J. Amer. Soc. Hort. Sci. 125(4):454–459. 2000.

RAPD Inheritance and Diversity in Pawpaw (*Asimina triloba*)

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Additional index words. Annonaceae, dominant inheritance, allele frequency, Nei's genetic identity and distance, cluster analysis

Abstract. Twelve, 10-base primers amplified a total of 20 intense and easily scorable polymorphic bands in an interspecific cross of PPF1-5 pawpaw [*Asimina triloba* (L.) Dunal.] x RET (*Asimina reticulata* Shuttlew.). In this cross, all bands scored were present in, and inherited from, the *A. triloba* parent PPF1-5. Nineteen of the 20 bands were found to segregate as expected (1:1 or 3:1) based on chi-square goodness-of-fit tests, and were subsequently used to evaluate genetic diversity in populations of *A. triloba* collected from six states (Georgia, Illinois, Indiana, Maryland, New York, and West Virginia) within its natural range. Analysis of genetic diversity of the populations revealed that the mean number of alleles per locus was $A = 1.64$, percent polymorphic loci was $P = 64$, and expected heterozygosity was $H_e = 0.25$. No significant differences were found among populations for any of the polymorphic indices. Partitioning of the population genetic diversity showed that the average genetic diversity within populations was $H_s = 0.26$, accounting for 72% of the total genetic diversity. Genetic diversity among populations was $D_{st} = 0.10$, accounting for 28% of the total genetic diversity. Nei's genetic identity and distance showed a high mean identity of 0.86 between populations. Genetic relationships among the populations examined by unweighted pair-group mean clustering analysis separated the six populations into two primary clusters: one composed of Georgia, Maryland, and New York, and the other composed of Illinois, Indiana, and West Virginia. The Georgia and Indiana populations were further separated from the other populations within each group. This study provides additional evidence that marginal populations within the natural range of *A. triloba* should be included in future collection efforts to capture most of the rare and local alleles responsible for this differentiation.

The genus *Asimina* Adans. is the only temperate representative of the tropical Annonaceae, the largest primitive family of flowering plants, containing ≈130 genera and 2000 species (Cronquist, 1981). The genus *Asimina* includes nine species, which are indigenous to the United States (Kral, 1960). The best-known species is *Asimina triloba*, the North American pawpaw, which is the largest tree fruit native to the United States. Pawpaw fruit have an oblong shape with green skin and a taste that can be described as a very sweet blend of banana (*Musa ×paradisiaca* L.), mango (*Mangifera indica* L.), and pineapple [*Ananas comosus* (L.) Merr.] flavors with a mild pleasant fruit aroma. Its fruit quality, nutritional value, natural defense compounds (annonaceous acetogenins) and landscaping appeal, make pawpaw of great potential for commercial development as a new alternative fruit tree crop and ornamental in the United States and elsewhere (Johnson et al., 1996; Jones and Layne, 1997; Layne, 1996).

The natural range of *A. triloba* covers most of eastern North America, ranging from northern Florida to southern Ontario, Canada, and as far west as eastern Nebraska (Darrow, 1975). Generally, all *Asimina* species are diploids, 2n = 2x = 18. One possible exception is *A. pygmaea* (Bartr.) Dunal, for which chromosome counts have not been reported and triploids and hybrids are known to exist (Bowden, 1948; Kral, 1960). Pawpaw reproduces predominantly by asexual means through root suckering, which usually results in a typical small tree or bush of extensive thickets in lowland drainage basins near river flood plains (Willson and Schemske, 1980). The flowers are strongly protogynous, strictly outcrossing, and mainly fly or beetle-pollinated (Faegri and van der Piji, 1971). Low fruit set has been observed in nature, and attributed to inefficient dispersal of pollen and/or a lack of pollinators (Lagrange and Tramer, 1985; Willson and Schemske, 1980). Seed dispersal and germination is also generally poor in the wild (Peterson, 1991). Given these characters, breeders and germplasm custodians are concerned about the amount of genetic diversity retained in existing cultivars and germplasm collections.

In 1994, Kentucky State University (KSU) was approved by the U.S. Department of Agriculture (USDA) National Clonal Germplasm Repository System to serve as the national repository for *Asimina* species. A comprehensive research program for developing pawpaw as a new commercial tree fruit for Kentucky and the United States has been underway since 1990. Research priorities have included germplasm collection and evaluation, cultivar trials, development of cultural recommendations, clonal propagation, and breeding for cultivar improvement (Layne, 1996). For a plant species at an early stage of domestication, such as pawpaw, maintaining high levels of genetic diversity is essential for the long-term success of a breeding program and for maintaining a diversely valuable source of useful genes in the germplasm repository. However, little research has been conducted to investigate genetic diversity in pawpaw at the molecular level. Rogstad et al. (1991) studied genetic variation among pawpaw populations using one minisatellite DNA repeat (M13)

Received for publication 1 June 1999. Accepted for publication 3 Mar. 2000. This research was supported by USDA 1890 Institution Research Capacity Building Grant 92-38814-7485 originally awarded to M.B. Callaway. We would like to thank R. Neal Peterson, founder of the PawPaw Foundation, for the controlled cross material and Glen N. Johnson and Kristel B. Davis for skilled technical assistance with DNA extraction and PCR amplification. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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and concluded that clonal propagation and inbreeding might have contributed to the low to moderate variability found in pawpaw. More recently, genetic variation was studied in domesticated pawpaw cultivars (Huang et al. 1997) and natural populations (Huang et al., 1998) using isozyme markers. These studies suggested that moderate levels of genetic diversity exist in both cultivated as well as wild populations. However, these previous studies included only a limited number of loci. Random amplified polymorphic DNAs (RAPDs) offer the potential to generate large numbers of randomly distributed markers. Thus, RAPDs may reflect more accurately information on diversity of the species.

One major goal of ongoing research projects at the National Clonal Germplasm Repository for *Asimina* species at KSU is to develop a genetically rich pawpaw germplasm collection for conservation and for cultivar improvement in a long-term breeding program. Knowledge concerning levels and structuring of genetic diversity in pawpaw will allow plant breeders and custodians to formulate collecting priorities and sampling strategies for their conservation and breeding programs. The goals of the present investigation were to 1) identify a set of Mendelian RAPD markers in *A. triloba*, 2) use these markers to assess levels of genetic diversity in existing germplasm collections held at the KSU pawpaw repository, and 3) use this information to formulate a sampling strategy for further field collection.

Materials and Methods

Plant materials. Dormant stem cuttings from various pawpaw trees located at the USDA National Clonal Germplasm Repository for *Asimina* species in Frankfort, Kentucky were harvested in late Fall 1996, placed in separate bags, and shipped overnight to the USDA Forest Service, Southern Institute of Forest Genetics, Saucier, Mississippi. The cuttings represented individuals (n = 34) from an interspecific cross [PPF1-5 (*A. triloba*) x RET (*A. reticulata*)] (Table 1), and wild selections from six populations (10 to 18 trees per population) located in different states within the natural range of *A. triloba* (Table 2). PPF1-5 and RET are an advanced selection developed by the PawPaw Foundation and an accession collected from the wild in Florida, respectively. All stem cuttings were placed in water in a greenhouse and leaf tissue was collected from newly emerged shoots.

DNA extraction. Total nucleic acids were isolated from ≈2 g of leaf tissue using a modification of the cetyltrimethylammonium bromide (CTAB)-based procedure outlined in Wagner et al. (1987). The RNA component of these individual extracts was removed by incubation in the presence of RNase A as described in Ausubel et al. (1987). Oligonucleotide 10-base primers were obtained from Operon Technologies Inc., Alameda, Calif.

RAPD amplification. DNA amplification was based on the protocol reported by Williams et al. (1990). The reaction consisted of the following in 24 μL total volume: 6.25 ng template DNA, 1 μL primer DNA (5 μM stock), 3.6 μL dNTPs (1 mM stock), 2.4 μL 10× *Taq* DNA polymerase reaction buffer (500 mM KCl, 100 mM Tris-HCl, 1.0% Triton X-100, 15 mM $MgCl_2$), and 0.8 U *Taq* DNA polymerase. Reactions were loaded in flexible microtitre plates and overlaid with 25 μL of mineral oil. Microtitre plates were placed in preheated (85 °C) programmable temperature cyclers (PTC-100; MJ Research, Watertown, Mass.) and covered with mylar film. The DNA samples were amplified using the following thermal profile: 5 s at 95 °C; 1 min 55 s at 92 °C; followed by 45 cycles of 5 s at 95 °C, 55 s at 92 °C, 1 min at 35 °C, and 2 min at 72 °C; followed by 7 min at 72 °C. The reactions ended with an indefinite hold at 4 °C.

Electrophoresis. Amplification products were electrophoresed in 2% agarose gels and TAE buffer (40 mM Tris base, 20 mM sodium acetate, 2.0 mM EDTA, glacial acetic acid to pH 7.2) for

Table 1. Single-locus segregation and chi-square tests for 20 RAPD markers identified in the interspecific cross PPF1-5 (*A. triloba*) x RET (*A. reticulata*).

	Parents		Progeny			
	RAPD banding	RAPD	Phenotype (genotype)		Expected	
Locus	phenotype	genotype	1 (+/+ or +/–)	0 (–/–)	ratio	χ^2
A01_1075	1 x 1	+/– x +/–	26	8	3:1	0.04
A01_0900	1 x 0	+/– x –/–	15	19	1:1	0.47
A01_0575	1 x 0	+/– x –/–	12	22	1:1	2.94
A10_1050	1 x 0	+/– x –/–	14	20	1:1	1.06
A10_0700	1 x 0	+/– x –/–	16	18	1:1	0.12
A11_1000	1 x 0	+/– x –/–	17	17	1:1	0.00
A11_0950	1 x 0	+/– x –/–	9	25	1:1	7.53*
A11_0850	1 x 0	+/– x –/–	14	20	1:1	1.06
A11_0425	1 x 0	+/– x –/–	15	19	1:1	0.47
B07_2800	1 x 0	+/– x –/–	17	17	1:1	0.00
B07_1675	1 x 1	+/– x +/–	27	7	3:1	0.35
C08_0400	1 x 1	+/– x +/–	26	8	3:1	0.04
C12_0650	1 x 0	+/– x –/–	12	22	1:1	2.94
C15_1300	1 x 0	+/– x –/–	16	18	1:1	0.12
D05_0500	1 x 0	+/– x –/–	13	21	1:1	1.88
D20_0750	1 x 0	+/– x –/–	22	12	1:1	2.94
E05_0750	1 x 0	+/– x –/–	12	22	1:1	2.94
E12_1050	1 x 0	+/– x –/–	17	17	1:1	0.00
E12_0700	1 x 0	+/– x –/–	18	16	1:1	0.12
E14_0850	1 x 0	+/– x –/–	14	20	1:1	1.06

*Significant at $P = 0.05$.

Table 2. Null homozygous genotypes and allele frequencies of RAPDs in six *A. triloba* populations[z].

	Maryland (N = 17)		Georgia (N = 12)		Indiana (N = 18)		New York (N = 18)		West Virginia (N = 18)		Illinois (N = 10)	
Locus	q^2	q_{est}	q^2	q_{est}	q^2	q_{est}	q^2	q_{est}	q^2	q_{est}	q^2	q_{est}
A01-1075	0.53	0.73	0.42	0.65	0.72	0.85	0.71	0.84	0.28	0.53	0.83	0.91
A01-0900	0.35	0.59	0.17	0.41	0.06	0.25	0.06	0.25	0.38	0.62	0.83	0.91
A01-0575	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	0.91
A10-1050	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	0.91
A10-0700	0.71	0.84	0.58	0.76	0.06	0.25	0.35	0.59	0.67	0.82	1.00	1.00
A11-1000	0.00	0.00	0.00	0.00	0.41	0.64	0.00	0.00	0.00	0.00	0.40	0.63
A11-0950	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
A11-0850	0.83	0.91	1.00	1.00	0.56	0.75	1.00	1.00	0.56	0.75	0.79	0.89
A11-0425	1.00	1.00	0.67	0.82	1.00	1.00	1.00	1.00	0.77	0.88	1.00	1.00
B07-2800	0.28	0.53	0.17	0.42	0.22	0.47	0.35	0.59	0.00	0.00	0.34	0.59
B07-1675	0.20	0.45	0.17	0.42	0.50	0.71	0.00	0.00	0.17	0.41	0.17	0.42
C08-0400	0.44	0.66	0.17	0.42	0.38	0.62	1.00	1.00	0.22	0.47	0.83	0.91
C12-0650	0.62	0.79	1.00	1.00	0.56	0.75	0.24	0.49	0.94	0.97	0.50	0.71
C15-1300	0.06	0.25	0.00	0.00	0.28	0.53	0.00	0.00	0.67	0.82	0.83	0.91
D05-0500	0.12	0.36	1.00	1.00	0.06	0.25	0.17	0.41	0.34	0.58	0.83	0.91
D20-0750	0.06	0.26	0.67	0.82	0.34	0.58	0.12	0.34	0.56	0.75	0.50	0.71
E05-0750	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	0.91
E12-1050	0.12	0.35	0.25	0.51	0.00	0.00	0.35	0.59	0.00	0.00	0.00	0.00
E12-0700	0.00	0.00	0.00	0.00	0.38	0.62	0.29	0.54	0.45	0.67	0.83	0.91
E14-0850	0.62	0.79	0.17	0.42	0.28	0.53	0.64	0.80	0.56	0.75	0.34	0.59

[z]q^2 is the proportion of RAPD null homozygotes in the N sampled individuals of the population, and q_{est} is the indirect estimate of RAPD null frequencies corrected for dominance according to Lynch and Milligan (1994).

≈3.5 h at 3 V·cm^{-1} (150 V). A total of 3.0 μL loading buffer (10× TAE, 50% glycerol, and 0.25% bromophenol blue) was added to each reaction prior to electrophoresis. After electrophoresis, the gels were stained with ethidium bromide (0.4 μg·mL^{-1}) for 45 min, washed in distilled water for 1.0 h, and photographed under ultraviolet light using a camera and instant film (Polaroid MP-4 with Polaroid 667 film; Polaroid, Cambridge, Mass.).

Marker scoring and data analysis. One hundred RAPD primers (Operon Technologies, kits A–E) were used for initial screening against both parents and five progenies from the interspecific cross to identify segregating RAPD markers and verify reproducibility. Each sample was amplified at least two times. Twelve primers that amplified a total of 21 reproducible polymorphic bands were then selected to characterize the remainder of the family and the populations. RAPD banding phenotypes were scored as 1 (band present) or 0 (band absent) and the corresponding RAPD genotypes were assigned as +/+ or +/– and –/–, respectively. Segregating RAPD markers were identified by the manufacturer primer code corresponding to the 10-base primer responsible for their amplification, followed by a four digit number indicating the product size in base pairs (Table 1). Chi-square tests were used to determine the goodness-of-fit of these segregating polymorphisms to their expected Mendelian ratios (n = 34). Markers were tested for linkage using chi-square tests and confirmed using the software package JoinMap version 2.0. Population genetic analyses were performed among the populations assuming Hardy-Weinberg equilibrium (HWE) (average F_{is} = –0.087 with 17 isozyme loci in the populations), although some individual isozyme loci were found to have deviated from HWE in these populations (Huang et al., 1998). Allele frequencies were estimated indirectly using the homozygous null genotypes (–/–) and corrected for dominance according to Lynch and Milligan (1994) (Table 2). A set of intra- and inter-population genetic statistics were estimated and corrected for small sample size (Nei, 1978) and small number of populations (Nei, 1986) using GeneStat-pc 3.3 computer program (Lewis, 1994). Some of these statistics included the percentage of polymorphic loci (*P*) (95% criterion), the mean number of alleles per locus (*A*), expected heterozygosity (H_e), total genetic diversity (H_T), genetic diversity within populations (H_s), genetic diversity among populations (D_{st}), and the relative magnitude of genetic differentiation among populations ($G_{st} = D_{st}/H_T$) (Nei, 1987). Nei's (1978) genetic distances and identities were calculated for all pairwise combinations of populations. A dendrogram was constructed based on the matrix of the distances using unweighted pair-group mean analysis (UPGMA).

Results

Twelve 10-base primers amplified a total of 20 reproducible polymorphic bands in the interspecific cross PPF1-5 (*A. triloba*) x RET (*A. reticulata*). All of these bands were present in the *A. triloba* parent PPF1-5. Nineteen of the 20 bands were found to segregate consistent with expected Mendelian ratios (segregate 1:1 or 3:1) based on chi-square goodness-of-fit tests (Table 1), and used subsequently to evaluate genetic diversity in the populations (Table 2). Averaged across populations, the mean number of alleles per locus was *A* = 1.64 (ranging from 1.53 to 1.79), percentage of polymorphic loci *P* = 64 (ranging from 53 to 79), and expected heterozygosity was H_e = 0.25 (ranging from 0.22 to 0.29). No significant differences were found among populations for any of the polymorphic indices (Table 3). Partitioning of the population genetic diversity showed that genetic diversity within populations, H_s = 0.26, accounted for 72% of the total genetic diversity. Genetic diversity among populations, D_{st} = 0.10, accounted for 28% of the total genetic diversity. This result was

Table 3. RAPD diversity in six *A. triloba* populations based on 19 RAPD loci.

Population	Sample size	Polymorphic index[z]		
		Mean no. of alleles/locus (*A*)	Polymorphic locus (%) (*P*)	Expected heterozygosity (H_e)
Maryland	17	1.63	63	0.26
Georgia	12	1.58	58	0.24
Indiana	18	1.63	63	0.29
New York	18	1.53	53	0.23
West Virginia	18	1.68	68	0.25
Illinois	10	1.79	79	0.22
Means	16	1.6	60	0.25

[z]Figures within columns are not significantly different ($P = 0.05$).

reflected by Nei's (1978) $G_{st} = 0.28$, which measures the proportion of the genetic diversity attributable to population differentiation (Table 4). Nei's (1978) genetic identity and distance show that a high mean identity of 0.86 existed among the populations (Table 5). Genetic relationships among the populations were examined further by UPGMA clustering analysis and represented by a dendrogram (Fig. 1). The dendrogram separates the six populations into two primary clusters which somewhat reflects their geographic locations within the natural range of *A. triloba*. A more eastern cluster containing Maryland, New York and Georgia versus a more western cluster composed of Illinois, Indiana, and West Virginia. Populations on the fringe of the natural range of pawpaw (Georgia and Indiana) were separated further from the others within their respective groups.

Table 4. Genetic variability statistics (Nei 1987) for 19 RAPD loci scored on *A. triloba* populations collected from six states within its natural range.[z]

Locus	H_T	H_S	D_{ST}	G_{ST}
A01_1075	0.380	0.351	0.029	0.076
A01_0900	0.522	0.402	0.120	0.230
A01_0575	0.030	0.028	0.002	0.058
A10_1050	0.030	0.028	0.002	0.058
A10_0700	0.501	0.317	0.184	0.368
A11_1000	0.370	0.160	0.210	0.567
A11_0850	0.097	0.087	0.010	0.100
A11_0425	0.097	0.087	0.010	0.100
B07_2800	0.507	0.423	0.084	0.165
B07_1675	0.497	0.407	0.090	0.181
C08_0400	0.456	0.358	0.098	0.215
C12_0650	0.349	0.289	0.060	0.172
C15_1300	0.539	0.228	0.311	0.577
D05_0500	0.519	0.337	0.182	0.350
D20_0750	0.507	0.413	0.094	0.185
E05_0750	0.030	0.028	0.002	0.058
E12_1050	0.388	0.247	0.141	0.363
E12_0700	0.543	0.272	0.271	0.500
E14_0850	0.468	0.431	0.037	0.079
Means	0.359	0.258	0.102	0.283

[z]H_T = total genetic diversity, H_S = genetic diversity within populations, D_{ST} = genetic diversity between populations, and G_{ST} = the relative magnitude of genetic differentiation among populations.

Discussion

Proven reproducibility of RAPD banding phenotypes and confirmed Mendelian inheritance of RAPD loci are a prerequisite for application of RAPD data in studies of population genetics. Nineteen of the 20 RAPD loci identified in this study provide the first set of RAPD markers by polymerase chain reaction (PCR)-based protocols that can be used to assess genetic diversity in *A. triloba*. RAPD-based estimates of genetic diversity are higher than those based on isozymes [(Huang et al. 1998); $P = 64\%$ (RAPDs) vs. 43.5% (isozymes) and H_e: 0.249 (RAPDs) vs. 0.172 (isozymes)]. These results are in accordance with other studies that have compared levels of differentiation based on allozyme markers with those based on RAPD markers (Aagaard et al. 1998; Szmidt et al. 1996). Higher RAPD-based estimates might result from an inherently higher rate of detectable mutations (nucleotide substitution rates for noncoding sequences are generally higher than for coding sequences), and weaker degree of selective constraint. Previous studies challenge whether high levels of genetic variation could be maintained in *A. triloba* due to its clonal reproductive habit and low seed set in nature (Rogstad et al., 1991). This study provides additional evidence to support the contention that moderate to high levels of genetic diversity exist in *A. triloba* (Huang et al. 1997; 1998).

Partitioning of the RAPD genetic diversity within and between populations revealed a larger proportion of between population differentiation than was suggested using isozymes, indicating a more structured genetic distribution among populations of

Table 5. Estimates of Nei's (1978) unbiased genetic identities (above diagonal) and distances (below diagonal) between *A. triloba* populations.

Population	Population					
	Maryland	Georgia	Indiana	New York	West Virginia	Illinois
Maryland		0.93	0.87	0.94	0.89	0.83
Georgia	0.08		0.81	0.87	0.88	0.79
Indiana	0.14	0.21		0.86	0.88	0.86
New York	0.06	0.14	0.16		0.83	0.80
West Virginia	0.11	0.13	0.13	0.19		0.91
Illinois	0.19	0.23	0.15	0.22	0.10	

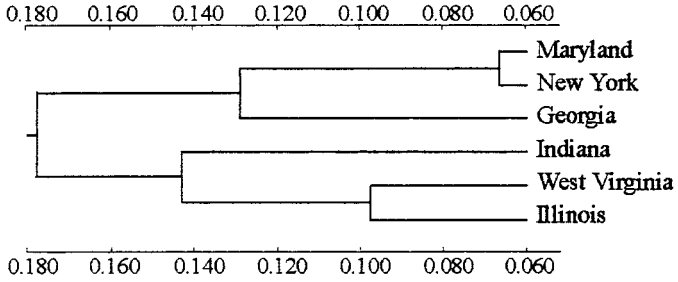

Fig. 1. RAPD-based UPGMA dendrogram of Nei's (1978) genetic distances among six populations of *A. triloba*.

A. triloba. About 28% of the RAPD genetic variation (Nei's G_{st} = 0.28) resided among populations. While isozyme data suggested that the populations were generally in HWE, and that relatively low levels of genetic diversity existed between populations (Nei's G_{st} = 0.118) (Huang et al., 1998), the higher RAPD-based estimate is reasonable for a temperate species such as *A. triloba* with life history characteristics such as a wide geographic range, insect outcrossing, ingested seed dispersal, and mixed sexual and asexual reproduction (Hamrick et al., 1989). The ability of RAPD markers to provide more detailed information on population structure may be due to a more random distribution of RAPD markers throughout the genome of *A. triloba*. Based on two-point linkage analyses, only two pairs of RAPD markers were linked (Table 6).

It appears as if gene flow may have an important role in attributing to genetic differentiation between populations. Estimates of gene flow, *Nm* = 0.61 [number of migrants exchanged between populations per generation; Wright (1931)], indicate a very low rate of gene exchange among the populations. According to Wright's (1931) estimate, gene flow rates less than four migrants per generation are not sufficient to prevent population differentiation due to genetic drift. The low *Nm* value may relate to the reproductive biology of *A. triloba*, which is characterized by low efficiency of cross-pollination, scarce insect pollinators, poor fruit production, and poor germination of selfed seeds in wild populations (Norman and Clayton, 1986; Norman et al., 1992; Walker, 1971).

Although we estimated a low level of migration, which should lead to high population differentiation, Nei's (1978) genetic identities were high for all pairwise comparisons (mean = 0.86). The highest genetic identity was between the Maryland and New York populations (0.94) and the lowest was between the Illinois and Georgia populations (0.79). These estimates are typical for conspecific populations based on isozyme data (Crawford, 1989). Although a summary of RAPD-based mean range of genetic identities for conspecific populations is not currently available, RAPDs have been used to evaluate genetic similarity in cultivated *Annona* L., which is a related genus in the family Annonaceae (Ronning et al. 1995). In this study, 84% identity was observed between conspecific cultivars and 65% to 79% among interspecific hybrids. High RAPD-based genetic identities among conspecific cultivars have also been reported in other horticultural crops; papaya (*Carica papaya* L.) (70% to 95%), sorghum [*Sorghum bicolor* (L.) Moench] (79% to 96%), muscadine grape (*Vitis rotundifolia* Michx.) (85% to 93%), and witchhazel (*Hamamelis japonica* Siebold & Zucc.) (72% to 93%); (*H. mollis* Oliv.) (68% to 98%)] (Marquard et al., 1997; Qu et al., 1996; Stiles et al., 1993; Tao et al., 1993). On average, 85% genetic identity of RAPDs should be an acceptable criterion for conspecific relationships within *A. triloba*.

The contradiction between low levels of migration and high genetic identities may be partially explained by a temporal change in migration rates. Recent human activities on the natural landscape, such as large scale timber harvesting, tend to lower rates of migration due to forest fragmentation. However, the effects of lower rates of migration are often not detectable for some time, due to the long-lived nature of forest tree species. In other words, migration rates may have been higher in the recent past, explaining the current (high) genetic identities between and among populations. However, not enough time (generations) may have elapsed to allow for higher levels of population differentiation through random genetic drift.

The RAPD-based UPGMA dendrogram presented in Fig. 1 is similar to the dendrogram based on isozyme data (Huang et al., 1998). Significant differences in morphology, reproductive performance, and isozyme allele frequencies among *A. triloba* populations in the extreme southern and northern regions of the natural range have been documented (Huang et al., 1998; Lagrange and Tramer, 1985). The RAPD-based UPGMA dendrogram provides additional evidence that populations within the natural range of *A. triloba* exist that are more distantly related, suggesting that future collection efforts focus in fringe areas of the range to capture most of the rare and local alleles responsible for this differentiation. For examples, the band-present allele at marker A11_1000 was found only in the Illinois and Indiana populations, or the null allele at markers A01_0575, A10_1050, and E05_0750 were found only in the Illinois population. RAPD analysis together with morphological characteristics and isozyme analysis should prove useful for developing a genetically rich pawpaw germplasm collection for conservation and for cultivar improvement in long-term breeding programs.

Table 6. Contingency tables and chi-square tests for independent assortment between RAPD loci identified in the interspecific cross PPF1-5 (*A. triloba*) x RET (*A. reticulata*). Observed number of RAPD band phenotypes (n = 34) are the main entries, expected number are in parentheses. A11_1000-E12_1050 linked at 18.4 Kosambi centiMorgans, B07_1675-D05_0500 linked at 0.0 Kosambi centiMorgans.

	E12_1050		
A11_1000	Band present	Band absent	
Band present	3 (8.5)	14 (8.5)	$\chi^2 = 14.24$
Band absent	14 (8.5)	3 (8.5)	$P < 0.001$
	D05_0500		
B07_1675	Band present	Band absent	
Band present	6 (12.75)	21 (12.75)	$\chi^2 = 14.94$
Band absent	7 (4.25)	0 (4.25)	$P < 0.001$

Literature Cited

Aagaard, J.E., Krutovskii, K.V., and S.H. Strauss. 1998. RAPDs and allozymes exhibit similar levels of diversity and differentiation among populations and races of Douglas-fir. Heredity 81:69–78.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Stuhl. 1987. Current protocols in molecular biology. Wiley, New York.

Bowden, W.M. 1948. Chromosome numbers in the Annonaceae. Amer. J. Bot. 35:377–381.

Crawford, D.J. 1989. Enzyme electrophoresis and plant systematics, p. 146–164. In: D.E. Soltis and P.S. Soltis (eds.). Isozymes in plant biology. Dioscorides Press, Portland, Ore.

Cronquist, A. 1981. An integrated classification of flowering plants. Columbia Univ. Press, New York.

Darrow, G.M. 1975. Minor temperate fruits, p. 276–277. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.

Faegri, K. and L. van der Piji. 1971. The principles of pollination ecology. Pergammon Press, New York.

Hamrick, J.L. and M.J.W. Godt. 1989. Allozyme diversity in plant species, p. 43–63. In: A.H.D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir (eds.). Plant population genetics, breeding and genetic resources. Sinauer Associates, Inc., Publ., Sunderland, Mass.

Huang, H., D.R. Layne, and R.N. Peterson. 1997. Isozyme polymorphisms for identification and assessment of genetic variation in cultivated pawpaws [*Asimina triloba* (L.) Dunal]. J. Amer. Soc. Hort. Sci. 122:504–511.

Huang, H., D.R. Layne, and D.E. Riemenschneider. 1998. Genetic diversity and geographic differentiation in pawpaw [*Asimina triloba* (L.) Dunal] populations from nine states as revealed by allozyme analysis. J. Amer. Soc. Hort. Sci. 123:635–641.

Johnson, H.A., J. Gordon, and J.L. McLaughlin. 1996. Monthly variations in biological activity of *Asimina triloba*, p. 609–614. In: J. Janick (ed.). Progress in new crops. Amer. Soc. Hort. Sci. Press, Alex., Va.

Jones, S.C. and D.R. Layne. 1997. Cooking with pawpaws. Ky. State Univ. Pawpaw Ext. Bul. 001.

Kral, R. 1960. A revision of *Asimina* and *Deeringothamnus* (Annonaceae). Brittonia 12: 233–278.

Lagrange, R.L. and E.J. Tramer. 1985. Geographic variation in size and reproductive success in the pawpaw (*Asimina triloba*). Ohio J. Sci. 85:40–45.

Layne, D.R. 1996. The pawpaw [*Asimina triloba* (L.) Dunal]: A new fruit crop for Kentucky and the United States. HortScience 31:777–784.

Lewis, P.O. 1994. GeneStat-PC, v. 3.3. N.C. State Univ., Raleigh.

Lynch, M. and B.G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3:91-99.

Marquard, R., E.P. Davis, and E.L. Stowe. 1997. Genetic diversity among witchhazel cultivars based on randomly amplified polymorphic DNA markers. J. Amer. Soc. Hort. Sci. 122:529–535.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nei, M. 1986. Definition and estimation of fixation indices. Evolution 40:643–645.

Nei, M. 1987. Molecular evolution genetics. Columbia Univ. Press, New York.

Norman, E.M. and D. Clayton. 1986. Reproductive biology of two Florida pawpaws: *Asimina obovata* and *A. pygmaea* (Annonaceae). Bul. Torrey Bot. Club 113:16–22.

Norman, E.M., K. Rice, and S. Cochran. 1992. Reproductive biology of *Asimina parviflora* (Annonaceae). Bul. Torrey Bot. Club 119:1–5.

Peterson, R.N. 1991. Pawpaw (*Asimina*). In: J.N. Moore and J.R. Ballington (eds.). Genetic resources of temperate fruit and nut crops. Acta Hort. 290:567–600.

Qu, X., J. Lu, and O. Lamikanra. 1996. Genetic diversity in muscadine and American bunch grapes based on randomly amplified polymorphic DNA (RAPD) analysis. J. Amer. Soc. Hort. Sci. 121:1020–1023.

Rogstad, S.H., K. Wolff, and B.A. Schaal. 1991. Geographical variation in *Asimina triloba* Dunal (Annonaceae) revealed by the M13 DNA fingerprinting probe. Amer. J. Bot. 78:1391–1396.

Ronning, C.M., R.J. Schnell, and S. Gazit. 1995. Using randomly amplified polymorphic DNA (RAPD) markers to identify *Annona* cultivars. J. Amer. Soc. Hort. Sci. 120:726–729.

Stiles, J.I., C. Lemme, S. Soudur, M.B. Morshidi, and R. Manshardt. 1993. Using randomly amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars. Theor. Appl. Genet. 85:697–701.

Szmidt, A.E., Wang, X.R., and M.Z. Lu. 1996. Empirical asessment of allozyme and RAPD variation in *Pinus sylvestris* (L.) using haploid tissue analysis. Heredity 76:412–420.

Tao, Y., J.M. Manners, M.M. Ludlow, and R.G. Henzell. 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Moench). Theor. Appl. Genet. 86:679–688.

Wagner, D.B., G.R. Furnier, M.A. Saghai-Maroof, S.M. Williams, B.P. Dancik, and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proc. Natl. Acad. Sci. USA 84:2097–2100.

Walker, J.W. 1971. Unique type of angiosperm pollen from the family Annonaceae. Science 172:565–567.

Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18:6531–6535.

Willson, M.F. and D.W. Schemske. 1980. Pollinator limitation, fruit production, and floral display in pawpaw (*Asimina triloba*). Bul. Torrey Bot. Club 107:401–408.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:195–197.