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Investigation of Hybridization between *Asclepias speciosa* and *A. Syriaca* using Alkanes, Fatty Acids and Triterpenoids*

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Key Word Index—*Asclepias;* Asclepiadaceae; hybridization, triterpenoids; alkanes; fatty acids.

Abstract—Individuals of *Asclepias speciosa* and *A. syriaca* from Kansas and Nebraska were sampled and their alkanes, fatty acids and triterpenoids analyzed. Analysis of variance for 30 compounds yielded four significant and seven highly significantly quantitative differences between the taxa. No qualitative differences were found. Analyses of the components using Well's hybrid distance diagram and principal coordinate analysis confirmed that hybridization as well as introgression is occurring between these taxa in this region. Data from isozymes revealed that the two taxa are almost identical. These data, taken together, are indicative of a closer (perhaps conspecific) relationship between these taxa than previously suggested.

Introduction

The genus *Asclepias* is composed of approximately 140 species and Woodson recognized 108 species in North America [1, 2]. The North American species are generally herbaceous perennials with only a few annuals [1]. All *Asclepias* species cytologically investigated to date are diploids ($n = 11$) [1, 2]. Woodson [1] recognized hybrids between *Asclepias speciosa* Torr. and *A. syriaca* L., but did not consider hybridization to be of frequent occurrence. Stevens [3] reported that *A. speciosa* and *A. syriaca* were sympatric in western and central North Dakota, but that only *A. speciosa* occurred in western North Dakota. Putative hybrids were collected in western Minnesota and Stevens [3] noted that he had seen other putative hybrids in the area. The two taxa are difficult to distinguish morphologically, however, and Stevens [3] concluded that leaf shape was not a reliable character, and were best separated on flower size and number of flowers per umbel. Thomson and Wagner [4] reported hybrid swarms between *A. speciosa* and *A. syriaca* as well as backcrossing in western Minnesota. These hybrid swarms were found mostly on disturbed sites but Thomson and Wagner [4] suggested that introgression was occurring into the gene pools of both species. Artificial cross-polination of *A. syriaca* with pollinia from *A. speciosa* has been accomplished [5] and fruit pods obtained, although there was no mention of germination tests of the putative hybrid seed.

Asclepias syriaca appears to be a more aggressive, "weedy" species than *A. speciosa* in central Nebraska and Kansas. The former is often found in fallow fields, roadsides, and in disturbed areas near irrigation ditches, whereas the latter is less frequent and more often found with native grassland plants. The largest number of hybrid swarms were found in disturbed roadsides, on railroad right-of-ways and abandoned highways.

The purpose of this study was to examine a zone of sympatry between *A. speciosa* and *A. syriaca* in eastern Kansas and Nebraska to determine the extent of hybridization and backcrossing using chemical data. Because there are so few morphological characters that separate *A. speciosa* from *A. syriaca*, we have used alkanes, fatty acids and triterpenoids [6–8] for this analysis. In addition the genetic differentiation between these two species is discussed.

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Results and Discussion

The initial field observations revealed that *A. speciosa* had long coronas (9–13 mm) and few flowers per umbel (12–30) whereas *A. syriaca* had short coronas (3–5 mm) and many flowers per umbel (50–75) in agreement with Stevens' [3, 5] observations. Thirty alkanes, fatty acids and triterpenoids were present in amounts greater than 0.5% in the extracts and these were quantified in the GLC analyses of the non-polar extractives of 46 samples (Table 1). These 30 components, along with per cent yield (dry weight basis), were subjected to one-way analyses of variance (ANOVA 1). Of the 30 components statistically analyzed, four were significantly and seven were highly significantly different between the *A. speciosa* and *A. syriaca* groups used in ANOVA (Table 1). The composition of the extracts for *A. speciosa* from Kansas was essentially the same as previously reported [6] for *A. speciosa* collected near Salt Lake City, Utah. The closeness of *A. speciosa* and *A. syriaca* is revealed by their nearly identical alkanes, fatty acids and triterpenoids (Table 1). The two taxa differ principally in the amounts of triacontane, unknown 6, β-amyrin butyrate, α amyrin butyrate, and unknowns 10 and 13 (Table 1). Even so, these differences are strictly quantitative and rather minor. The 19 components with F ratios greater than 1.0 (F greater than 1.0 indicates that the variance between taxa was greater than the variance within the taxa) were then utilized for the computation of Well's hybrid distances and principal coordinate analysis.

A F-1 (F from ANOVA between the two taxa) weighted hybrid distance diagram (9, 10] was

TABLE 1. ANALYSIS OF VARIANCE FOR PERCENT YIELD FOR 30 NON-POLAR COMPOUNDS WHICH WERE PRESENT IN AMOUNTS GREATER THAN 0.5% OF THE TOTAL GLC COMPONENTS IN EITHER *A. SPECIOSA* OR *A. SYRIACA*

Component	Avg. % of GLC peaks *A. speciosa*	*A. syriaca*	F. ratio	Probability
% yield	6.50	6.23	0.36	0.56 ns
Palmitic acid	0.76	0.59	0.23	0.65 ns
Linolenic acid	0.76	0.65	0.08	0.77 ns
Squalene ($C_{30}H_{50}$)	0.10	0.52	7.41	0.02 *
Nonacosane ($C_{29}H_{60}$)	0.56	0.58	0.10	0.92 ns
Triacontane ($C_{30}H_{62}$)	0.77	0.17	29.64	0.002 **
Hentricontane ($C_{31}H_{64}$)	0.80	0.42	7.67	0.02 *
Campestrol	0.60	0.73	2.89	0.11 ns
Stigmasterol	0.58	0.61	0.05	0.83 ns
β-Amyrin	2.27	2.41	0.26	0.62 ns
β-Sitosterol	2.79	2.89	0.19	0.67 ns
Unknown 5	0.62	0.50	0.31	0.59 ns
α-Amryin	4.51	5.23	5.69	0.31 ns
Unknown 6	1.44	0.58	16.12	0.002 **
Unknown A2	0.80	0.29	8.37	0.01 **
β-Amyrin acetate	10.37	10.68	0.08	0.78 ns
ψ-Taraxsterol	1.30	0.75	6.52	0.02 *
α-Amyrin acetate	46.28	50.36	2.01	0.18 ns
Unknown 7	0.51	0.22	12.76	0.03 *
Unknown 8	0.56	0.40	2.08	0.17 ns
Oleanolic acid	2.34	2.00	1.74	0.21 ns
Ursolic acid	1.21	1.15	0.04	0.84 ns
Unknown 19	0.06	0.17	3.90	0.67 ns
β-Amyrin butyrate	1.42	1.00	35.67	0.0001 **
Unknown 10	0.66	0.16	24.11	0.0005 **
α-Amyrin butyrate	3.95	3.29	8.74	0.01 **
Unknown 11	0.76	0.54	0.85	0.625 ns
Unknown 13	0.55	0.05	31.28	0.0002 **
β-Amyrin hexanoate	1.92	2.01	0.28	0.61 ns
α-Amyrin hexanoate	6.96	7.88	1.90	0.19 ns
α-Amyrin palmitate	1.20	1.11	0.13	0.73 ns

F at (1, 13 df), probability at $P=0.05$. Components are listed in the order of their elution from a DB1 (methyl silicone) capillary column.

constructed using the aforementioned 19 components. In addition, a synthetic intermediate was created by averaging each of the 19 compounds using the mean values for *A. speciosa* and *A. syriaca* (denoted by F in all figures). The hybrid distance diagram (Fig. 1) showed that neither *A. speciosa* nor *A. syriaca* were entirely uniform based on these components. Both *A. speciosa* and *A. syriaca* populations contained individuals that, chemically, appeared to be intermediate in the hybrid distance diagram. This was in spite of the fact that the plants were quite uniform in their respective floral morphology. Many of the samples were intermediate in their chemistry (Fig. 1), but there is a decided shift toward putative backcrosses with *A. syriaca.*

In a comparison of multivariate methods for the detection of hybridization. Adams [9] found that the hybrid sunfish obtained by artificial crosses and putative natural hybrids of *Juniperus* [11] were separated in the second coordinate axis of principal coordinate analysis, with the first coordinate separating the parents. Principal coordinate analyses, using the 19 chemical components to generate F-1 (F from ANOVA of the two parents) weighted similarity measures between the 47 OTUs (46 individuals plus the synthetic intermediate), accounted for 32.68, 7.57, 6.04, 4.51 and 4.13% of the variation, respectively, for the first five eigenroots. Eigenvectors 3, 4, and 5 appear to asymptote and no biological pattern could be discerned. The first principal coordinate does clearly separate the parents (Fig. 2). The separation between the two species is greater (Fig. 2) than with the distance diagram (Fig. 1), and the heterogeneity of the chemistry of the two taxa is still evident. The synthetic intermediate (F) is intermediate on axis 1 as anticipated. Again, one sees essentially a continuum of individuals between *A. speciosa* and *A. syriaca* with more individuals tending towards *A. syriaca* in their chemistry (Fig. 2).

A correlation analysis of corona length, number of flowers per umbel and the chemical score on principal coordinate 1 revealed the following correlations:

Corona length × no. flowers/ umbel	= 0.82
Corona length × chemical score on PC1	= −0.71
No. flowers/umbel × chemical score on PC1	= 0.56

A 3-dimensional plot of the corona length, number of flowers/umbel and chemical score on principal coordinate 1 is shown in Fig. 3. Several individuals with long coronas and few flowers/umbel are intermediate in their chemistry. In contrast, very few individuals with short coronas and many flowers/umbel were

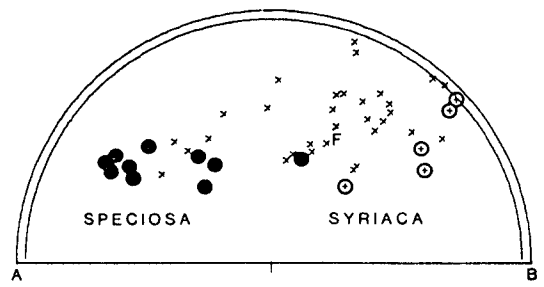

FIG. 1. WELL'S HYBRID DISTANCE DIAGRAM BASED ON 19 COMPOUNDS, F-1 WEIGHTED. Solid circles are individuals of the reference group used to represent *A. speciosa.* The open circles with a plus (+) inside are individuals of the reference group used to represent *A. syriaca.* The crosses are individuals not used to represent either of the taxa in ANOVA. F is the position of a synthetic individual, exactly intermediate in each compound between the means of *A. speciosa* and *A. syriaca.*

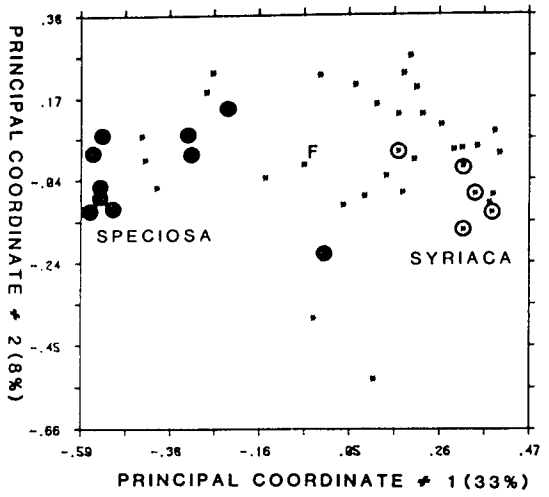

FIG. 2. PRINCIPAL COORDINATE ANALYSIS OF 47 OTUs BASED ON F-1 WEIGHTED, GOWER METRIC SIMILARITIES. The solid circles, open circles, and F, respectively, are individuals of *A. speciosa, A. syriaca* and a synthetic individual that is exactly intermediate between the means of the two taxa for each compound.

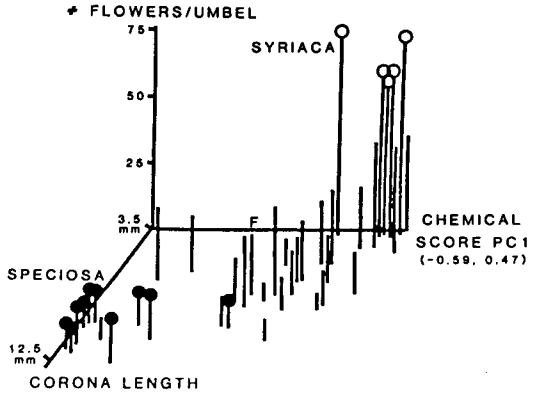

FIG. 3. A 3-DIMENSIONAL ORDINATION OF *ASCLEPIAS* INDIVIDUALS USING THEIR CHEMICAL SCORE ON PRINCIPAL COORDINATE 1, CORONA LENGTH ON COORDINATE 2 AND NUMBER OF FLOWERS PER UMBEL ON COORDINATE 3. The open circles, closed circles, and F represent reference individuals of *A. syriaca*, and *A. speciosa*, and an individual intermediate for each of the three characters. See text for discussion

intermediate in their chemistry. Several individuals with an intermediate number of flowers/umbel were strongly clustered with *A. syriaca* according to their chemistry. The recombinations of chemistry and morphology suggest genetic segregation and backcrossing.

This begs the question of the distinctness of these taxa as species. In another study of 15 populations of *A. speciosa* and four populations of *A. syriaca* (Price, in preparation) samples from the two taxa (which were separated by over 1000 miles) were found to be only slightly differentiated for isozyme characters based on pairwise population comparisons (Table 2). Hendrick's genetic similarity [12] for five isozymes showed that about a 10% lower average between *A. speciosa* and *A. syriaca* than within the taxa. Nei's distance measure [13] using nine isozymes showed no significant differences between the taxa (Table 2). No alleles or isozyme phenotypes were detected which were diagnostic for either *A. speciosa* or *A. syriaca.* One may conclude that *A. speciosa* and *A. syriaca* are not significantly differentiated for any of the isozyme systems which we examined.

The two taxa are very closely related; for without the flowers, it is almost impossible to classify individual specimens to the species. Woodson [1] placed these taxa in separate series based on corona character differences

TABLE 2. COMPARISONS OF ISOZYME SIMILARITIES WITHIN AND BETWEEN *ASCLEPIAS SPECIOSA* AND *A. SYRIACA*

Hedrick's genetic similarities§:

	Enzyme*					
Taxon comparison	ADH	EST	AAT	PGI	PGM	Average
speciosa-speciosa	0.5869[a]	0.786[a]	0.620[ab]	0.870[a]	0.796[a]	0.732[a]
speciosa-syriaca	0.5870[a]	0.771[a]	0.594[a]	0.860[a]	0.632[b]	0.689[b]
syriaca-syriaca	0.6541[a]	0.843[a]	0.769[a]	0.962[a]	0.701[ab]	0.786[a]

Nei's genetic similarity†:

	Enzyme locus					
Taxon comparison	ADH1	ADH2	EST1	EST2	AAJ1	
speciosa-speciosa	0.9982[a]	0.935[a]	0.992[a]	0.973[a]	0.860[a]	
speciosa-syriaca	0.9984[a]	0.952[a]	0.981[b]	0.985[a]	0.834[a]	
syriaca-syriaca	0.9984[a]	0.954[a]	0.986[ab]	0.997[a]	0.955	
Taxon comparison	AAT2	PGI1	PGM1	PGM2	Average	
speciosa-speciosa	0.960[a]	0.957[a]	0.9994[a]	0.996[a]	0.947[a]	
speciosa-syriaca	0.967[a]	0.956[a]	0.9997[a]	0.939[b]	0.949[a]	
syriaca-syriaca	0.987[a]	0.994[a]	0.9999[a]	0.886[c]	0.973[a]	

Data from Price, in preparation.

*Alcohol dehydrogenase, ADH; esterase, EST; aspartate aminotransferase, AAT; phosphoglucoisomerase, PGI; phosphoglucomutase, PGM.

†Nei's genetic similarity [13] calculated on the basis of allele frequencies for nine isozyme loci. Values with the same letter indicate no statistical difference (alpha = 0.10).

§Hedrick's genetic similarity [12] calculated on the basis of isozyme phenotypes for five different enzyme systems.

but he understood them to be closely related. Our data confirms [3–5] the fact that these two taxa freely hybridize (at least in the zone of sympatry studied), and that the morphological characters, alkanes, fatty acids, triterpenoids and isozymes are very similar between these two taxa. However, our observations indicate that the taxa sort themselves out on the basis of ecological preference. At present, they are maintaining their ranges in spite of hybridization and possible introgression in the sympatric areas of eastern Kansas and Nebraska. Additional study will be needed to determine the infraspecific variation in these taxa and relationships with other taxa of *Asclepias* before a definitive statement can be made as to whether *A. speciosa* and *A. syriaca* should be treated as subspecies of a single species.

Experimental

Based on the floral characters suggested by Stevens [3, 5] plants of *A. speciosa, A. syriaca* and putative hybrids were collected (aerial parts) in July 1983 in Kansas and Nebraska. The plants were air dried (60°) and divided into voucher specimens and material for extraction. The voucher specimens are deposited at the Kansas State University Herbarium.

The above ground foliage was ground in a Wiley Mill to pass a 2 mm sieve and then extracted 20 h with hexane in Soxhlet extractors. The hexane solvent was removed under reduced pressure. GLC analyses were performed on a Varian 1800 gas chromatograph (f.i.d.; 350°) using a J & W DB1 fused quartz capillary column (30 mm × 0.32 mm) with He (30 ml/sec). All analyses were performed in the split mode (25:1 split ratio) with the injector temperature at 275°. The oven temperature was programmed 160° to 340°: 8°/min for 12 min; 4°/min for 21 min; isothermal for 9 min. Peak areas were quantitated using a digital integrator. Peak identification follows the previous GC-MS analyses [6]. Several small components were found in this set of samples and coded as unknowns in this study.

The chemical data were coded and analyzed by one-way analysis of variance (ANOVA) with two populations (1 d.f.) and six samples of *A. speciosa* and five samples of *A. syriaca* (d.f. = 9). Principal coordinate analysis followed the formulation of Gower [14] using the Manhattan metric, scaled by the range (=Gower metric) [15] and weighted by F-1 (from ANOVA) as formulated by Adams [9, 16]. The Wells hybrid distances [10] were computed using F-1 character weights as formulated by Adams [9].

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