Aliso: A Journal of Systematic and Floristic Botany

Volume 12 | Issue 2 Article 13

1989

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ALISO
12(2), 1989, pp. 379–386

ALLOZYME VARIATION IN *HELIANTHUS PRAECOX* SSP. *HIRTUS*, A RARE SUNFLOWER FROM SOUTHERN TEXAS

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ABSTRACT

Allozyme data were used to evaluate the genetic status of a rare endemic sunflower from Texas, *Helianthus praecox* ssp. *hirtus*. Comprising approximately 200 plants, the taxon is presently known from a single population in the Carrizo Springs region of Southern Texas. Electrophoretic examination of 17 enzyme loci revealed that ssp. *hirtus* is genetically similar to, but distinct from, the two more widespread subspecies of *H. praecox*: ssp. *praecox* and ssp. *runyonii*. Levels of genetic variation in the single population of ssp. *hirtus* ($P = 0.47$, $A = 1.88$, and $H = 0.17$) are similar to those observed for other populations in the *H. praecox* complex ($P = 0.44$, $A = 1.68$, and $H = 0.15$). Thus, if isozyme loci are used as the one indicator of genetic variation in ssp. *hirtus*, it cannot be concluded that this taxon is endangered due to a loss of genetic variation. Nevertheless, due to the highly endemic status of ssp. *hirtus* serious conservation efforts must be made to ensure the long-term preservation of this taxon. Several management recommendations are given herein.

Key words: *Helianthus praecox* ssp. *hirtus*, Asteraceae, genetic variation, allozymes, endangered species.

INTRODUCTION

Electrophoretic data are widely used for obtaining genetic information about plant and animal populations. Such information has been used to: 1) describe levels of genetic variation within and among populations or species (Nevo 1978; Brown 1979; Hamrick, Linhart, and Mitton 1979; Karron 1987); 2) examine how genetic variation is distributed (Nevo 1978; Hamrick 1983; Loveless and Hamrick 1984); 3) examine relationships between closely related species (Gottlieb 1981; Crawford 1983); and 4) detect hybridization and introgression phenomena (Selander, Hunt, and Yang 1969; Levin 1975; Rieseberg, Soltis, and Palmer 1988*a*). Although the significance of electrophoretic evidence for studies of rare and endangered taxa has long been recognized, only recently has a concerted effort been made to apply allozyme data to questions in conservation biology (Chambers and Bayless 1983; Crawford, Stuessy, and Silva O. 1988; Rieseberg et al. 1988*c*). Here we use allozyme data to evaluate the genetic status of a highly endemic sunflower from Southern Texas, *Helianthus praecox* ssp. *hirtus*.

Helianthus praecox Engelm. & Gray comprises three subspecies (ssp. *praecox*, ssp. *runyonii* Heiser, and ssp. *hirtus* Heiser) confined to Southern Texas (Heiser 1956; Heiser et al. 1969). All three subspecies are obligate outcrossers and crosses among them yield highly fertile F_1 hybrids (Heiser 1956). Previous electrophoretic studies (Wain 1983) have revealed a high degree of genetic similarity between ssp. *praecox* and ssp. *runyonii* ($I = 0.94$), but the genetic affinities of ssp. *hirtus* have not been investigated electrophoretically. All three subspecies are allopatric and have a limited geographic distribution (Heiser 1956). Subspecies *praecox* occurs on Galveston Island, Texas, and the adjacent mainland, while subspecies *runyonii* is found on coastal prairies from Aransas County to Cameron County,

Texas. Subspecies *hirtus* is even more limited in distribution, apparently confined to a single population in the Carrizo Springs region of Southern Texas (Heiser 1956). The population at the type locality just east of Carrizo Springs on Texas Hwy 85 (*Heiser 3064*) appears to have been destroyed by urban development (Rieseberg unpublished). Nevertheless, extensive search in Carrizo Springs area by the senior author in the summer of 1987 revealed the presence of one extant population of ssp. *hirtus,* consisting of nearly 200 plants. The population occurs in sandy soil along Highway 83, approximately one mile south of Carrizo Springs.

Several factors may threaten the long-term existence of ssp. *hirtus.* First, tractor-drawn mowers that scalp Texas highway right-of-ways certainly inflict damage to the single known population each year. A second possible problem involves the two more common and numerous sunflower species in the area, *H. annuus* L. and *H. debilis* Nutt. ssp. *cucumerifolius* (T. & G.) Heiser. Both taxa hybridize readily with *H. praecox* (Heiser 1956) and if either taxon came in contact with the single known population of ssp. *hirtus,* loss of this population due to genetic assimilation would be possible. Third, due to the relatively small size of this population, ssp. *hirtus* could lack genetic variation in features critical for survival should conditions change in the Carrizo Springs area. Finally, there has been some question whether ssp. *hirtus* is a distinct taxonomic entity (J. Poole, pers. comm.). Although well-isolated geographically, ssp. *hirtus* is rather similar morphologically to ssp. *runyonii,* differing only in stem pubescence (Heiser et al. 1969). Because of the lack of unambiguous morphological or genetic evidence indicating its distinct taxonomic status, ssp. *hirtus* has been largely ignored by rare-plant biologists and managers at the Texas Natural Heritage Program (J. Poole, pers. comm.) and the U.S. Fish and Wildlife Service (K. Collins, pers. comm.).

The study described here examines allozyme variation within and among populations of the three subspecies of *H. praecox.* Two specific questions are addressed: 1) Is ssp. *hirtus* genetically distinct from ssp. *praecox* and ssp. *runyonii*? and 2) Are levels of genetic variation in ssp. *hirtus* comparable to those observed for more widespread *Helianthus* taxa?

MATERIALS AND METHODS

Plants

Achenes for isozyme studies were gathered from 11 populations of *H. praecox* (Table 1), including one population of ssp. *hirtus,* four populations of ssp. *praecox,* and five populations of ssp. *runyonii.* Achenes were collected from mature heads of 30–100 individuals per population and then pooled. No more than five achenes were removed from single heads to avoid biasing our population sample toward heads with a large number of achenes. In general, the achenes were germinated and enzymes were extracted from two- to three-week-old seedlings. GDH and ADH, however, were extracted from achenes placed on damp filter paper overnight. One hundred individuals were analyzed for the single population of ssp. *hirtus,* and approximately 30 individuals were analyzed per population of ssp. *praecox* and ssp. *runyonii.*

Electrophoresis

The detailed methods of electrophoresis and staining of enzymes we employed have been described elsewhere (Soltis et al. 1983; Rieseberg and Soltis 1988; Rieseberg, Soltis, and Soltis 1988). The following 12 enzymes were analyzed: acid

Table 1. Field collections of *Helianthus praecox* s. l.[1] used for enzyme electrophoresis. Several measures of genetic variation are also given, including percent polymorphic loci (*P*), mean number of alleles per locus (*A*), and mean heterozygosity (*H*).

Taxon/population	Locality	*P*	*A*	*H*
H. praecox ssp. *hirtus*:				
Rieseberg 1031	Dimmit Co., Texas	0.47	1.88	0.17
H. praecox ssp. *praecox*:				
Rieseberg 1061	Brazoria Co., Texas	0.29	1.29	0.11
Rieseberg 1063	Galveston Co., Texas	0.35	1.47	0.12
Rieseberg 1064	Galveston Co., Texas	0.35	1.47	0.09
Rieseberg 1065	Galveston Co., Texas	0.35	1.35	0.10
Mean		0.34	1.40	0.11
H. praecox ssp. *runyonii*:				
Rieseberg 1035	Bee Co., Texas	0.41	1.71	0.12
Rieseberg 1039	Cameron Co., Texas	0.41	1.53	0.13
Rieseberg 1043	Brooks Co., Texas	0.59	2.12	0.21
Rieseberg 1045	Kennedy Co., Texas	0.41	1.71	0.14
Rieseberg 1052	Nueces Co., Texas	0.65	2.24	0.22
Rieseberg 1056	Aransas Co., Texas	0.53	1.88	0.17
Mean		0.51	1.87	0.17

[1] Vouchers are deposited at RSA.

phosphatase (APH), alcohol dehydrogenase (ADH), aldolase (ALD), glutamate dehydrogenase (GDH), glyceraldehyde-3-phosphate dehydrogenase ([NADP]/G3PDH), isocitrate dehydrogenase (IDH), malic enzyme (ME), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), shikimate dehydrogenase (SKDH), and triosephosphate isomerase (TPI). Enzymes were resolved on 12% starch gels using the following buffer systems from Soltis et al. (1983): for ADH, GDH, and TPI, system 6; for PGI, ME, and ALD, a modification of system 8 (Rieseberg and Soltis 1987); for PGM, 6-PGD, and SKDH, system 9; and for [NADP]G3PDH, IDH, and APH, system 1.

Loci were designated sequentially with the most anodally migrating isozyme designated *1*, the next *2*, and so on. Likewise, alleles were designated sequentially with the most anodally migrating allozyme designated *a*. The genetic basis of enzyme variation in *Helianthus* is discussed elsewhere (Torres 1974; Torres and Diedenhofen 1976; Kahler and Lay 1985; Rieseberg and Soltis 1988).

Data Analysis

Genetic identity values (Nei 1972) were calculated for all pair-wise population comparisons using the computer program "GAP," developed by P. Pack (unpublished). Standard measures of genetic variation were also computed for all three taxa, including mean number of alleles per locus (*A*), proportion of loci polymorphic (*P*), and mean heterozygosity (*H*). A minimum alternate allele frequency of 0.01 defined a polymorphic locus.

RESULTS

Twelve enzymes and 17 loci were surveyed electrophoretically: *Aph*; *Adh1*; *Ald3*; *Gdh*; *[NADP]G3pdh1, 2*; *Idh2*; *Me*; *Pgi1, 2*; *Pgm1, 2*; *6-Pgd3*; *Skdh2*; and

Table 2. Mean allele frequencies in the three subspecies of *Helianthus praecox*: *hirtus* (PH), *praecox* (PP), *runyonii* (PR).

Locus-allele	Frequency			Locus-allele	Frequency		
	PH	PP	PR		PH	PP	PR
Aph-a	0.04	0.00	0.01	*6Pgd3-d*	0.00	0.00	0.03
b	0.95	0.77	0.74	*e*	0.00	0.00	0.00
c	0.02	0.00	0.02	*Pgi2-a*	0.02	0.00	0.01
d	0.00	0.23	0.23	*b*	0.00	0.00	0.01
Adh1-a	0.25	0.07	0.04	*c*	0.00	0.10	0.04
b	0.00	0.00	0.01	*d*	0.45	0.59	0.71
c	0.00	0.07	0.00	*e*	0.09	0.08	0.05
d	0.75	0.86	0.90	*f*	0.41	0.23	0.17
e	0.00	0.00	0.01	*g*	0.03	0.23	0.17
f	0.00	0.00	0.03	*Pgm1-a*	0.00	0.00	0.02
g	0.00	0.00	0.01	*b*	0.57	0.00	0.13
Gdh-a	1.00	1.00	0.96	*c*	0.43	1.00	0.86
b	0.00	0.00	0.04	*Pgm2-a*	0.03	0.00	0.05
G3pdh1-a	0.00	0.00	0.00	*b*	0.83	0.91	0.83
b	1.00	1.00	0.97	*c*	0.00	0.09	0.10
c	0.00	0.00	0.03	*d*	0.14	0.00	0.02
G3pdh2-a	0.00	0.00	0.00	*Skdh2-a*	0.02	0.00	0.00
b	0.00	0.00	0.00	*b*	0.36	0.76	0.42
c	1.00	1.00	0.99	*c*	0.57	0.00	0.13
d	0.00	0.00	0.01	*d*	0.05	0.24	0.38
Ihd2-a	1.00	1.00	0.97	*Skdh2-e*	0.00	0.00	0.16
b	0.00	0.00	0.03	*f*	0.00	0.00	0.01
Me-a	0.00	0.00	0.04	*Tpi1-a*	0.00	0.00	0.01
b	0.00	0.17	0.16	*b*	1.00	1.00	0.99
c	1.00	0.83	0.68	*Tpi2-a*	0.70	0.00	0.00
d	0.00	0.00	0.01	*b*	0.30	1.00	0.95
e	0.00	0.00	0.11	*c*	0.00	0.00	0.01
6Pgd3-a	0.00	0.07	0.02	*d*	0.00	0.00	0.01
b	0.00	0.00	0.01	*Tpi3-a*	0.18	0.00	0.00
c	1.00	0.93	0.94	*b*	0.96	1.00	0.97
				c	0.18	0.00	0.03

Tpi1, 2, 3. Two loci, *Ald3* and *Pgi1*, were monomorphic in all three subspecies of *H. praecox*. Variation was observed for the remaining 15 loci. Mean allele frequencies at polymorphic loci for each taxon examined are given in Table 2.

Genetic identities within and among the three subspecies of *H. praecox* were high (Table 3). Genetic identities among populations within each taxon were greater than 0.95 (no interpopulational genetic identity value is provided for ssp. *hirtus* since it consists of a single population). This value is similar to values reported among populations of other plant species (Gottlieb 1981; Crawford 1983). Mean identities among the three subspecies ranged from 0.905 between ssp. *praecox* and ssp. *hirtus* to 0.952 between ssp. *praecox* and ssp. *runyonii* (Table 3).

Values for the proportion of polymorphic loci (P), mean heterozygosity (H), and mean number of alleles per locus (A) were quite different in populations of the three subspecies of *H. praecox* (Table 1). Populations of ssp. *runyonii* and ssp. *hirtus* were characterized by high levels of genetic variability, whereas populations of ssp. *praecox* were genetically depauperate in comparison (Table 1).

Table 3. Mean genetic identities and ranges of identities among the three subspecies of *Helianthus praecox*.

Taxon	*H. praecox* ssp. *hirtus*	*H. praecox* ssp. *praecox*	*H. praecox* ssp. *runyonii*
H. praecox ssp. *hirtus*	*	0.905 (0.891–0.917)	0.908 (0.890–0.919)
H. praecox ssp. *praecox*	—	0.971 (0.950–0.982)	0.952 (0.922–0.980)
H. praecox ssp. *runyonii*	—	—	0.957 (0.928–0.995)

* No value given because single population analyzed.

DISCUSSION

Genetic Differentiation

The results indicate that for genes encoding soluble enzymes, ssp. *hirtus* is similar to, but genetically distinct from, ssp. *praecox* and ssp. *runyonii* ($I = 0.905$ and $I = 0.908$, respectively). Although these identity values are quite high (Table 3), they are lower than the genetic identity observed between ssp. *runyonii* and ssp. *praecox,* the two more widespread and morphologically distinct subspecies of *H. praecox* ($I = 0.952$). Furthermore, the genetic identity between ssp. *hirtus* and the other two subspecies of *H. praecox* is similar to values observed among other subspecific pairs in the *H. debilis/H. praecox* complex (Wain 1982, 1983). It should also be noted that the genetic identity of 0.952 between ssp. *praecox* and ssp. *runyonii* based on 17 loci examined herein, is almost identical to the value of 0.94 reported for these taxa by Wain (1983) based on a smaller number of loci (12).

Although no single allele completely differentiates ssp. *hirtus* from other populations of *H. praecox, Tpi2-a* occurs at a very high frequency (0.70) in ssp. *hirtus* (Table 2), but was not found in any other populations of *H. praecox.* Subspecies *hirtus* also is characterized by two other unique alleles that occur at much lower frequencies, *Skdh2-a* and *Tpi3-a* (Table 2). The presence of unique alleles that characterize ssp. *hirtus,* combined with morphological and geographic evidence (Heiser 1956), strongly suggest that ssp. *hirtus* is a unique genetic entity deserving subspecific status.

Genetic Diversity

Evolutionary theory predicts low levels of genetic polymorphism in plant species with small ranges and few individuals (Drury 1974; Karron 1987; Karron et al. 1988). This is due to the fixation of single alleles by genetic drift and founder effects (Wright 1931; Levin 1984) and to strongly directional selection toward genetic uniformity in a limited array of environments (Van Valen 1965; Babbel and Selander 1974). It follows that loss of genetic diversity due to these factors is much more likely to occur in restricted species rather than widespread species. Electrophoretic studies have generally confirmed this prediction (Karron 1987). Nevertheless, much more data are needed on rare-plant genetics and reproductive ecology before we can confidently say that there is a strong correlation between genetic diversity and range in such plants (e.g., see Lewin 1987).

Table 4. Percent polymorphic loci (*P*), mean number of alleles per locus (*A*), and mean heterozygosity (*H*) in several *Helianthus* taxa.

Taxon	Geographical distribution[1]	*n* Loci	*P*	*A*	*H*	Source
H. annuus	W	25	0.42	1.52	0.11	Rieseberg et al. (1988*b*)
H. bolanderi	W	25	0.39	1.53	0.11	Rieseberg et al. (1988*b*)
H. debilis:						
ssp. *debilis*	W	12	0.25	*	0.06	Wain (1982)
ssp. *tardiflorus*	R	12	0.21	*	0.05	Wain (1982)
ssp. *vestitus*	R	12	0.28	*	0.06	Wain (1982)
H. exilis	R	25	0.40	1.60	0.12	Rieseberg et al. (1988*b*)
H. praecox:						
ssp. *hirtus*	R	17	0.47	1.88	0.17	
ssp. *praecox*	R	17	0.34	1.40	0.11	
ssp. *runyonii*	W	17	0.51	1.87	0.17	

[1] R, restricted; W, widespread.
* Not reported by author.

Thus it is surprising that, while ssp. *hirtus* is highly restricted in distribution, its one known natural population appears to be no more depauperate genetically at isozyme loci than other populations in the *H. praecox* complex (Table 1). Levels of genetic variation in ssp. *hirtus* are almost identical to those found in ssp. *runyonii* and are much higher than those observed in ssp. *praecox*. Furthermore, slightly lower levels of genetic variation relative to values in ssp. *hirtus* have been observed for both widespread and restricted *Helianthus* taxa that have been examined electrophoretically (Table 4). Additional members of the *H. debilis*/*H. praecox* complex have also been analyzed electrophoretically (Wain 1983), but no estimates of genetic variability were given. Comparisons of genetic variability among the taxa listed in Table 4 may be slightly misleading because nonequivalent sets of loci were used in the studies compared, and it is well known that some loci tend to have more alleles than others (Gottlieb 1981). Nevertheless, it is clear than genetic diversity at isozyme loci in ssp. *hirtus* is high relative to both widespread and restricted *Helianthus* taxa.

The high level of genetic diversity in ssp. *hirtus* may be due to historical factors. It is possible that ssp. *hirtus* was once widespread and has only recently declined in range. Furthermore, it seems unlikely that this taxon has undergone recent bottlenecks, although a surprising increase in genetic variability was observed in a recent study of bottlenecked houseflies (Lewin 1987). Relatively high levels of genetic variation have also been observed for a number of other restricted species [e.g., *Capsicum cardenasii* Heiser & Smith (McLeod et al. 1983), *Gaura demareei* Raven & Gregory (Gottlieb and Piltz 1976), *Layia discoidea* Keck (Gottlieb, Warwick, and Ford 1985), and *Pinus longaeva* D. K. Bailey (Hiebert and Hamrick 1983)]. In contrast, the low levels of genetic variability in ssp. *praecox,* which is relatively restricted in distribution, are more consistent with the predicted level of genetic variation based on limited geographic distribution.

In summary, if isozyme loci are used as the one indicator of genetic variation in ssp. *hirtus,* it cannot be concluded that this taxon is endangered due to a loss of variation. Nevertheless, it would be unwise to assume that this taxon is variable

genetically for characters that would allow it to adapt to changes in its present environment. Regardless of the level of genetic variation, this is a highly endemic taxon that may consist of a single population and, as a result, be in great danger of extinction due to human interference, various demographic factors (Lande 1988), and interspecific hybridization.

Management Implications

It is clear from this study that ssp. *hirtus* is a unique genetic entity in great danger of extinction due to its extreme endemism. The following recommendations may contribute to the long-term preservation of *H. praecox* ssp. *hirtus*. First, further searches should be made in the Carrizo Springs area for additional populations of this taxon. Attempts should also be made to halt disruptive human activities near the single known population of ssp. *hirtus*. In particular, the Texas Highway Department should be encouraged to refrain from mowing in this area during the summer and early fall of each year. Additionally, other annual sunflowers such as *H. annuus* and *H. debilis* ssp. *cucumerifolius* should be removed from close proximity to the remaining population of ssp. *hirtus* to reduce the chance of hybridization and subsequent genetic assimilation. Finally, attempts should be made to establish additional populations of ssp. *hirtus* in the Carrizo Springs area—sites where interference by man is less likely and where other sunflower species are less numerous.

ACKNOWLEDGMENTS

This study was supported by NSF Grant BSR-8722643 to LHR. We thank Rhonda Rieseberg and Aaron Liston for helpful comments on the manuscript.

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