AA	BB	—	BB
Evers	Texas	AA	AB	AB	—	BB
John Garner	Texas	AA	AC	AA	—	BB
Nugget	Texas	AA	AA	BD	—	BB
Texas Prolific	Texas	AA	AA	AB	—	BB
Western	Texas	AA	AB	BB	—	AA

kansas is represented by only two accessions in the cultivar collection, and was not an area for the provenance collection, seed nuts were collected from Arkansas native pecans along the Arkansas, Red, St. Francis, and White rivers in 1994. Analysis of seedlings from those collections may contribute to our understanding of genetic diversity in the remaining populations of Arkansas native pecans. Seed collection sites for use in State Forest Commission conservation programs could be influenced by that information. Native pecan populations of Arkansas initiate bud growth during May, when the area typically receives heavy rainfall (Baldwin, 1975). Such weather creates pressure for the development of scab disease [*Cladosporium caryigenum* (Ell. et Lang.) Gottwald] (Demaree, 1924). If the trees have adapted to that pressure by developing resistance to the disease, Arkansas trees may provide a reservoir of useful genetic diversity that could contribute to the Pecan Breeding Program. Pecan cultivars originating in the humid southeastern United States have been the primary source of resistance to scab (Thompson and Grauke, 1991). Future cultivars may benefit from the inclusion of Arkansas native pecan germplasm into working collections of the Pecan Breeding Program.

Fig. 2. United States production of grafted and native pecan nuts in million kilograms, from 1944 to 1994, showing the 10-year moving average for each.

lina, South Carolina, and Alabama were subtracted from U.S. total native production, leaving only the records of states that produce true native pecans (Fig. 2). The records from 1944 to 1994 indicate a peak in native production during the early 1970s, followed by a steady decrease. Total native production is currently below that reported in the early 1940s.

As native pecan production has decreased, production from grafted pecans has increased (Fig. 2). The pattern of increasing grafted pecan production indicates a dynamic, growing, improved pecan industry, despite erratic production in the early years of this decade.

About 57% of U.S. grafted pecan production is due to just four cultivars: 'Stuart' (22%), 'Western Schley' (15%), 'Desirable' (11%), and 'Wichita' (10%) (Thompson, 1990). The trend of increasing grafted tree acreage and decreasing genetic diversity demands attention.

Records of native pecan production since 1934 were analyzed for four native pecan producing states: Texas (Fig. 3a), Oklahoma (Fig. 3b), Louisiana (Fig. 3c), and Arkansas (Fig. 3d). Despite the erratic pattern of production shown, only Arkansas shows a precipitous decrease in native production. Since Ar-

Summary

We have gained valuable information concerning the status of pecan trees in native populations, and regarding the efficiency of our collections in representing and preserving that diversity. This evaluation validates the strategy of collecting and evaluating seedlings from native populations as well as grafted cultivars. Native populations are largely untapped resources that are under severe threat in some areas. We will continue to collect and evaluate native pecan populations using the fastest and most reliable methods of assessing diversity. Seedlings found to have unusual patterns of diversity in those evaluations can be incorporated into the ex situ collection for further study. The native populations from which they originated may merit consideration as in situ reserves, where multidisciplinary research could address broader questions.

The designation of a core subset may increase access to the diversity of the cultivar collection, facilitating usage and management decisions. The core is not a full representation of the genetic diversity of pecan, given the recognition of alleles in the provenance collection, and the existence of native populations that have not been sampled. The preservation of one generation's allelic array is of no intrinsic value. However, access to a small, diverse, subset of the collection may facilitate development of improved methods of evaluation, or may influence choice of accessions for particular purposes. As we gain information concerning the genetic diversity of pecan, the core subset will be revised to reflect that information. We believe use of the core subset will contribute to the acquisition of information.

Fig. 3. Native pecan production in million kilograms, from 1934 to 1994, in (A) Texas, (B) Oklahoma, (C) Louisiana, and (D) Arkansas. Yearly production = $\blacktriangle$. Moving 10-year average = $\circ$.

Namkoong (1992) noted that, "To provide alternatives for forest management, we must first clarify genetic biodiversity concepts. In particular, since genetic diversity and genetic management systems fundamentally affect stand, species, and community diversities, we must describe the status, structure, and dynamics of genetic diversity within an ecological context." The National Plant Germplasm System is only one member of the multidisciplinary team addressing that challenge. The evaluation of ex situ collections should send us out to native populations with improved management options, and with further questions. We believe the partnership between the

repository's germplasm collections, the Pecan Breeding Program, and the pecan production industry offers an ideal situation for sustained progress.

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