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High Genetic Diversity and Low Population Structure in Porter's Sunflower (*Helianthus porteri*)

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Abstract

Granite outcrops in the southeastern United States are rare and isolated habitats that support edaphically controlled communities dominated by herbaceous plants. They harbor rare and endemic species that are expected to have low genetic variability and high population structure due to small population sizes and their disjunct habitat. We test this expectation for an annual outcrop endemic, *Helianthus porteri* (Porter's sunflower). Contrary to expectation, *H. porteri* has relatively high genetic diversity (H_e = 0.681) and relatively low genetic structure among the native populations (F_{ST} = 0.077) when compared to 5 other *Helianthus* species (N = 288; 18 expressed sequence tag–SSR markers). These findings suggest greater gene flow than expected. The potential for gene flow is supported by the analysis of transplant populations established with propagules from a common source in 1959. One population established close to a native population (1.5 km) at the edge of the natural range is genetically similar to and shares rare alleles with the adjacent native population and is distinct from the central source population. In contrast, a transplant population established north of the native range has remained similar to the source population. The relatively high genetic diversity and low population structure of this species, combined with the long-term success of transplanted populations, bode well for its persistence as long as the habitat persists.

Key words: *conservation genetics, gene flow, geographic isolation, granite outcrop,* Helianthus porteri, *transplant experiment*

Granite outcrops in the southeastern United States are rare and geographically isolated habitats that support edaphically controlled communities dominated by herbaceous plants (McVaugh 1943; Baskin and Baskin 1988). Granite outcrop plant communities have been well studied as a classic example of ecological succession (Burbanck and Platt 1964; Sharitz and McCormick 1973; Burbank and Phillips 1983). They harbor many endemic and rare plant species that require high light and tolerate the periodic droughts, characteristic of these shallow (0–25 cm) soil assemblages (Baskin and Baskin 1988). There is a general expectation that these rare and endemic species will have low genetic diversity due to the effects of genetic drift and inbreeding though it has been recognized that many factors (e.g., type of rarity, mating system, life history, and presence of any large localized populations) may be mitigating factors (Rabinowitz et al. 1986; Hamrick and Godt 1989; Barrett and Kohn 1991; Ellstrand and Elam 1993; Gitzendanner and Soltis 2000). The naturally disjunct spatial structure of granite outcrops also leads to an expectation of less gene flow, which would facilitate population differentiation due to genetic drift or local adaptation (Ellstrand and Elam 1993; Godt and Hamrick 1993). While there is some evidence supporting the expectation of relatively high genetic structure for several outcrop species in the southeastern United States and elsewhere, support for the expectation of relatively low genetic diversity is mixed (Murdy and Carter 1985; Wyatt et al. 1992; Godt and Hamrick 1993; Byrne & Hopper 2008; Koelling et al. 2011). Here we investigate genetic variation and population structure of the granite outcrop endemic, *Helianthus porteri*.

Helianthus porteri (A. Gray) Pruski (Pruski 1998) is a self-incompatible annual sunflower that is endemic to granite outcrops of the southeastern United States from eastern Alabama, through the piedmont of Georgia and South Carolina (McVaugh 1943; Shelton 1963). Granite outcrops can vary in size from a few square meters of exposed granite to large emergent monadnocks (e.g., Stone Mountain, GA). Although *H. porteri* is not found on all outcrops in the region, it can be locally abundant in the shallow soils of the annual–perennial vegetation zone. *Helianthus porteri* germinates in late March and continues growth through the hottest, driest

part of the season to flower and set seed through early fall (Shelton 1963; Mellinger 1972). Putative pollinators include bees, beetles, moths, and butterflies (Shelton 1963; S. Gevaert, personal observation), and seeds are primarily gravity dispersed though they can be moved short distances through water runoff (Houle and Phillips 1988). Populations of *H. porteri* that were transplanted in 1959 from the central part of the range (Mount Arabia in Georgia) to several unoccupied rock outcrops in blocks of sod (McCormick and Platt 1964; Mellinger 1972) still persist and are available for study.

In this article, we analyze genetic diversity and population structure of *H. porteri* and compare it with other *Helianthus* species using simple sequence repeat (SSR) markers derived from expressed sequence tags (ESTs). These markers were initially designed from cultivated sunflower (*H. annuus*) and subsequently used for population genetic analyses in wild sunflower (a widespread annual; also *H. annuus*) and several other species, including *H. verticillatus* (rare perennial), *H. angustifolius* (widespread perennial), *H. grosseserratus* (widespread perennial), and *H. niveus ssp. tephrodes* (rare annual/perennial; Ellis et al. 2006; Pashley et al. 2006; Ellis and Burke 2007; Mandel et al. 2012). We test the expectation that *H. porteri* has relatively low genetic diversity and high population structure when compared with more common and widespread species. Additionally, we investigate the long-term effect of transplantation on genetic diversity by comparing transplant populations and native populations of *H. porteri*.

Materials and Methods

Collection of Plant Materials and DNA Extraction

Helianthus porteri leaf material was collected from 24 individuals from each of 10 native populations and 2 transplanted populations (288 individuals total; PL and NC, known as Heggie's Annex and Rocky Place in Mellinger 1972) in 2009 (Table 1 and Figure 1). Populations varied in distance from each other, with Pine Louisville (PL; referred to as Heggie's Rock Annex in Mellinger 1972) and Heggie's Rock (HR) being the closest at 1.5 km apart and North Carolina (NC; referred to as Rocky Face, NC in McCormick and Platt 1964) being the furthest from all populations at 280 km from HR. Most populations sampled ranged from 5 to 15 km from their nearest neighbor. Some populations of *H. porteri* exist between those sampled but were not sampled in this study, largely due to the limited survival of individuals at the time tissue was harvested. Harvested leaves were frozen at −80 °C until DNA was extracted. Total genomic DNA was isolated using the cetyltrimethylammonium bromide method (Doyle and Doyle 1987) from 24 individuals at each of the

Table 1 Mean ± 1 SE for 12 populations of *Helianthus porteri* for 18 loci, the species grand mean

Population (arranged west to east)	Population coordinates	A	H_O	H_E	F_{IS}
CMR	33°14′15.12″ N 85°8′49.36″ W	5.944 bc (0.716)	0.427 (0.054)	0.561 b (0.062)	0.205 (0.059)
CW	33°25′25.78″ N 84°58′13.97″ W	5.333 c (0.505)	0.411 (0.062)	0.570 b (0.055)	0.225 (0.088)
PM	33°38′9.28″ N 84°10′13.58″ W	6.889 abc (0.646)	0.408 (0.059)	0.647 ab (0.055)	0.346 (0.073)
MA	33°40′0.57″ N 84°7′15.77″ W	8.056 a (0.777)	0.523 (0.063)	0.723 a (0.050)	0.270 (0.064)
SM	33°48′10.31″ N 84°8′39.11″ W	7.889 ab (0.893)	0.535 (0.067)	0.659 ab (0.059)	0.214 (0.072)
IBR	33°42′50.89″ N 84°1′21.25″ W	6.389 abc (0.622)	0.477 (0.063)	0.624 ab (0.056)	0.223 (0.075)
COM	33°42′37.69″ N 83°55′52.09″ W	6.889 abc (0.918)	0.477 (0.052)	0.615 ab (0.060)	0.186 (0.052)
WG	33°45′7.01″ N 83°49′42.48″ W	6.611 abc (0.611)	0.413 (0.047)	0.650 ab (0.052)	0.329 (0.061)
RS	33°53′13.14″ N 83°20′2.01″ W	6.611 abc (0.719)	0.415 (0.056)	0.592 b (0.054)	0.252 (0.073)
HR	33°32′35.35″ N 82°15′1.88″ W	5.556 c (0.776)	0.422 (0.057)	0.564 b (0.056)	0.262 (0.081)
Grand mean (averaged across populations)		6.565 (0.205)	0.456 (0.016)	0.621 (0.018)	0.242 (0.020)
Pooled species level		18.222 (2.292)	0.454 (0.049)	0.681 (0.056)	0.333 (0.049)
PL	33°32′29.80″ N 82°16′49.51″ W	5.667 c (0.572)	0.481 (0.057)	0.574 b (0.048)	0.183 (0.081)
NC	35°57′48.13″ N 81°7′4.86″ W	6.944 abc (0.508)	0.483 (0.048)	0.630 ab (0.053)	0.208 (0.045)

Values are averaged over all loci in each population. *A*, mean number of alleles per locus; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity; *FIS*, within population coefficient of inbreeding. The species-level pooled means are also given. The first 10 populations are native, while the 2 listed at the bottom are transplants.

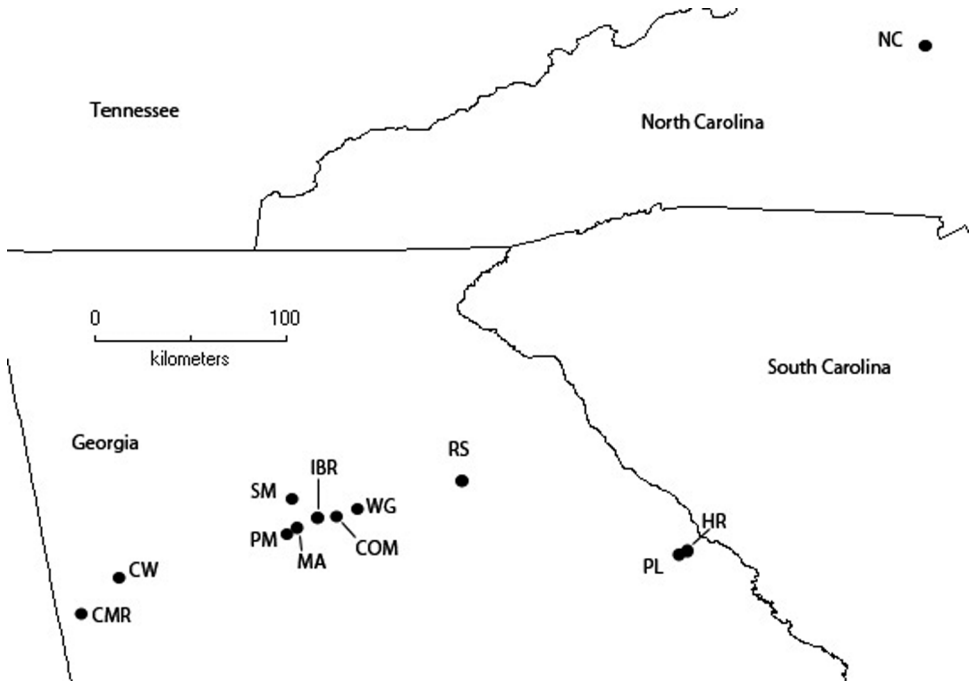

Figure 1. Locations of *Helianthus porteri* populations in the southeastern United States where seed was collected for this study: 10 native populations and 2 transplanted populations (PL and NC).

12 populations. In the smallest populations (COM, PL), collected individuals were separated by at least 0.5 m, while in the largest populations, one individual was sampled from each soil island (greater than 1 m apart) across the outcrop. All DNA samples were quantified using a NanoDrop 2000 (Thermo Scientific, Wilmington, DE).

Selection of Loci, PCR Protocols, and Genotyping

Eighteen of 22 EST–SSR loci initially developed for *H. annuus* were chosen as genetic markers for this study (Supplementary Table 1). These loci were previously determined to amplify successfully in *H. verticillatus*, *H. angustifolius*, *H. grosseserratus,* and *H. niveus* ssp. *tephrodes* (Ellis et al. 2006; Pashley et al. 2006).

SSR genotyping was performed using a modified version of the fluorescent labeling protocol of (Schuelke 2000) as detailed by Wills et al. (2005). Polymerase chain reaction (PCR) was performed in a total volume of 15 μL containing 10 ng of DNA, 30 mM Tricine pH 8.4-KOH, 50 mM KCl, 2 mM $MgCl_2$, 100 μM each of dNTP, 0.1 μM M13 forward (−29) sequencing primer labeled with either green dye (HEX), blue dye (FAM), or yellow dye (NED), 0.1 μM reverse primer, 0.01 μM forward primer, and 1 U of *Taq* DNA 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; and followed by 20 min at 72 °C.

PCR products were diluted 1:50 and visualized on an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, CA) with MapMarker 1000 ROX size standard (BioVentures Inc., Murfreesboro, TN) included in each lane to allow for accurate fragment size determination. GeneMarker (v. 1.70; SoftGenetics, State College, PA) was used to call allele sizes for all individuals.

Data Analysis

Descriptive population genetics statistics for the 10 native populations were calculated using GenAlEx (v. 6.2; Peakall and Smouse 2006): percentage of polymorphic loci, mean number of alleles per locus, and gene diversity, calculated as Nei's (1987) unbiased expected heterozygosity (H_e), including both pooled species-level values and as an average over loci and populations. Relationships among populations were graphically assessed via principal coordinate analysis (PCO; in GenAlEx) using pairwise genetic distances among all individuals in all 10 native populations of *H. porteri* with the covariance standardized method. Isolation by distance was tested with a Mantel test comparing a Nei's genetic distance matrix with geographic distance matrix (in GenAlEx). Population structure in *H. porteri* was investigated using the Bayesian clustering program STRUCTURE (v. 2.3.3; Pritchard et al. 2000), using an admixture model with correlated allele frequencies. For each analysis, $K = 1–11$ population genetic clusters were evaluated with 15 runs per K value, and the probability values were averaged across runs for each cluster. The initial burn-in period was set to 50 000 replicates with 10^6 Markov Chain Monte Carlo (MCMC) iterations. This analysis was repeated, and the results were found to be consistent across 15 runs. The ΔK method of Evanno

et al. (2005) was used to determine the most likely number of subdivisions in the dataset. Given that this method often only identifies the highest degree of structure in a dataset, we also examined the next most likely value of *K* (Coulon et al. 2008). Population structure was also examined using analysis of molecular variation (Excoffier et al. 1992) as implemented in GenAlEx to hierarchically partition genetic variation and estimate F_{ST} (Wright 1951). We then used the same analytical tools to assess the relationships between the native *H. porteri* populations and the transplant populations.

In order to evaluate the effects of geographic isolation and endemism on the population genetics of *H. porteri*, we compared genetic structure (F_{ST}) of the native populations of *H. porteri* with 2 perennial sunflower species (3 populations of both *H. verticillatus* and *H. angustifolius)* and 1 annual/perennial sunflower species (9 populations of *H. niveus subsp. tehprodes*). We also compared species-level genetic diversity (H_e) of *H. porteri* with 3 perennial sunflower species (3 populations of both *H. verticillatus* and *H. angustifolius*, and 5 populations of *H. grosseserratus*), an annual species (12 populations of *H. annuus*), and an annual/perennial species (9 populations of *H. niveus* subsp. *tehprodes*). These analyses were performed via 2-way analysis of variance(SAS v. 9.2; SAS Institute, Cary, NC) with species and locus as fixed effects. Data from the other sunflower species were collected by Ellis et al. (2006), Pashley et al. (2006), and Mandel et al. (2013), and they used the same set of EST–SSRs employed herein. Estimates of population genetic structure (F_{ST}) for *H. annuus* and *H. grosseserratus* were not available for this comparison because fewer individuals were sampled from a larger number of populations in those cases. For the F_{ST} comparison, we used only the common polymorphic loci and F_{ST} was log transformed to meet the assumption of normality of residuals and homogeneity of variance for residuals. For the H_e comparison, we used only the common polymorphic loci, and H_e was arcsine-transformed.

Results and Discussion

Genetic Diversity in the Native Populations

In terms of genetic diversity within the 10 native populations, 17 of the 18 EST–SSR loci were polymorphic in at least one population. The mean number of alleles per locus (*A)* was 18.222 ± 2.292 (standard error [SE]; $N = 240$, Table 1) for the species, with an effective number of alleles of 5.172 (± 0.945 SE; pooled). The values for expected heterozygosity (H_e) in each population ranged from 0.561 (CMR) to 0.723 (MA) with a population mean of 0.621 (± 0.018 SE) and a species-level (pooled) H_e of 0.681 (± 0.056 SE). This pattern of relatively high genetic diversity is similar to the granite outcrop endemic *Tradescantia hirsuticaulis*, also an obligate outcrossing species, which had higher than expected levels of genetic diversity (Godt and Hamrick 1993), but contrary to the results found in *Arenaria uniflora* (Wyatt et al. 1992), which harbored low levels of genetic diversity for both outcrossing and self-pollinating populations. Both *T. hirsuticaulis* and *A. uniflora* co-occur on the granite outcrops with *H. porteri*.

The comparison of *H. porteri* to 5 other *Helianthus* species (all outcrossing) using genetic markers common to all of the species in the analyses (Table 2) demonstrated that genetic diversity (H_e) of *H. porteri* was high and not significantly different from the widespread *H. annuus* (annual), *H. angustifolius (perennial),* or the rare *H. verticillatus* (perennial; $P > 0.05$) even though there was significant variation among species overall (Table 2; $F_{5,91} = 6.64$, $P < 0.0001$). Genetic diversity in *H. porteri* was greater than that of the rare *H. niveus ssp. tephrodes* (annual) and the widespread, though genetically depauperate, *H. angustifolius* (perennial). Thus, the characteristic of being a widespread versus rare or endemic species does not appear to be the dominant factor determining overall levels of genetic diversity among these congeners that share the same mating system (Gitzendanner & Soltis 2000).

The *H. porteri* populations differed significantly for *A* and H_e, but not H_o (Table 1). Populations on the western and eastern margins of the native range showed lower *A* and H_e than centralized populations ($F_{9,179} = 3.55$, $P < 0.001$). The differences in genetic diversity at the periphery may be a function of greater isolation from the range center, potentially resulting in more limited gene flow. All populations had at least 6 private alleles, of which SM (20), MA (18), and RS (16) had the most, and PM (7), HR (7), WG (6), and IBR (6) the fewest. Private alleles were found at 17 loci, with BL0010 (19) and BL0027 (15) having the most and BL0002 and BL0022 having 1 each. Eleven private alleles occurred at a frequency of 0.075 or greater, and 3 at a frequency greater than 0.25 (BL0010, allele 336, HR, 0.556; BL0010, allele 351, HR, 0.250; BL0030, allele 254, SM, 0.368).

Table 2 Species comparisons for genetic diversity (pooled species H_e) and genetic structure (F_{ST}; mean (± SE)) for 2 annual sunflowers *Helianthus porteri* and *H. annuus*, one annual perennial *H. niveus ssp. tephrodes*, and 3 perennial sunflowers, *H. angustifolius*, *H. verticillatus*, and *H. grosseserratus*

Species	Sample size (N)	Genetic diversity (H_e)	Genetic structure (F_{ST})	Species attributes
H. porteri	200	0.618 (0.050) a	0.117 (0.029)	Restricted annual
H. angustifolius	48	0.344 (0.046) bc	0.174 (0.039)	Common perennial
H. verticillatus	71	0.478 (0.048) ab	0.116 (0.039)	Rare perennial
H. grosseratus	56	0.437 (0.048) ab	N/A	Common perennial
H. annuus	12	0.581 (0.042) a	N/A	Common annual
H. niveus ssp. tephrodes	119	0.314 (0.040) c	0.172 (0.031)	Rare annual/perennial

Data for *H. grosseserratus* and *H. annuus* are not available for F_{ST}. Note that only 11 shared polymorphic loci (for all species) were used to calculate the values for the F_{ST} species comparison, whereas the F_{ST} values presented elsewhere for *H. porteri* (Table 1 and the text) accounts for all 18 loci available.

Table 3 Pairwise population F_{ST} values from the analysis of molecular variance and 999 permutations for *Helianthus porteri*

CMR	CW	PM	MA	SM	IBR	COM	WG	RS	HR	PL	NC	
0.000												CMR
0.081	0.000											CW
0.105	0.104	0.000										PM
0.095	0.093	0.037	0.000									MA
0.082	0.058	0.044	0.047	0.000								SM
0.073	0.064	0.063	0.071	0.017*	0.000							IBR
0.067	0.063	0.053	0.046	0.029*	0.033	0.000						COM
0.110	0.108	0.053	0.035	0.065	0.084	0.059	0.000					WG
0.126	0.110	0.079	0.074	0.078	0.083	0.077	0.075	0.000				RS
0.127	0.125	0.098	0.096	0.076	0.083	0.073	0.124	0.112	0.000			HR
0.144	0.139	0.104	0.090	0.093	0.105	0.087	0.130	0.114	0.024*	0.000		PL
0.112	0.110	0.098	0.090	0.075	0.085	0.066	0.110	0.093	0.103	0.117	0.000	NC

Both native and transplant populations (shaded) are included. Population abbreviations are shown in the first row and last column, with populations following a west (CMR) to east (NC) geographical gradient. All values are significantly different from zero at $P < 0.001$, unless noted as $^*P < 0.01$.

Six loci were consistently out of Hardy–Weinberg equilibrium: BL0002, BL0003, BL0004, BL0020, BL0023, and BL0025. In all populations (averaged across all loci), estimates of F_{IS} were greater than zero. The smallest population (in terms of both population size and surface area covered), COM, had the lowest average F_{IS} value (0.186). Several possible explanations (not necessarily mutually exclusive) exist for this tendency toward positive F_{IS} values in this self-incompatible species. First, the presence of null alleles at these loci could lead to positive estimates of F_{IS}. However, given that the primers for these loci were designed from genic regions and are transferable across a variety of sunflower taxa, it seems unlikely that null alleles are a major contributor to the elevated F_{IS}. Second, some degree of biparental inbreeding or mating between genetically related individuals (Nason and Ellstrand 1995) may be occurring in populations of *H. porteri* despite their self-incompatibility. This could be due to a combination of the patchy occurrence of suitable habitats (soil depressions) within granite outcrops and limited seed dispersal, resulting in the clustering of related individuals, or to possible correlations in flowering time that could increase the likelihood of mating among related individuals. Finally, unrecognized substructuring of populations could have produced a Wahlund effect, or an apparent heterozygote deficit, due to our having treated multiple subpopulations as one larger population (Wahlund 1928). This would result in an overestimate of H_e and thus positive estimates of F_{IS}.

Population Structure in the Native Populations

Despite the isolated nature of the granite outcrops, populations of *H. porteri* demonstrated a low level of population differentiation ($F_{ST} = 0.077$, $P < 0.001$). Pairwise population measures of F_{ST} were all significantly different from zero (Table 3, $P < 0.01$), with the greatest seen between CMR and HR ($F_{ST} = 0.127$), CMR and RS ($F_{ST} = 0.126$), CW and HR ($F_{ST} = 0.125$), and WG and HR ($F_{ST} = 0.124$). The CMR and HR populations represent the outermost sampled locations in our study (western and eastern edges); still, we did not find evidence for significant isolation by distance ($R = -0.009$, $P = 0.48$).

Consistent with the finding of no isolation by distance, the PCO analysis showed no clear geographical patterning in population differentiation (Figure 2a). The ΔK method of Evanno et al. (2005) showed that $K = 2$ was the most likely number of subdivisions in our dataset. The STRUCTURE results for $K = 2$ generally agree with the F_{ST} results, e.g., CMR and CW are highly similar, whereas HR is quite distinct (Figure 3a). We also examined the next most likely value of K which was $K = 5$. The resulting 5 groupings of populations roughly corresponded to 1) Camp Meeting Rock (CMR) and Coweta (CW); 2) Mount Arabia (MA); 3) Panola Mountain (PM), Stone Mountain (SM), Irwin Bridge Road (IBR), and Costley Mill (COM); 4) Walnut Grove (WG) and Rock and Shoals (RS); and (5) Heggie's Rock (HR) (Figure 3b). Taken together, these results indicated that *H. porteri* exhibits less in the way of clear geographic structuring compared with other granite outcrop endemics *Tradescantia hirsuticaulis* (Godt and Hamrick 1993) and *Arenaria uniflora* (Wyatt et al. 1992).

Finally, we also compared measures of F_{ST} in *H. porteri* to that for other sunflower species using the same set of genetic markers. The F_{ST} for *H. porteri* did not differ significantly from 3 other sunflower species (*H. niveus ssp. tephrodes*—rare, annual/perennial; *H. angustifolius*—common, perennial; *H. verticillatus*—rare, perennial; F_{ST} species effect, $F_{3,36} = 1.45$, $P = 0.2433$). Thus, despite the apparent geographic isolation of *H. porteri* populations among granite outcrops, we did not find support for the expectation of elevated population structure in this endemic species.

The relatively low level of population genetic structure in *H. porteri* documented here is consistent with the results of previous studies on population differentiation in plant traits and performance in this species. Native populations have been found to differ greatly for plant growth and reproductive success both spatially and temporally, likely driven in large part by precipitation and other edaphic factors (Cumming 1969; Mellinger 1972; Shure and Ragsdale 1977; Gevaert 2011). Some populations experience severe mortality of plants prior to reproduction in severe drought years although the populations recover due to an abundant seed bank (Mellinger 1972; Houle and Phillips 1988; Gevaert 2011). Though population differences in edaphic characteristics and plant performance

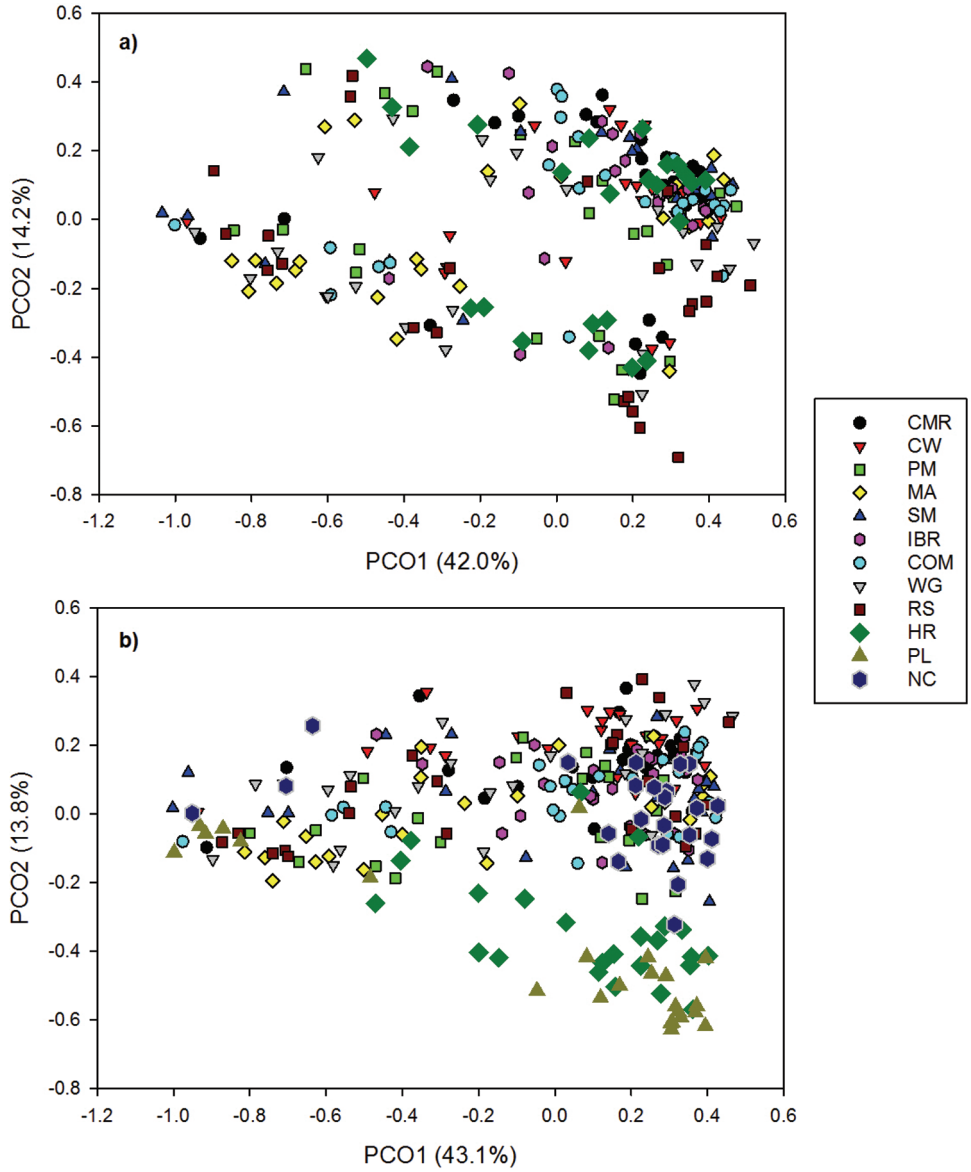

Figure 2. PCO(in GenAlEx) representing relationships among *Helianthus porteri* individuals in (a) 10 native populations (populations are presented in order of geographical location, CMR being farthest west and HR being farthest east) and (b) the 10 native populations plus 2 transplant populations (PL and NC), using genetic distances in the covariance standardized method. The legend shows all 12 populations despite the 2 transplant populations (PL, NC) only included in the second analysis (b). See Figure 1 for locations of populations.

suggest the potential for local adaptation, a comparison of these populations under common environment conditions demonstrated that genetically based differences in plant traits and responses to stress are relatively small and provide no support for local adaptation (Gevaert 2011).

Genetic Diversity and Population Structure of Transplant Populations

We also analyzed two *H. porteri* populations that were transplanted from the MA population in the central portion of the range as sod blocks into unoccupied outcrops in 1959 (Burbanck and Platt 1964; Mellinger 1972). When the transplant populations (PL, NC) were included in the population genetic parameters, measures of pooled species-level genetic diversity for all 12 populations ($A = 18.889 \pm 0.278$; $F_{IS} = 0.365 \pm 0.058$; $H_e = 0.685 \pm 0.055$; $F_{ST} = 0.084$) were not significantly different from the pooled species-level values for the 10 native populations (Table 1).

The PL transplant population is more genetically similar to the neighboring HR native population than to the source MA native population, suggesting that substantial gene flow has occurred since the PL population was established in 1959, based on several lines of evidence. First, PL and HR both

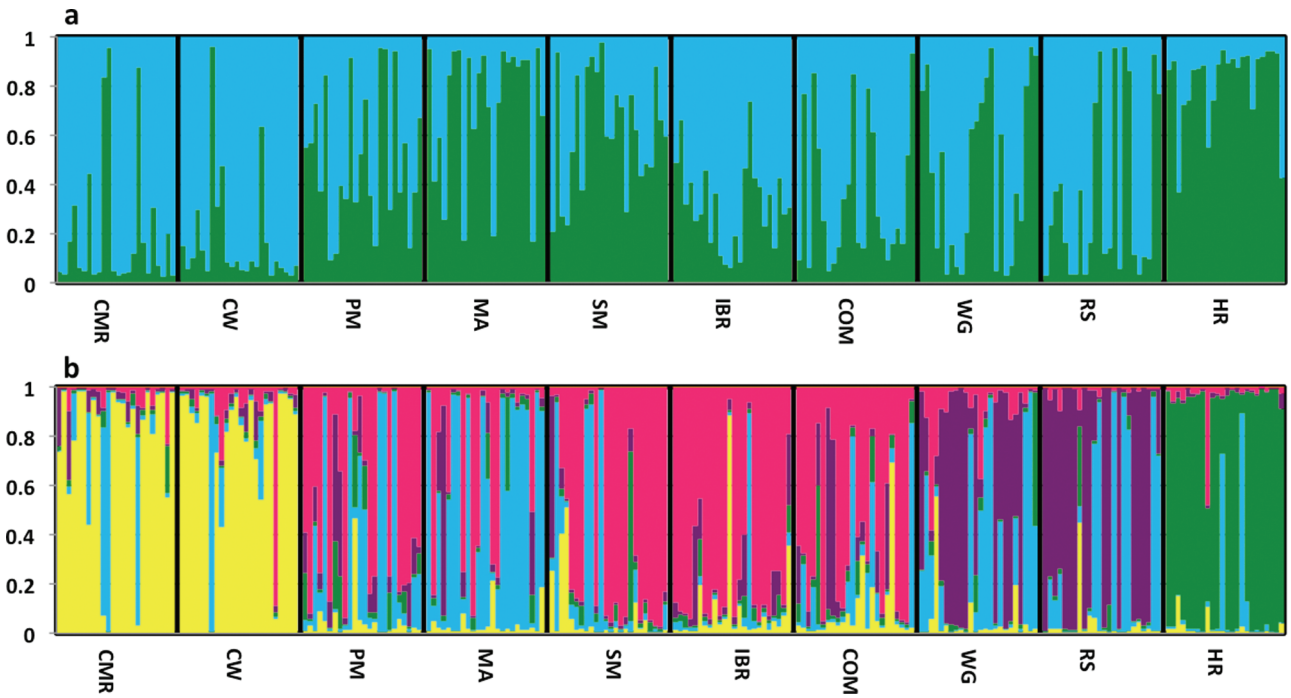

Figure 3. STRUCTURE analysis results with (a) $K = 2$ and (b) $K = 5$ genetic clusters for 10 native populations of *Helianthus porteri*. Bars for each individual indicate the average result across 15 independent iterations. Each individual is represented along the *x*-axis, with populations presented in order of geographical location, CMR being farthest west and HR being farthest east.

have a lower H_e and A than MA (Table 1). Second, the results of the pairwise F_{ST} analyses show PL to be most similar to HR ($F_{ST} = 0.024$), and least similar to all other populations (range: 0.087–0.144; Table 3). Third, when PL and NC are included in a PCO, the native HR and transplanted PL were found to be most similar to each other and most different from the other 10 populations, separating along PCO1 (PCO1: 43.09%, PCO2: 13.80%; Figure 2b). Fourth, a STRUCTURE analysis with all 12 populations shows PL and HR being greatly distinct from all other populations (Supplementary Figure 1). Fifth, PL and HR also share 2 alleles at the BL0010 locus in high frequency (both alleles together total more than 80%), that no other populations share. The population genetic results combined with the presence of these otherwise private alleles in PL suggest that gene flow has occurred between these 2 populations (Slatkin 1985), which are separated by ~1.5 km. Pollinators have been known to move pollen comparable distances in other plant species, including annuals, perennials, and tropical trees (Broyles et al. 1994; Pasquet et al. 2008; Jha and Dick 2010; Ashley 2010) and is the most likely mechanism for long distance gene flow among these populations as seeds are primarily gravity dispersed.

The NC transplant population is located far to the northeast of any known populations of *H. porteri* and thus not likely to be influenced by gene flow from other populations. Consistent with this expectation, NC remains similar to populations in the central portion of the range where the MA source population occurs (Supplementary Figure 1) even though it has been separated for 50+ generations since the initial transplant experiment in 1959. First, H_e and A for NC do not differ from that of MA (Table 1). Second, when PL and NC are included in a PCO, the genetic composition of the transplanted NC is more similar to the central populations including MA than to HR and PL (Figure 2b) even though this differentiation is not evident in the pairwise F_{ST} comparisons (Table 3).

Conclusions

Despite the apparent geographical isolation of populations across the range of *H. porteri*, we found high levels of genetic diversity within populations and low levels of genetic structure among populations of this species. These findings indicate that *H. porteri* is more similar to widespread, nondisjunct annual species, contrary to the expectations based on other granite outcrop species (Wyatt et al. 1992; Godt and Hamrick 1993; Byrne and Hopper 2008). This may be due to ongoing gene flow among populations, as evidenced by the genetic exchange between the transplanted PL population and the native HR population across a relatively small number of generations. The sporophytic self-incompatibility of *H. porteri* might also have contributed to this pattern by enforcing outcrossing, thereby enhancing the opportunity for gene flow among populations.

Intervening populations (either extant and unsampled or recently extinct) might also have served as a conduit for gene flow among the sampled populations. In fact, many

granite outcrop plant communities have declined or been extirpated in the recent past due to a variety of anthropogenic impacts including granite quarrying, recreational use, trash dumping, and covering of exposed granite by landowners (Allison 1992). If population extinctions occurred relatively recently, then it is possible that not enough time has passed for genetic drift to have affected the observed patterns of genetic diversity. In addition, it is worth noting that 5 of the 12 populations are quite large (thousands of individuals); as such, they may have been less susceptible to the effects of genetic drift (Ellstrand and Elam 1993). Ultimately, the relatively high genetic diversity and low population structure evident within and among populations of this species, combined with the long-term success of transplanted populations, bode well for its persistence as long as the habitat persists.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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