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Research Article

Determination of genetic relationships among populations of *Asclepias tuberosa* (Asclepiadaceae) based on ISSR polymorphisms

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Abstract. Asclepias tuberosa L., or butterflyweed, is a native plant species occurring throughout much of North America, and is a valued horticultural plant most known for its ability to attract butterflies. Three subspecies, *A.t. interior*, *A.t. rolfsii*, and *A.t. tuberosa*, have been identified based on leaf shape, but their overall genetic variability is not known. The current study investigated the genetics of this plant from populations located in six different geographic areas in the United States. Because there is very little knowledge of the genetics of this plant, as a first approach, analyses based on ISSR polymorphisms were used to determine genetic structure. A total of 115 ISSR bands, of which 96.5% were polymorphic, were scored from 82 samples. The Exact Test for population differentiation showed that populations from all six geographic locations were genetically distinct from one another. UPGMA analysis determined that the populations from the different geographic locations did not cluster into three different groups representing the three subspecies. These results do not support the separation of *A. tuberosa* into three subspecies. Instead, they suggest that each of the individual populations studied are relatively genetically isolated. However, there is potential for gene flow, which may allow the populations of *A. tuberosa* to maintain variability.

Introduction

Asclepias tuberosa L. has many common names including "butterflyweed" or "butterfly bush" and is a perennial that is familiar to wildflower enthusiasts and many gardeners. It is part of the milkweed or Asclepiadaceae family, occurring throughout much the world and includes over 280 genera and 2000 species. The *Asclepias* L. genus is composed (thus far) of 76 different species (91 accepted taxa) in the United States (US) and has been studied extensively because of their production of secondary metabolites and their complex reproduction, perhaps rivaling that of the Orchidaceae (Wyatt and Broyles, 1994; USDA, NRCS, 2007).

A. tuberosa is unlike other milkweeds in that it does not produce the milky sap that is indicative of the Asclepiadaceae. Buttterflyweed, however, is an attractive plant with showy flowers arranged in umbels and ranging in color from yellow to orange to red with or without variegation. The plants grow to a height of about one meter and are able to grow in a variety of habitats

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and do not require extensive care, although they prefer full sun and well-drained soil. Butterflyweed, as its name indicates, is primarily known as a food source for butterflies, including the monarch butterfly, and provides a place for the butterflies to lay their eggs. Because of its beauty, ability to attract butterflies and relative ease of maintenance, butterflyweed has become a desired horticultural product occurring in many butterfly gardens (Bush-Brown and Bush-Brown, 1980; Davis, 1993).

Historically, various Native American and European cultures have used the plant as a traditional medicine. The root has been used to primarily treat heart and lung problems, including pleurisy, a condition caused by an inflammation of the lungs and chest cavity. Therefore, this plant is also known as "pleurisy root" (Foster and Duke, 2000; Stevens, 2006). Indeed, the secondary metabolites produced in milkweeds are mostly toxic to organisms, including livestock and humans, but certain insects, including the monarch butterfly, have evolved a tolerance to the plant's toxic secondary metabolites, and even express the toxins to use them as a defense against predators (Agrawal and Malcolm, 2002; Stevens, 2006).

Like all *Asclepias* plants, *A. tuberosa* has a complex floral morphology, including the presence of a "hood" and use of pollinia, structures in which all of the pollen from an anther is transported as one unit (Wyatt and Broyles, 1994). *A. tuberosa* is self-incompatible and in another plant of the same genus, *A. exaltata*, it has been suggested that the self-incompatibility is post-zygotically controlled by a single locus (Lipow and Wyatt, 2000). Butterflyweed relies on insect cross-pollination for reproduction, and although *A. tuberosa* is associated with the Lepidoptera, other insects, especially Hymenoptera, are also primarily responsible for the cross-pollination that occurs in this species (Fishbein and Venable, 1996). Interestingly, according to the United States Department of Agriculture (USDA), the plant has apparently been disappearing in certain regions of the Northeast US and is protected in four different states, including Maine, New Hampshire, New York, and Vermont (USDA, NRCS, 2007).

A. tuberosa has a broad range in the US as well as in Mexico and southern Canada, overlapping with the range of the monarch butterfly. In the US it grows along its eastern portion from the Atlantic coast and into the west, to the prairies and towards the Rocky Mountains (USDA, NRCS, 2007). *A. tuberosa* has been divided into three subspecies occupying different geographic regions, with overlapping boundaries where subspecies distinctions are blurred (Woodson, 1947, 1954, 1962). The distribution of *A. t. rolfsii* is mainly in southeastern US; *A. t. tuberosa* occurs along the Atlantic Coast of the US from Massachusetts to the Carolinas; and *A. t. interior*, the dominant form, is distributed towards the midwest and prairie states (Woodson, 1962). These subspecies were classified mainly by observing leaf shape (Woodson, 1947). However, it is unknown whether differences in leaf shape are in and of themselves a reflection of overall genetic variability. There is some evidence to suggest that there is a significant genetic component to differences in leaf shape in *A. tuberosa*, although environmental effects also contribute as a major factor (Wyatt, 1977; Wyatt and Antonovics, 1981). Studies on *Arabidopsis* and other plants suggest that although leaf shape is controlled by many genes, even dramatic differences in leaf shape can be controlled by a single locus (for review, see Byrne et al., 2001 or Tsukaya, 2003). In addition, later observations of *A. tuberosa* have determined that since the changes in leaf shape are so continuous along the cline, subspecies distinctions, at least between *A. t. tuberosa* and *A. t. interior*, were arbitrarily made (Wyatt and Antonovics, 1981). Yet these subspecies distinctions remain, as do the differences in leaf shape (Wyatt and Antonovics, 1981; USDA, NRCS, 2007).

This study investigates whether populations of *A. tuberosa* from separate geographic regions of the US belong to different broad genetic groups, independent of leaf shape and therefore, of subspecies designations. As of yet, no genetic studies have been used to distinguish populations or establish relatedness among the *A. tuberosa* plants in the US. It is unknown whether the "exploitably vulnerable" native plants in New York (USDA, NRCS, 2007) are genetically distinct

from those that are abundant in a plains state such as Iowa, or a southern state such as Florida. Such knowledge may be of interest to those who may undergo conservation efforts in states where the plants are disappearing. In this study, we have investigated the genetics of butterflyweed populations from Florida, Iowa, Louisiana, New York, Oklahoma, and Tennessee. All are separated by hundreds, if not thousands of kilometers, and are positioned to represent a geographical sampling of the populations that are found throughout the US. This paper also reports on the types of relationships these populations have to one another, including whether or not they cluster into groups that may be representative of their presumed subspecies.

Since there are no prior studies on the genetics of *A. tuberosa*, DNA samples were analyzed from a select number of the plants from the six different locations in the US by determining inter-simple specific repeat (ISSR) polymorphisms (Zietkiewicz et al., 1994). ISSR analysis has become a well-known technique to study genetic diversity in a number of plants, including *Arabidopsis* (Godwin et al., 1997; Barth et al., 2002). Like other widely used techniques, ISSR analysis uses PCR to amplify non-coding hypervariable regions in the genome; the primers, however, are based on a sequence of di-, tri-, tetra-, and/or pentanucleotide repeats. ISSR analysis has advantages over other PCR-based methods of DNA analysis due to its low cost, ease of implementation, and no requirement of prior knowledge of the genome.

Materials and Methods

Plant material

Asclepias tuberosa leaf samples from established, flowering plants were collected from the following locations in six different states: roadsides and an undeveloped lot about twelve miles south and southwest of Gainesville, Florida (12 plants); the Brewer and Steele Prairies of Cherokee County (northwest), Iowa (16 plants); two different sites along a roadside within the Kisatchie National Forest, Catahoula Ranger District in central Louisiana (18 plants); Marshlands Conservancy in Westchester County, New York (12 plants); farmland and the Lexington Wildlife Management Area, in central Oklahoma (13 plants); and roadsides of Coffee County and Franklin County, Tennessee (11 plants). Pictures of the plants in flower (if possible) were taken to determine leaf shape.

Two to three leaves from each plant were collected into plastic bags containing silica beads, or in the case of the New York samples, the leaves were wrapped in aluminum foil and placed into a container with dry ice. The New York samples were then transported directly to Iona College where they were stored in a -70°C freezer until DNA isolation. All other samples in silica were shipped overnight to Iona College, whereupon they were also stored in the -70°C freezer until DNA extraction.

Genomic DNA extraction

Approximately 0.1g of leaf tissue was ground in liquid nitrogen and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) in a total final volume of approximately 100 μL. Random samples were quantified by measuring absorbance at 260nm. Most samples quantified were determined to have a DNA concentration of approximately 0.1 mg mL^{-1}.

ISSR analysis

Eighty-two plants, representing a random sample of 11-18 plants from each population were selected for ISSR analysis. Primers for the ISSR analysis were obtained from Primer set #9 from the University of British Columbia Laboratory (UBC, Vancouver, Canada). PCR conditions were as follows for a 20 μL reaction: ~100 ng DNA; 5μM primer, 200 μM each of the dNTPs; 1 unit of *Taq* DNA polymerase; 1x PCR buffer provided in the PCR Core System I & II kit (Promega, Madison, WI). The amplification reactions were run in one of two thermocyclers: Perkin-Elmer GeneAmp PCR System 2400 (Waltham, MA) or Eppendorf Mastercycler (Hamburg, Germany). Note that all samples for an individual primer were amplified in only one thermocycler. The following conditions were used for the amplification reactions: one cycle

for 5 minutes at 95°C for the initial denaturation; then 35 cycles of 95°C for 1 minute (denature), 50°C or 53°C for 1 minute (annealing) (Table 1), and 72°C for 2 minutes (polymerization); and finally one cycle of 72°C, 5 minutes (final polymerization). Duplicate reactions were run for reproducibility, where the duplicate reactions were completed at separate times in approximately half of the reactions. PCR bands were separated on 1.5% agarose gels with a 100 base pair ladder (Biorad, Hercules, CA) in 1X TBE, and stained with ethidium bromide (Sambrook and Russell, 2001).

Data analysis

Gels were photographed with a Polaroid camera (Waltham, MA) or with the Fotodyne Gel Imaging System (Hartland, WI) and the sizes of the fragments were estimated from the pictures of the gels. DNA bands from different samples were considered homologous if they were determined to have the same molecular weight. Each polymorphic band was scored for its presence (1) or absence (2). Genetic similarity between populations was determined using the Exact Test for population differentiation with 1,000 dememorization steps, 10 batches, and 2,000 permutations per batch (Raymond and Roussett, 1995). The genetic relatedness of the populations was analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Nei's distance measure (Nei, 1972). Bootstrap analysis was performed on the UPGMA using 1000 replicates to determine confidence in the branches of the dendogram. Both the Exact Test and UPGMA used were part of the software program, Tools for Population Genetic Analyses (TFPGA) (Miller, 1997). Variation within and among populations was determined by Analysis of Molecular Variance (AMOVA) with 1000 permutations as part of the Arlequin 3.1 software program (Weir and Cockerham, 1984; Excoffier et al., 1992; Excoffier, et al., 2005). Comparison of geographic and genetic distance was performed with the Mantel test as part of the TFPGA program (Miller, 1997).

Results

ISSR diversity

After initially screening 37 ISSR primers, the following eight primers were determined to produce clear and reproducible bands to be used in the ISSR analysis: UBC_{808}, UBC_{809}, UBC_{842}, UBC_{847}, UBC_{861}, UBC_{864}, UBC_{868}, and UBC_{878}. Only clearly recognizable and reproducible bands that occurred in at least 5% of the samples were used in the analysis (those bands occurring in less than 5% of the samples were usually present in only one plant and disregarded). A total of 115 amplified DNA bands were used in this analysis. Each primer amplified between 10 and 19 DNA fragments, ranging in size from 200-2700 base pairs. A very large percentage, 96.5% (111/115), of the fragments were polymorphic among all 82 samples tested, with each primer displaying 92-100% polymorphic bands (Table 1). No two individuals possessed identical ISSR banding patterns.

Table 1. Primers for ISSR analysis (the molecular weights were rounded to the nearest ten), and the percentage of fragments found to be polymorphic.

Primer (UBC)	Primer sequence	Annealing temp (°C)	Fragment size range (in base pairs)	Number of ISSR fragments	Percentage of fragments found to be polymorphic
808	$(AG)_8C$	53	380-1950	19	100%
809	$(AG)_8C$	53	220-860	10	100%
842	$(GA)_8YG$	53	200-1080	17	94%
847	$(CA)_8RC$	53	580-1900	16	100%
861	$(ACC)_6$	50	740-2700	13	92%
864	$(ATG)_6$	50	450-1650	10	100%
868	$(GAA)_6$	53	280-1730	13	92%
878	$(GGAT)_4$	53	510-1650	17	94%
Total			200-2700	115	96.5%

Exact test for population differentiation

An Exact Test for population differentiation (Raymond and Rousset, 1995) was used to determine whether the populations in the different geographic regions were genetically distinct from one another. Pairwise matrix analyses using the Exact Test showed that genetic differences among all six populations were significant ($p < 0.001$) (Table 2). Interestingly, for the Louisiana and Iowa populations, samples from each of the geographic locations were collected from two separate and relatively isolated sites. Samples from the Louisiana population were collected from two different patches (nine samples each) within the Kisatchie National Forest, where they were separated from each other by approximately one-half kilometer. Samples from the Iowa population were collected from two separate prairies (seven samples and nine samples each), located approximately 20 kilometers from each other. When the samples from the two sites were separated into different populations, the Exact Test analyses showed that the samples from each of the two sites had significant genetic differences ($p < 0.001$ for the two Louisiana sites, $p < 0.001$ for the two Iowa sites). Therefore, each of the Iowa and Louisiana populations were subdivided into two separate populations (total = eight populations) (Table 2). Although samples from the Florida and Oklahoma populations were also collected from two separate sites, the Exact Test showed that differences between the two sites for each of the Florida and Oklahoma populations were less significant ($p < 0.05$ and $p < 0.01$, respectively) than for those of the Louisiana and Iowa populations. Therefore, all samples within the Florida population were grouped into one population, as were the samples from the Oklahoma population.

UPGMA analysis

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis based on the ISSR polymorphisms of the eight separate populations from the six geographic areas was performed. The resulting dendogram did not show the populations from the six geographic areas branching into specific clusters (Figure 1). The Oklahoma and Tennessee populations, which are located in the center of the US, did form a branch, but the other populations attached to the dendogram at separate nodes. Not surprisingly, although the Iowa and Louisiana samples were separated into two populations each, the Iowa populations clustered together, and the Louisiana populations clustered together before joining the other populations in the dendogram. In all, five groups were identified from the dendogram (Figure 1). Bootstrap analysis using 1000 replicates had above 70% support at all nodes, suggesting a high confidence level for the dendogram (Hillis and Bull, 1993).

The UPGMA analysis showed that the plants did not cluster into three groups, as would be expected based on the three subspecies categorizations. To address this inconsistency, a simplified

Table 2. Analysis using the Exact Test for population differentiation based on ISSR polymorphisms. Matrix of combined probabilities for each pairwise comparison.

	Florida	Iowa – Brewer Prairie	Iowa – Syeele Prairie	Louisiana – Pop. A	Louisiana – Pop. B	New York	Oklahoma	Tennessee
Florida	*******							
Iowa – Brewer Prairie	`	*******						
Iowa – Steele Prairie	0.0000	0.0003	*******					
Louisiana – Pop. A	0.0000	0.0000	0.0000	*******				
Louisiana – Pop. B	0.0000	0.0000	0.0000	0.0000	*******			
New York	0.0000	0.0000	0.0000	0.0000	0.0000	*******		
Oklahoma	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*******	
Tennessee	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00000	*******

UPGMA analysis was used on the Iowa and Oklahoma plants that were definitely determined to have a leaf shape corresponding to *A. t. interior,* and compared them with Florida plants that were definitely determined to be *A. t. rolfsii*. Only plants that were clearly one shape or another were included in the analysis (Table 3). The plants were separated based on their overall leaf shape by using a key provided by Woodson (1954). The groupings coincided with what would be predicted by Woodson (1954) based on the locations of the expected "subspecies" (Table 3). For example, it was evident that most of the plants in the Iowa populations had a leaf shape that clearly corresponded to the *interior* subspecies, which is what would be predicted by Woodson (1954). If the plants were to group by subspecies alone, then the Iowa and Oklahoma populations should cluster. If they were to group according to our original UPGMA analysis (Figure 1), the Oklahoma and Florida populations should cluster. UPGMA analysis showed the three populations clustering according to our original UPGMA analysis (Figure 2), rather than by subspecies.

AMOVA

Hierarchical analysis of molecular variance (AMOVA) confirmed the results above, where if the eight populations were separated into five groups (Figure 1), genetic differences among the groups were significant ($P < 0.01$). The AMOVA determined that 80% of the variation occurred within populations, 9% among populations within groups, and 11% among groups (Table 4a). If plants were grouped according to subspecies (Table 3), the genetic differences among the groups became insignificant ($P > 0.50$). Here, the AMOVA calculated that 82% of the ISSR variation occurred within populations, while 18% occurred among populations within groups, and essentially 0% among groups (Table 4b) (Excoffier et al., 2005).

Geographic versus genetic distance

To determine whether geography contributed to genetic variability, a Mantel test was performed to compare geographic and genetic distance matrices. When all eight populations were taken into account, there was not a significant correlation between geographic and genetic distance (Mantel test: $r = 0.5408$; $P = 0.98$) (Miller, 1997).

Discussion

Genetic structure

When this study was initiated, considering the extremely large migratory range of the monarch

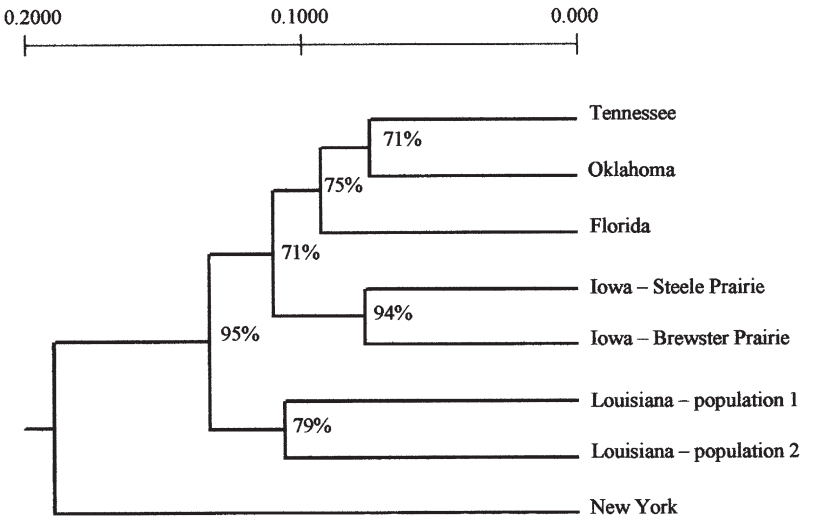

Figure 1. Dendogram based on UPGMA analysis of ISSR polymorphisms of eight populations of A. tuberosa from six different areas throughout the US. Numbers at the nodes indicate bootstrap values; the ruler at the top represents genetic distance based on Nei's genetic distance (1972).

butterfly, which coincides well with the range of *A. tuberosa*, it was hypothesized that there was the possibility that there was an extensive amount of gene flow throughout the US, even over large distances (Brower, 1996). It was also suggested that the westerly winds may have increased gene flow such that genotypes occurring in the west of the US would dominate over the others (Woodson, 1962). The Exact Test for population differentiation analyses seemed to show, however, that based on ISSR polymorphisms, individual populations were relatively genetically isolated from one another and that reproduction occurs mostly within populations rather than between populations. In fact, the Exact Test analyses showed that the two Louisiana populations, which were separated by approximately only 0.5 kilometers, and the two Iowa populations, which were separated by approximately 20 kilometers, were genetically distinct (Table 2). Though unexpected, this result was similar to studies performed on *A. exaltata*, where it was determined that although pollen may travel as far as one kilometer from its source, most of the pollination occurred within a few meters of the source plant (Broyles et al., 1994). In addition, pollination experiments performed on *A. tuberosa* showed greater success when plants within a population were crossed with each other than with plants from different populations (Wyatt, 1976).

These results, however, do not preclude that there can be exchange of genetic material over long distances. ISSR analysis of populations from the six distant geographic locations indicates that *A. tuberosa* is highly polymorphic, where the AMOVA determined that most of the variation (~80%) occurs within populations rather than among populations (Table 4). This result is most probably a reflection of the fact that *A. tuberosa* is an outcrossing species. A paternity exclusion analysis on pollen dispersal of *A. exaltata* has shown that a significant number of plants (~10%) within a population resulted from pollen outside of the population (Broyles and Wyatt, 1991). In addition, although butterflyweed pollen is transported mainly by insects, the seeds are dispersed by wind. It has been suggested that *A. syriaca* may have expanded its range southward by dispersing seeds with the aid of human and motor vehicle activity (Wyatt et al., 1993). Since many butterflyweed plants occur alongside roads, there could be the possibility that seeds may travel long distances, being blown by wind generated by moving cars. There is also anecdotal evidence that humans may have an effect in transporting genetic material by collecting seeds from roadsides and/or buying seeds from nurseries and planting them in their own gardens, far from their original source. Small, yet significant, cross-pollination from sources outside of a particular population of butterflyweed may very well contribute to its variability.

Genetic relationships

When the eight populations from the six regions of the US underwent UPGMA analysis, the resulting dendogram showed that the populations did not cluster into distinct groups (Figure 1). The two Iowa populations clustered at a single node, as did the two Louisiana populations, supporting the notion that geographic distance contributes to overall genetic relatedness. In addition, the Tennessee and Oklahoma populations were the only populations from different geographic regions that clustered at a node. They are

Table 3. Number of individuals from the Florida, Iowa, and Oklahoma populations that were categorized into one of the three subspecies. Numbers refer to only those plants that clearly showed a leaf shape indicative of the subspecies (not all plants were categorized due to unclear leaf shape or lack of data).

	Number of plants with a clearly defined leaf shape	*A. tuberosa* ssp. *interior*	*A. tuberosa* ssp. *rolfsii*	*A. tuberosa* ssp. *tuberosa*
Florida	8	0	8	0
Iowa:				
a. Brewer Prairie	a. 6	a. 6	a. 0	a. 0
b. Steele Prairie	b. 9	b. 9	b. 0	b. 0
Oklahoma	10	9	0	1

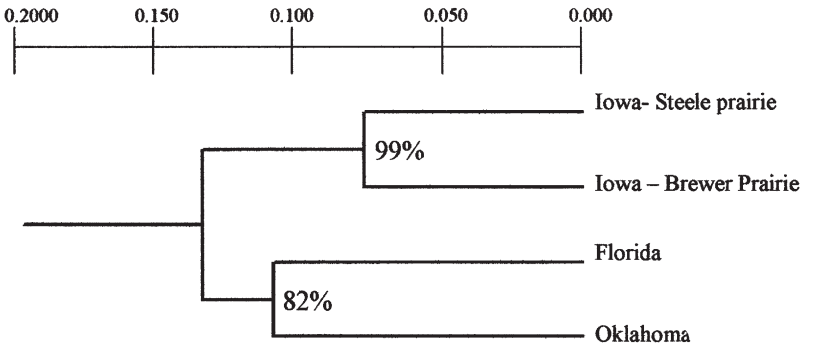

Figure 2. Dendogram resulting from UPGMA analysis of ISSR polymorphisms of a subset of plants that segregate into one of two subspecies and belong to a geographic population. Genetic distance (Nei, 1972) is shown on top and bootstrap values are given at each node.

next to each other geographically (relative to the other regions) and located in the middle of the *A. tuberosa* range, perhaps indicating that there was more gene flow between these two geographically close populations than with the other populations. A pairwise comparison of matrices using the Mantel test indicated, however, that overall, there was not a significant correlation between geographic distance and genetic distance. In fact, other than the relationships just mentioned above, populations from each geographic area seemed to occupy individual nodes (Figure 1), further indicating that the populations are genetically distinct and relatively isolated.

For example, the UPGMA analysis showed the New York population to be in its own group, completely separate from the other populations. Its relative presumed genetic isolation may be explained by the fact that the plant samples were collected from a geographically isolated stand. Indeed, the closest stand that could be identified was at least 45 kilometers northwest, in another protected part of the county. In addition, it has been shown that the primary pollinators in the New York stand were bees with no observed butterfly activity, suggesting that reproduction occurred solely within this population (Lodato, 1987). Over time, its genetic isolation may have caused the population to evolve independently from other organisms of its species. As populations become sparser in the northeastern portion of the US, genetic isolation, enhanced by geographic isolation of individual stands, may accelerate *A. tuberosa* evolution in these areas.

Table 4. AMOVA of populations grouped according to: a. clusters from the dendogram of the original UPGMA analysis (Figure 1); b. subspecies designations.

a.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation (%)	P-value
Among five groups (see Figure 1):	4	196.9	1.52	11.03	0.0078
Among populations within groups:	3	70.2	1.30	9.40	0.0000
Within populations:	74	811.2	10.96	79.56	0.0000

b.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation (%)	P-value
Among two groups: 1. ssp. *interior* - OK and IA (Brewer and Steele Prairies) 2. ssp. *rolfsii* - FL	1	29.1	-0.13	-0.99	0.501
Among populations within groups:	2	58.2	2.47	18.93	0.0000
Within populations:	27	289.6	10.73	82.05	0.0000

The role of geographic distance in *A. tuberosa* population genetics is unclear. The dendogram from the UPGMA analysis suggests that it does have a role, but the Mantel test does not show a significant correlation between geographic and genetic distance. Investigating the genetic variability of populations over a range of geographic distances, rather than just within a local area or among populations that are separated by hundreds of kilometers, might shed more light on this matter. Additional studies investigating populations along a transect or along a specific insect migratory route may be warranted.

Subspecies classifications

In general, these results do not support the classification of *A. tuberosa* into separate subspecies, even though leaf shapes appear to remain distinct among populations (Table 3). Our UPGMA analyses determined that populations did not cluster according to subspecies, even when leaf shape (the criterion used to separate out subspecies) was taken into account. Plants from the Oklahoma population that were determined to be *A. t. interior* clustered with plants from the Florida population that were determined to be *A. t. rolfsii*, rather than with the plants from the Iowa populations that were also determined to be *A. t. interior* (Figure 2). In addition, the AMOVA did not indicate significant differences between a group that included both the Iowa and Oklahoma populations and a group composed of the Florida population (Table 4b). These results suggest that at least *A. t. interior* and *A. t. rolfsii* do not represent genetically distinct evolutionary groups.

Further research

This paper reports on the genetic relatedness of eight populations from six geographic areas in the US. In general, our results suggest that each of the individual populations is relatively genetically isolated where most of the pollinations occur within the population. However, variability is maintained within these populations, most probably because *A. tuberosa* is an outcrossing species with potential for gene flow from other populations. Many questions remain, including the contribution of genes associated with leaf shape to overall genetic variability, the extent of gene flow among populations, the role of geography in genetic isolation, and the relationships to populations located in other parts of the *A. tuberosa* range. We are planning on further studies to include more populations throughout the US, as well as populations occurring within the same regions of the populations included in this study. In fact, samples that have been collected from an additional 14 populations throughout the US will be studied. We are planning to develop chloroplast markers and/or develop SSRs (microsatellites), to support results obtained by ISSR analysis.

As native plants begin to disappear from certain parts of the US, while others from different "subspecies" interbreed, and horticultural varieties of unknown origin are being planted by gardeners, it may be possible that the genetics of *A. tuberosa* may change in a relatively short amount of time.

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