Genetic Diversity and Geographic Differentiation in Pawpaw [*Asimina triloba* (L.) Dunal] Populations from Nine States as Revealed by Allozyme Analysis

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ABSTRACT. As a new National Clonal Germplasm Repository for *Asimina* species at Kentucky State University (KSU), of major concern to us is the genetic variation within our germplasm collection. The present study investigated the extent of genetic diversity for the pawpaw germplasm in our collection and the geographical pattern of genetic diversity among populations using isozyme markers. Allozyme diversity was high in *Asimina triloba* (L.) Dunal (Annonaceae) collected from all nine different states, as is typical for temperate woody perennial, widespread and outcrossing plant species. Averaged across populations, mean number of alleles per locus (A), percent polymorphic loci (P), effective number of alleles per locus (A_e), and expected heterozygosity (H_e) were 1.54, 43.5, 1.209, and 0.172, respectively. Significant deviations from Hardy-Weinberg equilibrium were found in nine populations at an average of 4.8 loci. Observed heterozygosity was higher than expected. Partitioning of genetic diversity showed that 88.2% resided within populations. The proportion of genetic diversity among populations ($G_{st} = 0.118$; $F_{ST} = 0.085$) was either lower than or within the range of those species with similar ecological and life-history traits. The mean genetic identity among populations was high ($I = 0.988$). An analysis using UPGMA clustered most populations as one major group, with the southernmost (Georgia) and the westernmost (Illinois) populations readily separated from the main group. The relationships discovered by principal component analysis (PCA) were similar to those revealed by UPGMA. In addition, PCA separated the northernmost population (New York) from the major group. Sampling strategies for future germplasm collection of *A. triloba* are also discussed.

The pawpaw (*Asimina triloba*) is the largest tree fruit native to the United States, growing wild in 26 eastern states and provinces ranging from northern Florida to southern Ontario, Canada, and as far west as eastern Nebraska (Darrow, 1975; Kral, 1960). The genus *Asimina* is the only temperate representative of the tropical Annonaceae (custard apple family) and comprises nine species (Kral, 1960). *Asimina triloba* and *A. parviflora* (Michx.) Dunal (the dwarf pawpaw) are widespread and inhabit rich, mesic woodlands. The other seven species are restricted to Florida, and the extreme southern portions of Georgia and Alabama (Callaway, 1990). All species are diploid [except *A. pygmaea* (Batt.) Dunal, for which counts have not been reported] and have 18 chromosomes ($2n = 2x = 18$) (Bowden, 1948). Pawpaw has tremendous potential for commercial development as a new crop for many reasons: 1) pawpaw fruit are an excellent food source, high in Vitamin C, potassium, magnesium, iron, copper, manganese, and

several essential amino acids (Jones and Layne, 1997; Peterson et al., 1982); 2) pawpaw produces natural defense compounds (annonaceous acetogenins) in leaf, bark and twig tissues; the compounds possess both highly potent antitumor and pesticidal properties (Johnson et al., 1996; Oberlies et al., 1997a, 1997b; Ratnayake et al., 1992); and 3) pawpaws are ideally suited for the residential edible landscape because of their lush, tropical appearance, attractive conical growth form, size, fall color, and delicious fruit (Layne, 1996).

In 1994, Kentucky State University (KSU) was approved by the USDA-ARS-National Plant Germplasm System to serve as the National Clonal Germplasm Repository for *Asimina* species as a satellite site of the NCGR in Corvallis, Ore. (Layne, 1996). Of initial concern to us was the genetic variation within our germplasm collection. Low levels of genetic variation could hinder the domestication of pawpaw and its development as a new crop.

The pawpaw is a small deciduous tree which naturally occurs in the forest understory of lowland drainage basins adjacent to streams and rivers where it can form extensive thickets (pawpaw patches) because of its predominantly clonal reproductive habit of root suckering (Willson and Schemske, 1980). The strongly protogynous flowers are mainly fly or beetle-pollinated (Faegri and van der Pijl, 1971; Zimmerman, 1941). Fruit production is low and seed dispersal and germination is generally poor in the wild (Lagrange and Tramer, 1985; Willson and Schemske, 1980). Previous work on pawpaw using M13 DNA probe suggested that genetic variation is very low within populations (Rogstad et al., 1991), but species distributed across as wide geographic range as pawpaw might still maintain significant genetic variability among populations (Hamrick and Godt, 1989). Our recent study of allozyme diversity in cultivated pawpaws indicated that they possess an average allozyme diversity similar to other long-lived woody

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perennials of widespread geographic range in temperate regions with insect-pollinated outcrossing breeding systems, secondary asexual reproduction and animal-dispersed seed (Huang et al., 1997). However, little has been reported on the genetic diversity and geographic variation in native pawpaw populations (Huang et al., 1997). An understanding of genetic diversity among and within pawpaw populations is needed to formulate appropriate sampling and management strategies for the KSU *Asimina* spp. repository (Marshall and Brown, 1975). The purpose of the present investigation was 2-fold: 1) to determine the extent of allozyme diversity in the native collection of pawpaw held at KSU and 2) to determine any geographical pattern of genetic diversity among our samples from different sites.

Materials and Methods

PLANT MATERIAL. All samples were taken from 3-year-old seedling trees in the KSU repository. The more than thirty seedlings per single population were grown from open-pollinated seeds that were previously collected from specific sites located in nine different states within the natural range of *A. triloba* (Table 1): Georgia (Taylor county), Kentucky (Lexington, Fayette county), Virginia (Boyce, Fredrick county), West Virginia (Shirley, Tyler county), Indiana (Salem, Union county), Illinois (Benton, Franklin county), Maryland (Wye, Talbot county), Pennsylvania (Lancaster, Lancaster county), and New York (Ithaca, Tompkins county). Tree seed lots from each of the original trees within each population were randomly planted and separated from each other in the repository. For isozyme analysis, random samples of 1-year-old dormant twigs were taken from three to five trees from each seed lot and genotype of the original tree was inferred based on our best judgement. Twenty-five to fifty trees of each of nine populations were used for allozyme analysis (Table 1). The samples were kept in Zip-lock bags at 4 °C until electrophoresis was conducted within two months.

ALLOZYME ANALYSIS. Enzymes were extracted as described by Huang et al. (1994). Allozymes were separated by isoelectric focusing on polyacrylamide gels (pH 4 to 9) as described by Mulcahy et al. (1981). Gels were assayed for acid phosphatase (ACP; EC 3.1.3.2), alcohol dehydrogenase (ADH; EC 1.1.1.1), aconitate hydratase (ACO; EC 4.2.1.3), adenylate kinase (ADK; EC 2.7.4.3), dihydrolipoamide dehydrogenase (DDH; EC 1.8.1.4), esterase (EST; EC 3.1.1.-), formate dehydrogenase (FDH; EC 1.2.1.2), fructose-bisphosphate aldolase (FBA; EC 4.1.2.13), fructose-bisphosphatase (FBP; EC 3.1.3.11), fumarate hydratase (FUM; EC 4.2.1.2), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.42), glutamate dehydrogenase (GDH; EC 1.4.1.2), glyceraldehyde-3-phosphate dehydrogenase (G3PDH; EC 1.2.1.12), hexokinase (HEX; EC 2.7.1.1), isocitrate dehydrogenase (IDH; EC 1.1.1.41), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.40), mannose phosphate isomerase (MPI; EC 5.3.1.8), peroxidase (PRX; EC 1.11.1.7), phosphoglucomutase (PGM; EC 5.4.2.2), phosphogluconate dehydrogenase (PGD; EC 1.1.1.44), phosphoglucoisomerase (PGI; EC 5.3.1.3), shikimate dehydrogenase (SKD; EC 1.1.1.25), and triosephosphate isomerase (TPI; EC 5.3.1.1). Gels were stained according to Wendel and Weeden (1989). Twenty-three presumptive loci were scored: *Acp-1*, *Acp-2*, *Acp-3*, *Adh*, *Ddh-1*, *Ddh-2*, *Ddh-3*, *Est-1*, *Est-2*, *Fdh*, *Mdh-1*, *Mdh-2*, *Me*, *Pgi-1*, *Pgi-2*, *Pgm*, *Prx-1*, *Prx-2*, *Prx-3*, *Prx-4*, *Skd-1*, *Skd-2*, and *Tpi*. Allele designation at these loci followed Huang et al. (1997).

DATA ANALYSIS. A set of measures of intra- and interpopulation genetic statistics was generated using the BIOSYS-1 computer

program (Swofford and Selander, 1981) including percent polymorphic loci (P) (95% criterion), mean number of alleles per locus (A), effective number of alleles per locus (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), total genetic diversity (H_T), genetic diversity within populations (H_s), genetic diversity between populations (D_{st}), relative magnitude of genetic differentiation among populations ($G_{st} = D_{st}/H_T$) (Nei, 1987), and χ^2 test for heterogeneity of allele frequencies among populations. F statistics were also used including the fixation index of individuals within populations (F_{IS}), the fixation index with respect to the total population (F_{IT}) and the proportion of genetic differentiation (F_{ST}) (Wright, 1978). Nei's (1978) genetic distance and identity, as well as the modified Roger's genetic distance (Wright, 1978), were calculated for each pair of populations. A dendrogram was constructed based on the matrix of Roger's distance using UPGMA (unweighted pair-group method using arithmetic averages). Principal component analysis (PCA) was performed based on allele frequency data of populations using PC-SAS for Windows (SAS Institute, Cary, N.C.).

Results and Discussion

GENETIC DIVERSITY IN *A. triloba*. Of the 24 enzyme systems investigated and 23 presumptive loci scored in this study, 49 alleles were detected across populations. Forty-three alleles were found at seventeen polymorphic loci. Averaged across populations, A, P, A_e , and H_e were 1.54, 43.5, 1.209, and 0.172, respectively (Table 2). No significant differences in A, P, A_e , and H_e were observed among populations. The allozyme diversity discovered in this study of *A. triloba* is within the range of temperate woody perennial, widespread and outcrossing plant species. The results also agree with our recent study on genetic variation in cultivated pawpaws (Huang et al., 1997). In comparison to average values of other seed plant species with similar ecological and life-history characteristics (Hamrick and Godt, 1989), *A. triloba* has a higher H_e than the average of long-lived woody perennials, species with wide geographic ranges, insect outcrossing species and species with sexual and asexual reproduction systems (Table 2). *Asimina triloba* has fewer A, when compared to the average of long-lived woody perennials and widespread species; and lower P, when compared to the average of long-lived woody perennials (Table 2). The high level of genetic diversity we observed in *A. triloba* contradicts an earlier report by Rogstad et al. (1991), who found relatively low levels of variability in *A. triloba* populations using one minisatellite DNA repeat (M13). They concluded that clonality and inbreeding might contribute to the low to moderate variability in *A. triloba*. However, several studies of *A. triloba*'s reproductive biology have demonstrated that selfed seed is rare, germinates poorly and the resulting seedlings do not compete well in growth with cross-pollinated seedlings (Norman et al., 1992; Willson and Schemske, 1980).

For the nine populations examined, an average of 4.8 loci (ranging from two to seven per population) out of 17 polymorphic loci deviated significantly from Hardy-Weinberg expectations (data not shown). Observed heterozygosities were higher than that expected under Hardy-Weinberg equilibrium in all populations (Tables 1 and 2). Heterozygote excess was also reflected by negative mean F_{IS} and F_{IT} values (Table 3). A possible explanation for the excess of heterozygotes is likely a combination of *A. triloba*'s predominant clonal reproduction habit which could produce skewed gene frequencies, high outcrossing rates and natural selection against homozygotes. While clonal plant species are commonly found to maintain a moderate level of genetic diversity,

Table 1. Allozyme allele frequencies and contingency χ^2 analysis of heterogeneity of allele frequencies in pawpaw populations from nine states.

Locus ^z	Population									χ^2
	GA (25) ^y	KY (52)	VA (25)	WV (50)	IN (51)	IL (50)	MD (48)	PA (25)	NY (52)	
<i>Acp-2</i>										3.03 ^{ns}
<i>a</i>	0.542	0.568	0.500	0.600	0.500	0.625	0.556	0.688	0.614	
<i>b</i>	0.458	0.432	0.500	0.400	0.500	0.375	0.444	0.313	0.386	
<i>Acp-3</i>										0.00 ^{ns}
<i>a</i>	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	
<i>b</i>	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	
<i>Ddh-2</i>										215.41 ^{**}
<i>a</i>	1.000	0.023	0.050	0.150	0.143	0.000	0.000	0.000	0.000	
<i>b</i>	0.000	0.841	0.950	0.850	0.857	1.000	1.000	1.000	0.818	
<i>n</i> ^x	0.000	0.136	0.000	0.000	0.000	0.000	0.000	0.000	0.182	
<i>Ddh-3</i>										25.36 ^{ns}
<i>a</i>	0.167	0.091	0.150	0.200	0.190	0.000	0.083	0.125	0.227	
<i>b</i>	0.833	0.909	0.850	0.725	0.738	1.000	0.917	0.813	0.773	
<i>c</i>	0.000	0.000	0.000	0.075	0.071	0.000	0.000	0.063	0.000	
<i>Est-1</i>										30.85 [*]
<i>a</i>	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.023	
<i>b</i>	1.000	0.955	1.000	1.000	1.000	1.000	1.000	1.000	0.886	
<i>n</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	
<i>Est-2</i>										34.25 ^{**}
<i>a</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	
<i>b</i>	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.864	
<i>n</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	
<i>Fdh</i>										11.26 ^{ns}
<i>a</i>	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.955	
<i>b</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	
<i>Mdh-2</i>										49.19 ^{**}
<i>a</i>	0.250	0.023	0.000	0.150	0.000	0.333	0.028	0.000	0.000	
<i>b</i>	0.750	0.977	1.000	0.850	1.000	0.667	0.972	1.000	1.000	
<i>Me</i>										37.61 ^{**}
<i>a</i>	0.250	0.159	0.000	0.100	0.071	0.000	0.083	0.188	0.023	
<i>b</i>	0.750	0.750	0.800	0.775	0.929	0.875	0.861	0.813	0.977	
<i>c</i>	0.000	0.091	0.200	0.125	0.000	0.125	0.056	0.000	0.000	
<i>Pgi-2</i>										27.70 ^{**}
<i>a</i>	1.000	0.909	0.950	0.875	1.000	0.708	0.861	0.813	1.000	
<i>b</i>	0.000	0.091	0.050	0.125	0.000	0.292	0.139	0.188	0.000	
<i>Pgm</i>										23.56 [*]
<i>a</i>	0.917	1.000	0.950	1.000	0.929	1.000	0.833	1.000	1.000	
<i>b</i>	0.083	0.000	0.050	0.000	0.071	0.000	0.167	0.000	0.000	
<i>Prx-1</i>										45.71 ^{**}
<i>a</i>	0.417	0.477	0.550	0.500	0.476	0.333	0.611	0.500	0.341	
<i>b</i>	0.500	0.523	0.450	0.500	0.476	0.667	0.389	0.500	0.568	
<i>c</i>	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>n</i>	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.091	
<i>Prx-2</i>										1.03 ^{ns}
<i>a</i>	0.417	0.455	0.500	0.475	0.429	0.500	0.417	0.500	0.455	
<i>b</i>	0.583	0.545	0.500	0.525	0.571	0.500	0.583	0.500	0.545	
<i>Prx-3</i>										93.68 ^{**}
<i>a</i>	0.625	0.591	0.500	0.575	0.500	0.333	0.583	0.500	0.500	
<i>b</i>	0.375	0.409	0.500	0.425	0.500	0.333	0.417	0.500	0.500	
<i>n</i>	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	
<i>Prx-4</i>										31.42 [*]
<i>a</i>	0.083	0.205	0.100	0.050	0.000	0.083	0.056	0.063	0.023	
<i>b</i>	0.917	0.705	0.900	0.800	0.929	0.875	0.944	0.938	0.932	
<i>c</i>	0.000	0.091	0.000	0.150	0.071	0.042	0.000	0.000	0.045	
<i>Skd-2</i>										5.61 ^{ns}
<i>a</i>	1.000	0.977	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
<i>b</i>	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Tpi</i>										8.75 ^{ns}
<i>a</i>	0.958	1.000	1.000	1.000	1.000	0.958	1.000	1.000	0.955	
<i>b</i>	0.042	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.045	

^zSix loci, *Acp-1*, *Adh*, *Ddh-1*, *Mdh-1*, *Pgi-1*, *Skd-1* were fixed in the sample examined (i.e., monomorphic loci).^ySample size.^x*n* = Null allele.^{ns},*,**Nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively.

Table 2. Allozyme variability for nine pawpaw populations in comparison to those plant species with similar life history characteristics.²

Population	Parameter ^y				
	A	P	A _e	H _o	H _e
Georgia	1.5 (0.1)	43.5	1.217	0.257 (0.074)	0.178 (0.047)
Kentucky	1.7 (0.1)	43.5	1.224	0.259 (0.076)	0.183 (0.045)
Virginia	1.5 (0.1)	47.8	1.193	0.265 (0.083)	0.162 (0.045)
West Virginia	1.6 (0.2)	47.8	1.239	0.280 (0.079)	0.193 (0.046)
Indiana	1.5 (0.1)	43.5	1.192	0.246 (0.080)	0.160 (0.046)
Illinois	1.5 (0.1)	39.1	1.220	0.250 (0.074)	0.180 (0.050)
Maryland	1.5 (0.1)	43.5	1.186	0.227 (0.071)	0.157 (0.043)
Pennsylvania	1.4 (0.1)	39.1	1.192	0.255 (0.081)	0.161 (0.046)
New York	1.7 (0.2)	43.5	1.215	0.223 (0.077)	0.177 (0.044)
Mean (at population level)	1.54 (0.1)	43.5 (3.08)	1.209 (0.018)	0.251 (0.018)	0.172 (0.013)
At species level	2.10 (0.2)	47.8	1.221	0.250 (0.075)	0.181 (0.042)
Means ^x					
Long-lived woody perennials	1.79 (0.06)	50.0 (2.5)	1.79 (0.06)	NA ^w	0.149 (0.009)
Widespread	1.72 (0.07)	43.0 (3.3)	1.23 (0.02)	NA	0.159 (0.013)
Temperate	1.51 (0.03)	32.6 (1.3)	1.15 (0.01)	NA	0.109 (0.005)
Outcrossing (animal)	1.54 (0.03)	35.9 (1.8)	1.17 (0.01)	NA	0.124 (0.008)
Reproduction (sexual and asexual)	1.47 (0.06)	29.4 (3.3)	1.14 (0.02)	NA	0.103 (0.013)

²Standard errors are in parentheses.^yA = the mean number of alleles per locus; P = the percent polymorphic loci (95% criterion); A_e = the effective number of alleles per locus; H_o = the observed heterozygosity; H_e = the expected heterozygosity.^xMeans at population level for those plant species with similar characteristics (Hamrick and Godt, 1989).^wNA = not applicable.

a species that is predominately outcrossed could still maintain a relatively high genetic diversity (Ellstrand and Roose, 1987). Although little is known about the mechanisms operating to maintain the genetic variation in *A. triloba* populations, sexual recruitment might have played an important role. An alternative possibility is that nonrandom selection may have been involved in our pawpaw collection. Several of our accessions were received as seeds from pawpaw enthusiasts who may have purposely selected for the largest fruited and most vigorously growing trees (Callaway 1991, 1992).

GENETIC STRUCTURE AND GEOGRAPHIC DIFFERENTIATION. Partitioning of genetic diversity among the 17 polymorphic loci showed that 88.2% resided within populations. Nei's G_{st} and Wright's F_{ST} indicated that 8.5% to 11.8% of the genetic diversity was distributed among populations (Table 3). The proportion of genetic diversity found among *A. triloba* populations was lower than the average for those species with wide geographic range (21%), insect outcrossing species (24.3%), species with any seed dispersal mechanisms (12.4% to 27.7%) and reproduction modes (21.3% to

Table 3. Genetic variability statistics (Nei, 1987) and Wright's F statistics (Wright, 1978) for 17 polymorphic loci for nine pawpaw populations.

Locus	Genetic variability statistics ²				Wright's F statistics ^y		
	H _T	H _s	D _{st}	G _{st}	F _{IS}	F _{IT}	F _{ST}
<i>Acp-2</i>	0.491	0.491	0.000	0.000	-0.732	-0.708	0.014
<i>Acp-3</i>	0.502	0.502	0.000	0.000	-1.000	-1.000	0.000
<i>Ddh-2</i>	0.308	0.133	0.175	0.568	0.708	0.880	0.589
<i>Ddh-3</i>	0.289	0.272	0.017	0.059	-0.220	-0.163	0.046
<i>Est-1</i>	0.048	0.033	0.015	0.313	0.534	0.566	0.069
<i>Est-2</i>	0.041	0.028	0.013	0.317	1.000	1.000	0.095
<i>Fdh</i>	0.014	0.010	0.004	0.286	1.000	1.000	0.041
<i>Mdh-2</i>	0.141	0.135	0.006	0.043	0.033	0.207	0.180
<i>Me</i>	0.275	0.275	0.000	0.000	-0.173	-0.103	0.060
<i>Pgi-2</i>	0.158	0.158	0.000	0.000	-0.231	-0.109	0.099
<i>Pgm</i>	0.080	0.076	0.004	0.050	-0.129	-0.043	0.076
<i>Prx-1</i>	0.528	0.528	0.000	0.000	-0.610	-0.566	0.027
<i>Prx-2</i>	0.498	0.498	0.000	0.000	-0.862	-0.854	0.004
<i>Prx-3</i>	0.524	0.524	0.000	0.000	-0.724	-0.655	0.040
<i>Prx-4</i>	0.231	0.210	0.021	0.091	0.001	0.052	0.052
<i>Skd-2</i>	0.007	0.005	0.002	0.286	-0.023	-0.003	0.020
<i>Tpi</i>	0.027	0.027	0.000	0.000	-0.045	-0.015	0.029
Mean	0.245	0.230	0.015	0.118	-0.087	-0.030	0.085

²H_T = the total genetic diversity; H_s = the genetic diversity within populations; D_{st} = the genetic diversity between populations; G_{st} = the relative magnitude of genetic differentiation among populations.^yF_{IS} = the fixation index of individuals within populations; F_{IT} = the fixation index with respect to the total population; F_{ST} = the proportion of genetic differentiation.

Table 4. Allozyme estimates of Nei's (1978) unbiased genetic identity (below diagonal) and Nei's (1978) unbiased genetic distance (above diagonal) among nine pawpaw populations.

Population	1	2	3	4	5	6	7	8	9
1) Georgia		0.045	0.047	0.037	0.039	0.062	0.054	0.052	0.049
2) Kentucky	0.956		0.000	0.000	0.003	0.011	0.001	0.000	0.004
3) Virginia	0.954	1.000		0.000	0.000	0.009	0.000	0.000	0.001
4) West Virginia	0.964	1.000	1.000		0.000	0.008	0.002	0.000	0.004
5) Indiana	0.961	0.997	1.000	1.000		0.016	0.001	0.000	0.000
6) Illinois	0.940	0.989	0.991	0.992	0.984		0.011	0.005	0.015
7) Maryland	0.948	0.999	1.000	0.998	0.999	0.989		0.000	0.006
8) Pennsylvania	0.950	1.000	1.000	1.000	1.000	0.995	1.000		0.000
9) New York	0.952	0.996	0.999	0.996	1.000	0.985	0.994	1.000	

22.5%) and temperate species (24.6%). But it was similar to other long-lived woody perennials (7.6%) and wind-pollinated outcrossing species (9.9%) (Table 3) (Hamrick and Godt, 1989).

Nei's (1978) genetic identity and distance were estimated for all 36 pairwise populational comparisons (Table 4). The mean identity for pairwise comparisons of the nine *A. triloba* populations was 0.988, ranging from 0.940 to 1.000. This is typical of conspecific populations based on allozyme data (Crawford, 1989). Genetic relationships among populations were further examined by UPGMA of Roger's genetic distance (Wright, 1978) and by PCA. The former revealed that most populations clustered as one major group except the southernmost (Georgia) and westernmost (Illinois) populations, which readily separated from the major group (Fig. 1). The data were also subjected to UPGMA using Nei's genetic distance (1978) and three other genetic distance indices (Swofford and Selander, 1981); these dendrograms produced were topographically identical.

The relationships revealed by PCA were similar to those revealed by UPGMA, except that PCA separated the northernmost population (New York) from the major group of populations sampled. More than 88% of all allelic variation among populations resided in the first five principal components. The first three components, which accounted for 28%, 22%, and 16% of total variation (Table 5), respectively, divided the nine populations into one major group and three single populations (Fig. 2). The northernmost population (New York) separated from this group along the first principal component as a result of frequencies for *Est-1n*, *Est-2a*, *Est-2b*, *Est-2n*, *Fdh-a*, *Fdh-b*, and *Prx-1n*. On the second principal component, the westernmost population (Illinois) separated from this group as a result of frequencies for *Pgi-2a*, *Pgi-2b*, *Prx-1b*, *Prx-2a*, *Prx-2b*, *Prx-3a*, and *Prx-3n*. On the third principal component, the southernmost population (Georgia) separated as a result of frequencies for *Ddh-2a*, *Ddh-2b*, *Mdh-2a*, *Mdh-2b*, *Prx-1c*, *Tpi-a*, and *Tpi-b* (Tables 1 and 5; Fig. 2). The southernmost (Georgia), westernmost (Illinois) and northernmost (New York) populations sampled for this study separated from the major population cluster, indicating that these marginal populations have a different genetic composition of allele frequencies. Lagrange and Tramer (1985) found a significant difference in tree morphology and reproductive performance of several *A. triloba* populations between the extreme southern and northern regions. The different allele frequencies present among the populations obviously corroborate such a separation.

Limited gene flow may have contributed to population divergence in the major group of populations. This is reflected by the indirect estimate of gene flow rate, $N_m = (1 - F_{ST})/4F_{ST} = 2.69$, which is probably not sufficient to prevent population differentiation due to genetic drift. The reproductive biology of *Asimina* has been characterized by low efficiency of cross-pollination, scarce

insect pollinators, poor fruit production and poor germination of selfed seeds (Norman, and Clayton, 1986; Norman et al., 1992; Walker, 1971). Although *A. triloba* is continuously distributed in the eastern United States, low efficiencies of pollen and seed dispersal are likely major factors contributing to such population differentiation in this widespread species.

A contingency χ^2 analysis for heterogeneity of allele frequencies across populations showed significance at 10 of 17 polymorphic loci, suggesting that local alleles were uniquely present in certain populations (Table 1). This agrees with the general notion that clonal populations are likely to have unique genetic composition (Parker, 1979; Ellstrand and Roose, 1987). The importance of local alleles should not be overlooked when a conservation plan or sampling strategy is considered for further genetic enhancement of the KSU repository. For instance, *Est-2a* and *Fdh-b* were only detected in the New York population, *Est-1a* only in the Kentucky and New York populations and *Ddh-2a* only in the more southerly states. These local alleles will be considered in formulating a sampling strategy for future germplasm collection.

SAMPLING STRATEGY FOR A GENETICALLY RICH AND DIVERSE PAWPAW GERMLASM COLLECTION. For a plant species such as pawpaw in its early stage of domestication, most genetic resources for breeding and germplasm conservation remain in wild populations of known rich genetic diversity and proven adaptability to specific ecogeographic sites. The KSU pawpaw germplasm repository was established in 1994 (Layne, 1996). Extensive exploration for germplasm and collection from wild populations in the natural range of *A. triloba* is currently being planned. Estimates of genetic diversity and geographic differentiation in the current study have provided valuable information upon which we will formulate future germplasm exploration and sampling strategies. First, to capture most of the genetic variation and encompass most

Fig. 1. UPGMA dendrogram of allozyme genetic distance based on modified Roger's genetic distance among nine pawpaw populations.

Table 5. Percent variation explained and the alleles with the highest loadings on the first three principal components (PCs).

PC % variation explained					
1		2		3	
28.41		21.64		15.96	
PC loadings					
<i>Est-1n</i>	0.2878	<i>Prx-1b</i>	-0.2878	<i>Prx-1c</i>	-0.3216
<i>Est-2a</i>	0.2878	<i>Prx-3d</i>	-0.2864	<i>Ddh-2a</i>	-0.3109
<i>Est-2b</i>	-0.2878	<i>Pgi-2a</i>	0.2619	<i>Tpi-a</i>	0.2980
<i>Est-2d</i>	0.2878	<i>Pgi-2b</i>	-0.2617	<i>Tpi-b</i>	-0.2980
<i>Fdh-a</i>	-0.2878	<i>Prx-2a</i>	-0.2362	<i>Ddh-2b</i>	0.2941
<i>Fdh-b</i>	0.2878	<i>Prx-2b</i>	0.2362	<i>Mdh-2a</i>	-0.2836
<i>Prx-1-d</i>	0.2744	<i>Prx-3a</i>	0.2611	<i>Mdh-2b</i>	0.2836

of the rare, local alleles, a survey is needed throughout the entire range of *A. triloba*, since the range of *A. triloba* is environmentally heterogeneous. Based on geography, climate and ecological conditions within its native range, 11 distinct regions need to be considered in developing a genetic sampling strategy, namely: prairie region in Missouri and Illinois, upper Mississippi Valley,

Great Lakes, Potomac and Suequehanna Valleys, Ozark highlands, Ohio Valley, southern Appalachian Highlands, Carolina coastal plain, east Texas, lower Mississippi Delta, southern Alabama and Georgia, and northern Florida (R. Neal Peterson, personal communication). Second, to use information on the partitioning of genetic diversity in *A. triloba* to formulate a collecting strategy, our data suggest that a special emphasis should be focused on locally common alleles in its native range (Lamboy et al., 1996; Marshall and Brown, 1975). The current study demonstrated that marginal populations may contain unique alleles. These locally common alleles could be associated with certain adaptive characteristics, which are interesting to plant breeders for cultivar improvement. To this point, special attention might need to be emphasized in the extreme southern region where *A. triloba* and *A. parviflora* naturally coexist. Unique phenotypes of pawpaw and natural hybrids of *A. triloba* x *A. parviflora* might be found (Peterson, 1991). Finally, morphological traits, which may be economically important to horticulturists for cultivar improvement, need to be exploited and evaluated in conjunction with molecular markers, to determine whether the genetic diversity patterns observed in the neutral markers reflect similar patterns in the horticulturally useful alleles.

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Fig. 2. Principal components analysis of allozyme frequencies in nine pawpaw populations: (a) plot of PRIN. 2 vs. PRIN. 1 and (b) plot of PRIN. 3 vs. PRIN. 1.

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