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ISOZYMES OF AN AUTOPOLYPLOID SHRUB, *ATRIPLEX CANESCENS* (CHENOPODIACEAE)

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ABSTRACT.— Diploid, tetraploid, and hexaploid populations of *Atriplex canescens* ($x=9$) were examined for 18 isozyme systems. Of 24 interpretable loci, only one locus (Per_1) was polymorphic. Another locus (Per_2) showed a dosage effect. Genetic distance values, D, ranged from near 0 to 0.05, which are in the normal range for local species races. Results from clonal ramets gave identical results. The data and analyses support an essentially autopolyploid origin for the polyploid populations examined.

Atriplex canescens (Pursh) Nutt. is a widely distributed shrub in western North America. It occurs in chromosome races of $2x-12x$. Tetraploids ($4x=2n=36$) are most common over a majority of the range of the species, but diploids and hexaploids are not infrequent in some widely distributed areas (Stutz and Sanderson 1979, McArthur and Freeman 1982). Higher polyploids are restricted in distribution. Stutz et al. (1975) presented meiotic evidence (2.18 IVs/cell, range of 0–6) indicating that the $4x$ populations are autopolyploid. Diploid ($2x$) *A. canescens* is essentially dioecious; $4x$ and $6x$ forms are trioecious—♀, ♂ and [♀, ♂] (McArthur 1977, McArthur and Freeman 1982). As might be expected in an outcrossing species, much morphological variation is evident within and between populations (McArthur et al. 1983). We undertook a study to examine isozyme patterns among polyploid levels, genders, and ecologically separated subpopulations of *A. canescens*.

Materials and Methods

We selected four populations (Table 1) for study. One, Kingston Canyon, was divided into two subpopulations because of strikingly different ecological conditions (plant communities, slope, moisture relationships, soils—McArthur et al. unpublished data) and because Freeman et al. (1976) showed that plants of the related species *A. confertifolia* tend to segregate ♀ versus ♂ on environmentally different sites. Seven plants from each sexual state were randomly sampled from each population. Thus, we sampled 14 plants from the Little Sahara Sand Dunes, 21 from Spanish Fork Canyon, 35 from Kingston Canyon, and 21 from near Grantsville (Table 1).

Plant material for isozyme analysis consisted of actively growing leaves from rooted cuttings (McArthur et al. 1984) growing in a greenhouse. Isozyme procedures followed Leonard et al. (1981) using a vertical polyacrylamide preformed gradient gel (Pharmacia PAA 4/30)[3]. We also followed Leonard et al. (1981) for isozyme staining of all systems except for shikimate DH (Linhart et al. 1981) and NADP-MDH (Henderson 1966). Eighteen isoenzyme systems were examined (Table 2). For heterozygous loci (only peroxidase Per_1 in this study) the "dose" of each allele for polyploid plants was detected visually by observation of staining intensity of two to five replicated gels.

Standard genetic distance, D, was calculated using the formula $D = -\log_e I$, where

$$I = \frac{J_{xy}}{J_{xx}J_{yy}}$$

and J with its subscripts is the probability that alleles under consideration are identical (Hartl 1980). Allele frequency differences fo-

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TABLE 1. Characteristics of sampled populations of *Atriplex canescens*.

Collection sites	Site descriptions	Chromosome numbers[a]	Sexual states
Little Sahara Sand Dunes, Juab Co., Utah	Large rolling sand dunes	2x	♀,♂
Spanish Fork Canyon, Utah Co., Utah	Steep (> 50°) canyon slope. Rocky, unconsolidated soil.	4x	♀,♂, [♀,♂]
Kingston Canyon, Piute Co., Utah	Two subpopulations: (1) alluvial fan on canyon floor; (2) steep (> 50°) talus slope.	4x	♀,♂, [♀,♂]
Near Grantsville, Tooele Co., Utah	Valley floor, sedimentary clay soils.	6x	♀,♂, [♀,♂]

[a]Little Sahara Sand Dune population and Grantsville population determined by Stutz et al. (1975 and 1979, respectively). We did counts for several plants each at Spanish Fork Canyon and Kingston Canyon following the methods of Stutz et al. (1975) and confirmed the counts for the other locations.

TABLE 2. Isozyme systems tested.

Isozyme[a]	Success	Genetics
Peroxidase	Yes	1 locus, polymorphic 1 locus, monomorphic
Glutamate DH	Yes	1 locus, monomorphic
Malate DH	Yes	1 locus, monomorphic[b]
Shikimate DH	Yes	2 loci, monomorphic
Indole Phenol Oxidase	Yes	2 loci, monomorphic
NADP - MDH	Yes	1 locus, monomorphic
LAP	Yes	2 loci, monomorphic
PGI	Yes	1 locus, monomorphic
PGM	Yes	2 loci, monomorphic
G-6-PDH	Yes	6 loci, monomorphic[a]
Amylase	Yes	2 loci, monomorphic[a]
Esterase	Yes	1 locus, monomorphic many loci, not interpretable
RBPC[c]	Yes	1 locus, monomorphic[b]
ADH	No	—
Catalase	No	—
Alkaline Phosphatase	No	—
Acid Phosphatase	No	—
GOT	No	—

[a]See Henderson 1966, Leonard et al. 1981, and Linhart et al. 1981 for full isozyme name descriptions except for RuDPC, which is Ribulose diphosphate carbohylase.
[b]Additional loci may be present but were difficult to resolve and appeared to be monomorphic.
[c]From our total protein analysis—apparently RBPC.

the Per_1 locus were analyzed using the Student-Newman-Keuls multiple range test following analysis of variance procedures (Woolf 1968).

RESULTS AND DISCUSSION

Of the 13 systems (24 interpretable loci) we were able to analyze, only one locus was polymorphic (Table 2). The Per_1 locus had slow, *s*, and fast, *f*, alleles. Under our experimental conditions (see Leonard et al. 1981) the Per_1–*s* allele migrates about 35 mm and the Per_1–*f* allele about 41 mm from the origin.

The isozyme data support the Stutz et al. (1975) and Stutz and Sanderson (1979) suggestion that *A. canescens* forms an autopolyploid complex. The preponderance of monomorphic loci, identical in each locus in each population, suggests genetic homogeneity inherent in autopolyploid complexes. In a similar study, Oliver and Ruiz Rejon (1980) also found identical isozymes at various polyploid levels in the apparent autoploid *Muscari atlanticum* (Liliaceae). In their study, they also found that esterase isozymes stained more intensely with increasing polyploid levels. We found an analogous situation with our Per_2 locus (54 mm from origin): all *A. canescens* plants had Per_2 monomorphically and showed a distinct dosage effect. Diploids stained lightly, tetraploids darker, and hexaploids darkest of all. Visual observation of Per_2 from a few plants is enough to ascertain the population ploidy level.

TABLE 3. Genetic distance, D, among the *Atriplex canescens* populations.

Collection sites	Distance values				
	1	2	3	4	5
Little Sahara Sand Dunes (1)	—				
Kingston Canyon slope (2)	.003	—			
Kingston Canyon flat (3)	.004	.000	—		
Spanish Fork Canyon (4)	.008	.000	.000	—	
Grantsville (5)	.024	.011	.011	.007	—

Ordinarily, polyploids have high levels of isozyme heterozygosity (Hamrick et al. 1979, Hunziker and Schaal 1983). That *Atriplex canescens* does not supports its probable autoploid condition. Autoploidy aside, it is another example, following Ledig and Conkle 1983), that some woody long-lived perennials have more isozymic homozygosity than previously thought, e.g., Hamrick et al. 1979. Sanderson and Stutz (1984) have recently discovered that diploids have a consistently different flavonoid pattern than do tetraploids for both *Atriplex canescens* and *A. confertifolia*. Their data can be interpreted as meaning the two ploidy levels have different flavonoid physiology.

Table 3 shows that genetic distance values among *A. canescens* populations are minimal but are in the range (D = nearly 0 to 0.05) that Nei (1976) suggested for local races of a species. All tetraploid populations had D values of nearly 0. The hexaploid population was set further from the tetraploid populations than was the diploid population. The hexaploid population may, interestingly, have some introgression from *A. tridentata* (Stutz et al. 1979), whereas the tetraploid populations appear to be strict autopolyploids. We point out that our genetic distance values reflect variation at only one of the 13 loci examined.

We examined two or more clonal ramets for isozymes from each plant and obtained identical results in each case. These results are similar to those of Sternberg (1976), who showed that separated clones of *Larrea tridentata* maintained identical isozyme patterns.

There was no statistically significant frequency difference of the Per_1 locus among the sexual phenotypes of *A. canescens* at the study sites (Table 4) even though these phenotypes differ in morphological and physiological characteristics (McArthur and Freeman 1982; McArthur et al. 1984; McArthur et al. unpublished data). However, in four of the five populations ♂'s have a higher frequency of the Per_1–*s* allele than do ♀'s. The change in frequency of the Per_1–*s* allele from diploid to tetraploid to hexaploid is interesting and warrants further attention.

Interesting, too, is that *A. canescens* is dioecious or trioecious. These sexual systems have long been considered to have evolved due to inbreeding depression (Grant 1975, Lloyd 1982). Given the monomorphic isozyme data for loci that are normally highly polymorphic (Table 2), it is difficult to see how inbreeding depression, in this case, could be as potent as is required to create the dioecious state. Krohne et al. (1980) discounted inbreeding depression as the driving force in the gynodioecious breeding system of *Plantago lanceolata*.

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LITERATURE CITED

FREEMAN, D. C., L. G. KLICKHOFF, AND K. T. HARPER. 1976. Differential resource utilization by the sexes of dioecious plants. Science 193:597–599.

GRANT, V. 1975. Genetics of flowering plants. Columbia University Press, New York. 514 pp.

HAMRICK, J. L., Y. B. LINHART, AND J. B. MITTON. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Sys. 10: 173–200.

HARTL, D. L. 1980. Principles of population genetics. Sinauer Associates, Inc., Sunderland, Massachusetts. 488 pp.

HENDERSON, N. S. 1966. Isozymes and genetic control of NADP-Malate Dehydrogenase in mice. Arch. Biochem. Biophys. 117:28–33.

TABLE 4. Frequency of the Per_1-s allele.

Population	Mean ± se	Frequency ♀	♂	[♀,♂]
Little Sahara Sand Dunes (2x)	.921 ± .057A[a]	.850	1.00	—
Kingston Canyon slope (4x)	.647 ± .071B	.643	.667	.625
Kingston Canyon flat (4x)	.643 ± .081B	.625	.667	—[b]
Spanish Fork Canyon (4x)	.583 ± .052B	.536	.643	.571
Grantsville (6x)	.258 ± .040C	.286	.238	.250

[a]Different letters after mean frequency values indicate significantly different ($p < .01$) means.
[b][♀,♂] ramets from Kingston Canyon flat were not available for study at the same time as the other populations, consequently they were not included.

HUNZIKER, J. H., AND B. A. SCHAAL. 1983. Isozyme variation in diploid tropical and octoploid subtropical-temperate species of *Bulnesia*. J. Hered. 74: 358–360.

KROHNE, D. T., I. BAKER, AND H. G. BAKER. 1980. The maintenance of the gynodioecious breeding system in *Plantago lanceolata* L. Amer. Midland Nat. 103:269–279.

LEDIG, F. T., AND M. T. CONKLE. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey Pine (*Pinus torreyana* Parry ex Carr.) Evolution 37: 79–85.

LEONARD, R. L., E. D. MCARTHUR, D. J. WEBER, AND B. W. WOOD. 1981. Electrophoresis of isoenzymes of 16 western shrubs: technique development. Great Basin Nat. 41:377–388.

LINHART, Y. B., M. L. DAVIS, AND J. B. MITTON. 1981. Genetic control of allozymes of shikimate dehydrogenase in ponderosa pine. Biochem. Genetics 19: 641–646.

LLOYD, D. C. 1982. Selection of combined versus separate sexes in seed plants. Amer. Nat. 120:571–585.

MCARTHUR, E. D. 1977. Environmentally induced changes of sex expression in *Atriplex canescens*. Heredity 38:97–103.

MCARTHUR, E. D., A. C. BLAUER, AND G. L. NOLLER. 1984. Propagation of fourwing saltbush (*Atriplex canescens* [Pursh] Nutt.) by stem cuttings. Pages 261–264 *in* A. R. Tiedemann, E. D. McArthur, H. C. Stutz, K. L. Johnson, and R. Stevens (compilers), The biology of *Atriplex* and related chenopods, Proceedings, Wildland Shrub Symposium. USDA Forest Service Gen. Tech. Rep. INT-172. 309 pp.

MCARTHUR, E. D., AND D. C. FREEMAN. 1982. Sex expression in *Atriplex canescens*: genetics and environment. Bot. Gaz. 143:476–482.

MCARTHUR, E. D., R. STEVENS, AND A. C. BLAUER. 1983. Growth performance comparisons among 18 accessions of fourwing saltbush (*Atriplex canescens*) at two sites in Central Utah. J. Range Manage. 36:78–81.

NEI, M. 1976. Mathematical models of speciation and genetic distance. Pages 723–765 *in* S. Karlin and E. Nevo, eds., Population genetics and ecology. Academic Press, New York. 832 pp.

OLIVER, J. L., AND M. RUIZ REJON. 1980. The relation between isozymes and ploidy level: its application to biogeographical studies of *Muscari atlanticum* (Liliaceae). Taxon 29:27–32.

SANDERSON, S. C., AND H. C. STUTZ. 1984. Flavonoids of diploid and polyploid *Atriplex confertifolia*. Pages 34–38 in A. R. Tiedemann, E. D. McArthur, H. C. Stutz, K. L. Johnson, and R. Stevens (compilers), The biology of *Atriplex* and related chenopods, Proceedings of Wildland Shrub Symposium. USDA Forest Service Gen. Tech. Rep. INT-172. 309 pp.

STERNBERG, L. 1976. Growth forms of *Larrea tridentata*. Madroño 23:408–417.

STUTZ, H. C., J. M. MELBY, AND G. K. LIVINGSTON. 1975. Evolutionary studies of *Atriplex*: a relic *gigas* diploid population of *Atriplex canescens*. Amer. J. Bot. 62:236–245.

STUTZ, H. C., C. L. POPE, AND S. C. SANDERSON. 1979. Evolutionary studies of *Atriplex*: adaptative products of the natural hybrid 6*n A. tridentata* × 4*n A. canescens*. Amer. J. Bot. 66:1181–1193.

STUTZ, H. C., AND S. C. SANDERSON. 1979. The role of polyploidy in the evolution of *Atriplex canescens*. Pages 615–621 *in* J. R. Goodin and D. K. Northington, eds., Arid land plant resources, Proceedings of International Arid Lands Conference, Texas Tech University, Lubbock. 724 pp.

WOOLF, C. M. 1968. Principles of biometry. D. Van Nostrand Company, Princeton, New Jersey. 359 pp.