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RESEARCH ARTICLE

Genetic diversity and population structure in the rare Algodones sunflower (*Helianthus niveus* ssp. *tephrodes*)

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Abstract Assessing levels and patterns of population genetic variation is an important step for evaluating rare or endangered species and determining appropriate conservation strategies. This is particularly important for ensuring the preservation of novel genetic variation in wild relatives of crops, which could provide beneficial alleles for plant breeding and improvement. In this study, we evaluate the population genetics of *Helianthus niveus* ssp. *tephrodes* (the Algodones sunflower), which is an endangered, wild relative of cultivated sunflower (*H. annuus* L.). This rare sunflower species is native to the sand dunes of the Sonoran Desert in southern California, southwestern Arizona, and northern Mexico and is thought to harbor beneficial alleles for traits related to drought tolerance. We genotyped nine populations of this species with a set of simple-sequence repeat markers derived from expressed sequence tags (EST-SSRs) and investigated levels of genetic diversity and population structure, in *H. niveus* ssp. *tephrodes*. We also compared our results to findings from five related sunflower species that have been analyzed with these same markers, including annuals and perennials that range from rare to widespread. The Algodones sunflower harbors lower levels of standing genetic variation, but similar levels of population structure as compared to other sunflower species. We also discovered that a disjunct population from northern Mexico was genetically distinct from populations elsewhere in the range. Given the occurrence of such a genetically unique population, our recommendations include population surveys of the southern portion of the range in hopes of bolstering the existing germplasm collection.

Keywords Conservation · Endangered species · Genetic diversity · *Helianthus* · Sunflower

Introduction

It has been argued that germplasm collections not only play an important role in the protection of native biodiversity (Falk 1987; Primack 2002), but can also serve as valuable sources of alleles for ongoing plant breeding and crop improvement efforts (e.g., Tanksley and McCouch 1997; Hajjar and Hodgkin 2007). Indeed, the gene pools of crop relatives often harbor beneficial alleles (e.g., Crute and Pink 1996; Bouzidi et al. 2002; Wilson et al. 2000; McCouch et al. 2007; Nevo and Chen 2010) and, in the case of rare and endangered species, such alleles are at risk of extinction. Given that germplasm collections are only as useful as the genetic variation that they contain, knowledge of the amount and distribution of genetic variation present within a species is critical for the development of appropriate sampling and management strategies (CPC 1991). For example, in species with high levels of population structure, collection efforts should focus on maximizing the number of populations sampled in order to preserve as much genetic diversity as possible (Richards et al. 2007). In contrast, in species with minimal population structure, fewer population collections can be made while still

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preserving a comparable fraction of the genetic diversity present within the species (Richards et al. 2007). Similarly, if in situ preservation is the goal, care should be taken to ensure the protection of the most genetically distinct populations or habitats, especially when resources are limited. Finally, population genetic approaches can also be used to identify unique populations that are likely to harbor novel (and potentially beneficial) alleles.

While population geneticists have successfully correlated patterns of population genetic variation with factors such as geographic range, these correlations are far from perfect. Indeed, while there is an overall trend for species with restricted ranges to exhibit reduced levels of genetic diversity (Hamrick and Godt 1989, 1996; Gitzendanner and Soltis 2000; Nybom 2004), there are a number of notable exceptions to this trend (e.g., Vogelmann and Gastony 1987; Ranker 1994; Young and Brown 1996; Ellis et al. 2006). For life history traits, clear patterns in terms of genetic diversity differences between annuals and perennials have not been shown. Some studies have found that annual species harbor lower levels of diversity as compared to perennials while others have found the reverse (see Hamrick and Godt 1989, 1996; Nybom 2004 for examples of both). Given the above, it seems clear that an understanding of the amount and distribution of genetic diversity within a given species requires direct investigation, as opposed to reliance on broad trends. Comparisons of population genetic parameters derived from such studies with those from close congeners can also be used to provide context for interpreting the resulting data and are a useful way to gauge potential losses of genetic diversity (e.g., Karron 1987, 1991; Baskauf et al. 1994; Gitzendanner and Soltis 2000). Here, we investigate patterns and levels of genetic variation within and among populations of the endangered Algodones sunflower (*Helianthus niveus* [Benth.] Brendegee ssp. *tephrodes* [A. Gray] Heiser) and compare our results to those derived from five congeners.

The Algodones sunflower is a wild relative of the cultivated sunflower, *H. annuus* L. This dicotyledonous species is native to the Algodones Dunes in the Sonoran desert, which exhibit harsh desert conditions including low mean annual rainfall (~5–7 cm/year) and high summer temperatures often exceeding 43 °C daily (Norris and Norris 1961; AGFD 2005). The Algodones Dunes are also home to some twenty plant species (Lukenbach and Bury 1983) including at least six rare and endangered plant species, such as Peirson's milk-vetch (*Astragalus magdalenae* var. *peirsonii*) and the perennial herb sandfood (*Pholisma sonorae*) (CNDDB 2012). Importantly, the Algodones sunflower exhibits leaf characteristics related to drought tolerance in a variety of plant species, including dense pubescence, reduced leaf size, and increased specific leaf area (Turner and Begg 1981; Chaves et al. 2003), suggesting that it might be a valuable source of alleles for breeding programs aimed at improving performance under drought conditions in sunflower. Little is known about the demographic history of the Algodones sunflower (AGFD 2005); however, the Algondones Dunes have recently experienced habitat destruction resulting from recreational vehicle activity (McGrann et al. 2005; Willoughby 2005). This habitat destruction from off-road vehicle use is listed as a substantial threat to the species (AGFD 2005), and the Algodones sunflower has been denoted as a species of special concern by the United States Fish and Wildlife Service (http://www.fws.gov/) and listed as endangered in the state of California (http://www.dfg.ca.gov/). Due to its rarity as well as its potential to serve as a source of beneficial alleles for sunflower breeding, seed collections from a number of *H. niveus* ssp. *tephrodes* populations have been made and deposited in the National Plant Germplasm System (http://www.ars-grin.gov/npgs/). These collections, the first of their kind for this species, add to more than 2,000 wild *Helianthus* accessions, including collections from several other rare or endangered sunflower species.

Here, we report the results of a population genetic analysis of available, wild-collected populations of *H. niveus* ssp. *tephrodes* from southern California and northern Mexico. Specifically, we estimated levels of genetic diversity and the extent of population structure in this rare species and compared the results to those from five other *Helianthus* species. This work utilized simple-sequence repeat markers that were developed from publicly-available sunflower expressed sequence tags (i.e., EST-SSRs) and which have previously been shown to be transferable across the genus *Helianthus* (Ellis et al. 2006; Pashley et al. 2006; Gevaert 2011). By surveying a common set of markers across multiple species, we were able to control for locus-specific effects when comparing estimates of genetic diversity or population structure, thereby resulting in increased statistical power for detecting differences amongst species (Ellis and Burke 2007).

Methods

Study species

Helianthus niveus ssp. *tephrodes* (the Algodones sunflower; hereafter referred to as HNIV) is a self-incompatible wild sunflower species that is native to the Algodones Dunes of the Sonoran Desert in southern California, southwestern Arizona [known from one locale in Yuma County (AGFD 2005)], and northern Mexico (Jepson and Hickman 1993; Seiler and Rieseberg 1997). While HNIV has been described as both an annual and a perennial, it is likely a facultative perennial when winter conditions are

allowable, as it has been observed flowering throughout the winter (LF Marek, personal communication). This species is not known to clonally propagate, as has been seen in certain other perennial wild sunflower species. Germination typically occurs from December to late March (LF Marek, personal communication) and is thought to be driven by seasonal winter rain events (Bowers 1996). Average height ranges from 0.5 to 1.5 m tall, and plants of this species exhibit a dense pubescence on their leaves, which are light green to light grey/green in color. Phylogenetic analyses (Timme et al. 2007) have concluded that HNIV belongs in the section *Helianthus* and is sister to a clade containing *H. petiolaris* Nutt., *H. deserticola* S. Watson, and *H. paradoxus* Heiser. HNIV has a diploid chromosome number of $2n = 34$.

Plant materials and genotyping

Achenes (i.e., single-seeded fruits) from nine HNIV accessions (hereafter referred to as populations) were obtained from the USDA North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA (Fig. 1a). A map depicting the known range of HNIV is also provided in Fig. 1b (Rogers et al. 1982). Eight populations (HNIV 1-3, 5-9) were collected in southern California and the other is from northern Mexico (HNIV 4). The populations studied here represent nine of the ten populations housed by the NCRPIS from California and Mexico; seeds from an Arizona collection from Yuma County were not available for distribution. These populations were collected by representatives from the NCRPIS and deposited in the National Plant Germplasm System. Full collection details can be accessed using the Germplasm Request Information Network (http://www.ars-grin.gov/). Briefly, sampled HNIV populations ranged in size from 20 to 150 plants. The sampling strategy included collecting an equal number of heads per individual and sampling all plants in small populations or a random subset in larger populations (LF Marek, personal communication). Achenes from each population were germinated in petri dishes on moist filter paper. Following germination, seedlings were reared in pots in the University of Georgia greenhouses, and DNA was extracted using the Qiagen (Valencia, CA) DNeasy Plant Mini Kit following the manufacturer's protocol.

All samples were genotyped using 22 EST-SSRs developed for *H. annuus* (HANN) and proven cross-transferable within the genus (Ellis et al. 2006; Pashley et al. 2006). EST-SSR genotyping was performed using the fluorescent labeling protocol of Schuelke (2000) as modified by Wills et al. (2005). PCR was performed in a total volume of 20 μL containing 5 ng of template DNA, 30 mM Tricine pH 8.4-KOH, 50 mM KCl, 2 mM $MgCl_2$, 125 μM of each dNTP, 0.2 μM M13 Forward (−29) sequencing primer labeled with either HEX, 6-FAM or NED, 0.2 μM reverse primer, 0.02 μM forward primer and 2 units of *Taq* polymerase. The PCR conditions were as follows: 3 min at 95 °C; 10 cycles of 30 s at 94 °C, 30 s at 65 °C and 45 s at 72 °C, annealing temperature decreasing to 55 °C by 1 °C per cycle, followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C, 45 s at 72 °C, followed by 20 min at 72 °C.

Amplicons were diluted 1:30 in deionized water and visualized using an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, CA) with MapMarker 1000 ROX size standards (BioVentures, Murfreesboro, TN) included in each lane to allow for accurate fragment size determination and two control individuals were included in each genotyping run to protect against artifactual variation in amplicon size between genotyping runs and to ensure precise genotyping calls. Alleles were called using the software package GeneMarker v. 1.51 (SoftGenetics, State College, PA).

In order to compare levels of genetic diversity between HNIV and other sunflower species, we included genetic data for these same 22 EST-SSRs from several other species. Genotypes of wild HANN individuals from 12 widespread populations and individuals of *H. porteri* (A. Gray) Pruski (HPOR) representing 12 populations served as comparisons for annual sunflower species. Wild HANN was previously genotyped by Pashley et al. (2006), and HPOR was previously genotyped by Gevaert (2011). Wild HANN is a geographically common species that also belongs to the section *Helianthus*, whereas HPOR is an endemic to granite outcrops in the southeastern United States and resides in the section *Divaricati*. Comparisons were also made to three perennial sunflower species also belonging to section *Divaricati*, including *H. angustifolius* L. (HANG), *H. grosseserratus* M. Martens (HGRO), and *H. verticillatus* Small (HVER). The former two are common, widespread species whereas the third is a rare, endangered sunflower and a candidate for the endangered species list (all three were previously genotyped by Ellis et al. 2006).

Population genetic analyses in *H. niveus* ssp. *tephrodes*

Measures of genetic diversity, including mean numbers of alleles per locus, observed heterozygosity, and Nei's (1978) unbiased gene diversity were calculated across the total sample for HNIV using GenAlEx v. 6.4 (Peakall and Smouse 2006). We also performed a test for Hardy–Weinberg equilibrium (HWE) using GenAlEx and tested for significant using a χ^2 test and sequential Bonferroni correction for multiple testing (Holm 1979). Rarefaction was used to compare allelic diversity across populations of HNIV and to account for differences in sample size among the populations (Hurlbert 1971; Petit et al. 1998; Kalinowski 2004). This analysis involved the use of HP-Rare

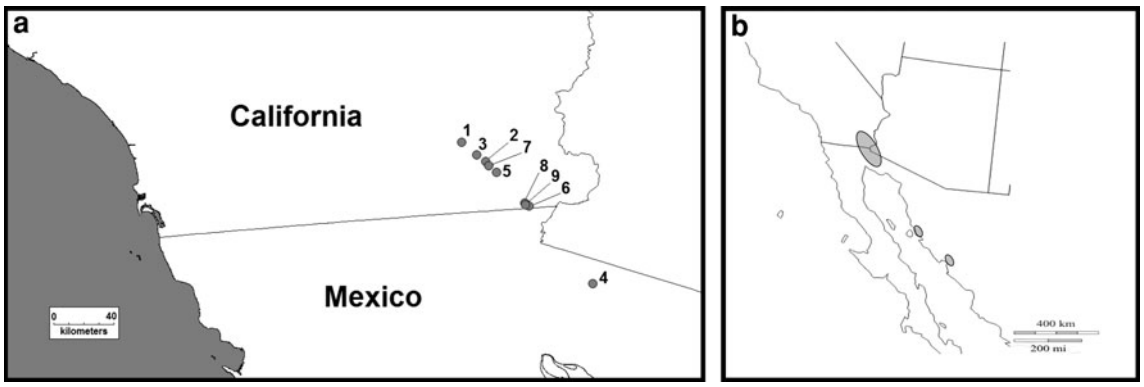

Fig. 1 **a** Map of locations of sampled populations. Numbers correspond to population names and order in Table 1. **b** Map of the known range of *Helianthus niveus* spp. *tephrodes* in the southwestern United States and northern Mexico following Rogers et al. (1982)

1.0 (Kalinowski 2005) to estimate allelic richness based on the genotypic data. Finally, for each population, we used the WorldClim database to gather information about the elevation, mean annual precipitation, and mean annual temperature using the latitude and longitude coordinates provided by NCRPIS for these populations (http://www.worldclim.org; Hijmans et al. 2005).

Population structure in HNIV was investigated using the Bayesian, model-based clustering algorithm implemented in the software package STRUCTURE (Pritchard et al. 2000). Briefly, individuals were assigned to K population genetic clusters based on their multi-locus genotypes. Clusters were assembled to minimize intra-cluster Hardy–Weinberg and linkage disequilibrium and, for each individual, the proportion of membership in each cluster was estimated. This analysis did not rely on prior population information (i.e., USEPOPINFO was turned off). For each analysis, $K = 1$–12 population genetic clusters were evaluated with 5 runs per K value, and the probability values were averaged across runs for each cluster. For each run, the initial burn-in period was set to 50,000 with 100,000 MCMC iterations. Preliminary analysis using the admixture model and either correlated or independent allele frequencies showed no appreciable differences between methods, so the analyses were performed assuming independent allele frequencies. Since the *DeltaK* method generally identifies the highest level (i.e., a hierarchy) of structure in the dataset (Coulon et al. 2008), we used a two-step approach for the STRUCTURE analyses. First, the most likely number of clusters was determined using the Delta$K$ method of Evanno et al. (2005). Next, we partitioned the STRUCTURE results according to the most likely K (based upon Delta$K$) and re-analyzed the data. The online program STRUCTURE Harvester was used to plot likelihood values and Delta$K$ (http://taylor0.biology.ucla.edu/struct_harvest/; Earl 2011).

Population structure was also investigated via analysis of molecular variation (AMOVA; Excoffier et al. 1992), as implemented in GenAlEx, to hierarchically partition genetic variation and estimate F_{ST} (Wright 1951). Statistical significance (i.e., H_0 = no genetic differentiation among the populations) was determined by performing 1,000 permutations. Genetic relationships among HNIV individuals were also examined graphically via principal coordinates (PCO) analysis using GenAlEx. To do this, a standard genetic distance matrix (Nei 1978) was first constructed based on the multi-locus genotypes. This distance matrix was then used for the PCO analysis, and the first two principal coordinates were graphed in two-dimensional space.

A test for isolation-by-distance (IBD) was performed using a Mantel test for matrix correspondence (Mantel 1967). Decimal degree coordinates for populations were obtained from NCRPIS for the nine accession collections. Using the Geographic Distance Matrix Generator from the American Museum of Natural History (http://biodiversityinformatics.amnh.org/open_source/gdmg/), we calculated the pairwise distances in km between all populations based on the decimal degree coordinates. Matrix correspondence between this geographic matrix and the Nei's genetic distance matrix (described above) was tested using GenAlEx (Smouse and Long 1992; Smouse et al. 1986).

Comparison to related species

We compared levels of genetic diversity and population structure in HNIV to five wild sunflower species including two annual (HANN and HPOR) and three perennial species (HANG, HGRO, and HVER). Collections for HANN were made across the United States, including populations from Arizona, Arkansas, California, Colorado, Iowa, Nebraska, North Dakota, Ohio, Texas, Utah, Washington, and Wyoming (Pashley et al. 2006). HPOR was collected across its known range in Georgia and North Carolina (Gevaert 2011). The three known populations of HVER were sampled as well as co-occurring populations of HANG at these

sites (Ellis et al. 2006). Samples from HGRO spanned much of its distribution from the central portion of the United States (Ellis et al. 2006). All of these species are diploid and self-incompatible. For these comparisons, expected heterozygosity values were used instead of unbiased measures because the latter values were not reported in these previous studies. ANOVAs were performed using JMP version 9 (SAS Institute, Cary, NC) to test for differences in measures of genetic diversity among these species. Loci which failed to amplify in a species from a previous study were not used. The main effects included in the ANOVA were species and locus (fixed effects) (i.e., the common EST-SSRs) with the dependent variables being either expected heterozygosities (averaged over populations within a species for each locus) or F_{ST} values (estimated by AMOVA for each locus). For calculations of F_{ST} only overlapping polymorphic loci across species was tested. Use of the same genetic markers in both taxa resulted in increased statistical power because locus-to-locus variation was explicitly accounted for in the model. All proportions were transformed with an angular transformation prior to analysis (Sokal and Rohlf 1995).

Results

Genetic diversity in *H. niveus* ssp. *tephrodes*

In total, we surveyed 119 HNIV plants from nine populations using 22 EST-SSRs. Of the 22 markers, 16 were polymorphic. Tests for HWE for the 16 polymorphic loci are summarized in Online Resource 1. While there were eight instances in which a marker showed a significant deviation from HWE in a particular population there were no consistent trends with regard to individual markers or populations. The following genetic diversity measures were calculated for the polymorphic loci. Species-wide diversity as measured by Nei's (1978) unbiased gene diversity, or expected heterozygosity (UH_e), across the total 119 sampled plants was 0.432 ± 0.042 (mean ± SE). The average species-wide observed heterozygosity (H_o) was 0.300 ± 0.049. The average number of alleles per polymorphic locus was 3.75 ± 0.359. The average population-level expected heterozygosity was 0.378 ± 0.018, and the average population-level observed heterozygosity was 0.304 ± 0.022. F_{IS} averaged 0.176 (0.036) on a per-population basis, ranging from −0.109 to 0.384. After adjusting for differences in sample sizes across populations via rarefaction, allelic richness values ranged from 1.97 to 2.40 alleles per polymorphic locus. All genetic diversity measures are reported in Table 1. A two-factor ANOVA (with population and locus as fixed effects) failed to detect a population effect on gene diversity (population $F_{8,120} = 1.52$, $P = 0.156$; locus: $F_{15,120} = 10.25$, $P < 0.0001$). Finally, allele frequency estimates for all markers/populations are given in Online Resource 2A whereas allele sharing across populations is summarized in Online Resource 2B.

Population structure in *H. niveus* ssp. *tephrodes*

With regard to population structure in HNIV, the Delta*K* method of Evanno et al. (2005) provided support for the presence of two genetically distinct clusters (i.e., $K = 2$), which largely separated the Mexican HNIV4 population from the remainder of the populations (Fig. 2a). Note that the one individual from population HNIV2 exhibits a genotype most consistent with HNIV4. While this could be a byproduct of long distance migration,

Table 1 Measures of genetic diversity in the nine *H. niveus* ssp. *tephrodes* populations based on the 16 polymorphic EST-SSRs surveyed herein

Accession	GRIN ID	E	P	T	*N*	A (SE)	H_o (SE)	UH_e (SE)	F_{IS} (SE)
HNIV1	AMES 27421	71	63	22.5	12	2.05 (0.128)	0.232 (0.051)	0.349 (0.055)	0.268 (0.094)
HNIV2	AMES 27850	106	63	22.4	16	2.21 (0.114)	0.312 (0.056)	0.366 (0.055)	0.104 (0.082)
HNIV3	AMES 27851	121	65	22.3	17	1.97 (0.099)	0.249 (0.063)	0.279 (0.051)	0.161 (0.121)
HNIV4	PI 613758	53	76	22.7	15	2.15 (0.139)	0.306 (0.072)	0.376 (0.063)	0.238 (0.117)
HNIV5	PI 650017	87	59	22.5	11	2.06 (0.160)	0.224 (0.062)	0.359 (0.063)	0.384 (0.112)
HNIV6	PI 650018	83	65	22.5	9	2.04 (0.153)	0.379 (0.071)	0.456 (0.054)	0.127 (0.119)
HNIV7	PI 650019	87	60	22.5	15	2.27 (0.128)	0.322 (0.060)	0.410 (0.058)	0.207 (0.109)
HNIV8	PI 650020	74	64	22.5	12	2.40 (0.128)	0.307 (0.063)	0.432 (0.051)	0.241 (0.104)
HNIV9	PI 650021	67	63	22.5	12	2.08 (0.128)	0.404 (0.075)	0.376 (0.054)	−0.109 (0.098)
Total					119	2.18 (0.143)	0.304 (0.021)	0.378 (0.018)	0.176 (0.036)

GRIN ID accession name from the GRIN National Genetic Resources Program, *E* elevation in meters, *P* mean annual precipitation in mm, *T* mean annual temperature in C. *N* number of individuals sampled, *A* allelic richness, H_o observed heterozygosity, UH_e Nei's (1978) unbiased measure of gene diversity, F_{IS} inbreeding coefficient

Values in parentheses are the SE of the means

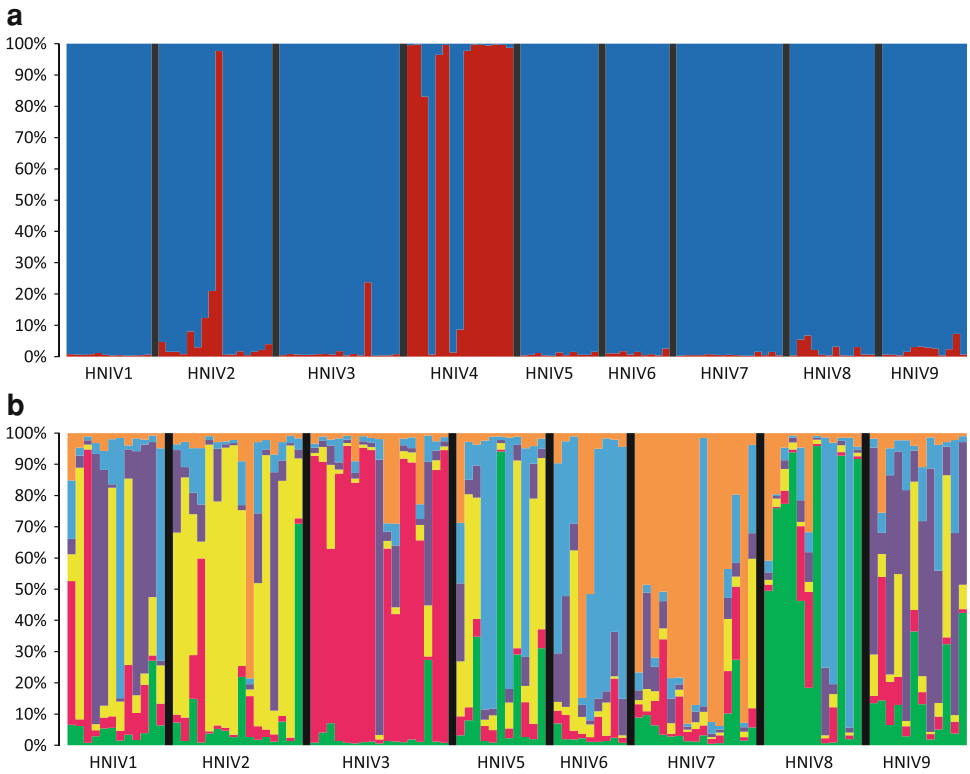

Fig. 2 **a** STRUCTURE results for $K = 2$ clusters for all populations including all nine populations. **b** STRUCTURE results for $K = 6$ clusters from the reduced data set (i.e., following removal of HNIV4)

another possible explanation is that this individual exhibits a rare (for HNIV2) genotype by chance—these (and other) populations share the majority of their alleles, albeit at different frequencies (Online Resource 2A, B). Given the large geographic distance separating these populations, the latter explanation is perhaps more likely. Next, following our two-step approach (see "Methods"), we removed the HNIV4 population and re-analyzed the data. In this case, Delta$K$ was the greatest for $K = 6$, resulting in somewhat distinct clusters for each of HNIV2, HNIV3, and HNIV7 (Fig. 2b). Plots of log likelihood and Delta$K$ values can be found in Online Resource 3.

The overall F_{ST} value as estimated from AMOVA was 0.143 ($P < 0.01$) for all sampled HNIV populations. Values of F_{ST} per locus ranged from 0.020 to 0.23, and all values fell within the 95 % confidence interval. Pairwise F_{ST} amongst the nine populations ranged from 0.072 to 0.300, and all values were significantly different from zero (all $P < 0.001$; Table 2). Consistent with the STRUCTURE results, the highest values occurred mostly between HNIV4 and the remainder of the populations. The PCO plot revealed broad overlap amongst many of the populations sampled here. However, in accordance with the STRUCTURE and pairwise F_{ST} results, HNIV4 was separated from all other populations (Fig. 3).

Finally, we found evidence for isolation-by-distance in the form of a significant correlation between genetic distance and geographical distance ($R^2 = 0.118$; $P < 0.01$). However, when the divergent HNIV4 population was removed, this correlation disappeared.

Comparison to related sunflower species

When compared to the two annual sunflowers (HANN and HPOR), HNIV had significantly lower levels of gene diversity. Gene diversity was also lower in HNIV as compared to the rare perennial species (HVER) and the widespread HGRO (species: $F_{5,91} = 9.63$, $P < 0.0001$, locus: $F_{16,91} = 2.61$, $P < 0.0001$). These values, along with the results of the post hoc Tukey–Kramer tests for among-species differences are listed in Table 3. Estimates of population structure did not differ amongst species as determined from a two-factor ANOVA (species: $F_{3,36} = 1.43$, $P = 0.175$, locus: $F_{14,36} = 1.22$, $P = 0.316$; Table 3). Note that, due to the previous method of population sampling (i.e., few and uneven individuals per population) in HANN and HGRO, F_{ST} was not calculated for all species (Ellis et al. 2006).

Discussion

In this study, we evaluated the population genetics of a rare/endangered relative of cultivated sunflower and compared our results to those from five related, self-incompatible (i.e., obligately outcrossing) sunflower species. In

Table 2 Pairwise F_{ST} estimates below the diagonal 1 and geographic distances in km above

	HNIV1	HNIV2	HNIV3	HNIV4	HNIV5	HNIV6	HNIV7	HNIV8	HNIV9
HNIV1	–	19	11.9	121.1	28.2	57.8	22.4	54.5	55.8
HNIV2	0.093	–	7.1	102.3	9.3	38.9	3.5	35.6	36.9
HNIV3	0.076	0.210	–	109.2	16.3	48.9	10.4	42.6	43.9
HNIV4	0.255	0.183	0.300	–	93	63.5	98.8	66.7	63.4
HNIV5	0.059	0.103	0.150	0.226	–	29.6	5.9	26.3	27.7
HNIV6	0.126	0.121	0.133	0.249	0.156	–	35.6	3.4	2.2
HNIV7	0.112	0.176	0.130	0.294	0.134	0.074	–	32.1	33.4
HNIV8	0.080	0.132	0.203	0.220	0.095	0.132	0.072	–	1.4
HNIV9	0.098	0.193	0.177	0.298	0.122	0.185	0.136	0.103	–

To with visualization, these values are color-coded with higher values in red and lower in blue

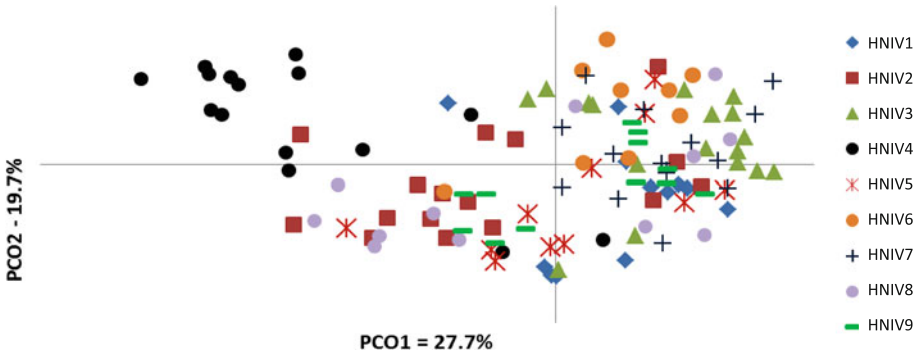

Fig. 3 Principal coordinates analysis of the nine sampled populations based on genetic distances amongst all sampled individuals

Table 3 Comparison of six sunflower species using a common set of EST-SSRs

Species	Abbr.	Life history	Status	*N*	Pops	H_e (SE)	TK	F	*P*	F_{ST} (SE)	F	*P*
H. niveus ssp. *tephrodes*	HNIV	Ann/Per	Rare	119	9	0.314 (0.040)	C	9.63	***	0.172 (0.031)	1.43	ns
H. annuus	HANN	Annual	Common	12	12	0.581 (0.042)	A			–		
H. porteri	HPOR	Annual	Endemic	288	12	0.685 (0.055)	A			0.117 (0.029)		
H. angustifolius	HANG	Perennial	Common	48	2/3[a]	0.344 (0.046)	BC			0.174 (0.039)		
H. grosseserratus	HGRO	Perennial	Common	56	5	0.437 (0.048)	AB			–		
H. verticillatus	HVER	Perennial	Rare	71	3	0.478 (0.046)	AB			0.116 (0.039)		

H_e measures had 17/22 loci in common and F_{ST} comparisons had 15/22 (making use of those loci which amplified in all species for H_e and only those that were polymorphic for F_{ST})

Values represent mean species-level measures of heterozygosity. Significant differences are based upon a two-factor ANOVA. *** $P < 0.0001$

TK column shows the within-model differences based upon a post hoc Tukey–Kramer Test. Data for the other species are from Ellis et al. (2006), Pashley et al. (2006), and Gevaert (2011)

N number of individuals sampled, *Pops* number of populations assayed, *ns* not significant

[a] The *H. angustifolius* collection was made spanning two of the *H. verticillatus* populations, and the widespread, continuous nature of *H. angustifolius* made population delineation less discrete

terms of population structure, HNIV exhibited low to moderate F_{ST} values, with the exception of a single divergent population (HNIV4 in Mexico), indicating relatively low levels of genetic divergence amongst populations. Moreover, there were no significant differences between HNIV and the other sunflower species under consideration. Interestingly, of the four sunflower species that have been evaluated for population structure with these same genetic markers, none have shown detectable differences in their partitioning genetic variation despite their differences in rarity and/or endemism. Although there is a trend for perennial sunflowers to have lower expected heterozygosity values (Table 3), this trend was not statistically significant.

Notably, HVER is an extremely rare species known to exist in only four populations in the southeastern United States, and these populations are separated by widely varying distances (ranging from 3 to 300 km; Ellis et al. 2006; Mandel 2010), but population structure was still comparable to that of a widespread congener (HANG). The other geographically restricted species, HPOR is limited to spatially isolated granite outcrops in the southeastern United States and shows similar (to the other sunflower species) F_{ST} values (Gevaert 2011). As suggested from the previous studies of rare sunflowers (Ellis et al. 2006; Gevaert 2011), if habitat loss and fragmentation occurred relatively recently, it may be that too little time has passed for there to have been a discernible effect on population genetic parameters. In this light, the perennial life history of HNIV (recall that the species exhibits facultative perenniality) may have also helped to mitigate the effects of rarity on population structure.

As expected for an endangered species with a restricted range, the Algodones sunflower harbored significantly lower levels of genetic diversity when compared to related sunflower species. Specifically, measures of gene diversity in HNIV were lower than those for both common (HANN) and rare (HPOR) annual sunflower species. These values were likewise lower for HNIV as compared to one common (HGRO) and one rare (HVER) perennial sunflower species, although gene diversity was comparable to that observed in the relatively common (but genetically depauperate) HANG. It should also be noted that that HNIV exhibited a larger fraction of monomorphic loci (6 of 22, with the next highest being HVER with 4 of 22; data not shown) as compared to the other species. Looking across populations, there was little variation in allelic richness, with all populations falling within the 95 % confidence intervals. Finally, in terms of inbreeding coefficients, populations of HNIV tended to exhibit positive F_{IS} values, potentially due to biparental inbreeding (Nason and Ellstrand 1995), which could have a negative effect on fitness.

Given the relatively low levels of standing genetic variation within HNIV, our findings underscore the importance of protecting this species through both in situ and ex situ conservation strategies. Despite the generally low/moderate estimates of population structure, the Mexican population (HNIV4) in particular was quite distinctive, as evidenced by both the elevated pairwise F_{ST} values and the PCO/STRUCTURE analyses (Table 2; Figs. 2, 3). Moreover, this population demonstrates marked phenotypic differences for flowering time as well as traits related to drought tolerance, including leaf pubescence, reflectance, size, and specific leaf area (E.F. Milton, unpublished data). Interestingly, a survey of environmental variables using WorldClim data for the HNIV populations showed that HNIV4 had the lowest elevation (53 m) and the highest mean precipitation (76 mm) of all populations surveyed. These data suggest that HNIV4 might be experiencing different selective pressures relative to populations found in the Imperial Sand Dunes.

While neutral genetic variation does not necessarily correlate strongly with adaptive genetic variation (e.g., Reed and Frankham 2001; McKay and Latta 2002), population genetic analyses such as the one described herein are a valuable first step for directing conservation efforts (DeSalle and Amato 2004). Given our findings, as well as the observed phenotypic differences between HNIV4 and the other populations surveyed, we recommend that this population be prioritized for conservation protection. More generally, relatively little is known about the distribution of this species in the southern portion of its range, so further exploration of this region in search of additional HNIV populations would be worthwhile, especially to points further south where this species is known to have previously occurred (Fig. 1b). Given the relatively low level of genetic diversity present within the existing germplasm collection of this species, supplemental collections may also be beneficial for the ex situ preservation of genetic diversity, both for possible future re-introductions as well as crop improvement efforts. This is especially true if additional genetically divergent populations can be identified. Finally, given its rarity and the potential for HNIV to provide beneficial alleles for sunflower breeding efforts, additional studies aimed at identifying functional variation within and among HNIV populations would be especially valuable.

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