Conserv Genet (2015) 16:1277–1293
DOI 10.1007/s10592-015-0739-8

RESEARCH ARTICLE

Environmental requirements trump genetic factors in explaining narrow endemism in two imperiled Florida sunflowers

Chase M. Mason[1] · Caitlin D. A. Ishibashi[1] · Ashley M. Rea[1] · Jennifer R. Mandel[2] · John M. Burke[1] · Lisa A. Donovan[1]

Received: 24 October 2014 / Accepted: 5 June 2015 / Published online: 10 June 2015
© Springer Science+Business Media Dordrecht 2015

Abstract The mechanisms generating narrow endemism have long been of interest to biologists, with a variety of underlying causes proposed. This study investigates the origins of narrow endemism of two imperiled Florida endemics, *Helianthus carnosus* and *Phoebanthus tenuifolius,* in relation to a widespread sympatric close relative, *Helianthus radula*, as well as other members of the genus *Helianthus*. Using a combination of population genetics and environmental niche modeling, this study compares evidence in support of potential mechanisms underlying the origin of narrow endemism, including environmental specialization versus inbreeding, loss of diversity, or other predominantly genetic factors. The two narrow endemics were found to be comparable in genetic diversity to *H. radula* as well as other widespread *Helianthus* species, with little to no evidence of inbreeding. Environmental niche modeling indicates that distributions of both narrow endemics are strongly related to temperature and precipitation patterns, and that both endemics are threatened with severe reductions in habitat suitability under projected climate change. Evidence indicates that genetic factors likely are not the cause of narrow endemism in these species, suggesting that these species are likely ecological specialists and thus historical narrow endemics. This makes both species vulnerable to climate change, and of immediate conservation concern.

Keywords Climate change · Genetic diversity · *Helianthus* · Inbreeding · Niche modeling · *Phoebanthus*

Introduction

Narrow endemics are taxa restricted to small geographic areas. These species are often of conservation interest due to their relative rarity in comparison to widespread species and the inherent risk of extinction associated with occupying only small geographic areas (Kruckeberg and Rabinowitz 1985). The origins of narrow endemism are not well understood, though several underlying causes have been proposed. These include range contractions in formerly widespread species, often due to habitat loss, as well as speciation events into isolated or specialized habitats (Kruckeberg and Rabinowitz 1985). These two opposing origins are directly related to species' environmental requirements, with the latter likely resulting in more narrow requirements than the former. Regardless of origins, narrow endemics have often been hypothesized to be genetically depauperate (Stebbins 1942; Hamrick and Godt 1996), with such species having reduced genetic diversity either as a result of adaptation to narrow ecological conditions or increased inbreeding of clustered populations in a small geographic area (Kruckeberg and Rabinowitz 1985). In this way, reduced genetic variation may represent either a contributing factor to narrow endemism or a consequence thereof. Understanding the relative importance of narrow environmental requirements and genetic variation in determining narrow endemic status is key to our understanding of this biological phenomenon and the management of threatened species.

The southeastern United States is a hotspot of plant endemism, with multiple geographic centers with high

Electronic supplementary material The online version of this article (doi:10.1007/s10592-015-0739-8) contains supplementary material, which is available to authorized users.

✉ Chase M. Mason
chasemason.evolution@gmail.com

[1] Department of Plant Biology, University of Georgia, 2502 Miller Plant Sciences, Athens, GA 30602, USA

[2] Department of Biological Sciences, University of Memphis, Memphis, TN 38152, USA

densities of narrowly endemic species (Estill and Cruzan 2001; Sorrie and Weakley 2001). The southeast has been a focal region for the study of the origins of narrow endemism, and in particular the potential role of glacial refugia in generating the patterns of species distributions seen today (Soltis et al. 2006; Avise 2000). In addition, the study of endemics in relation to their widespread close relatives has been highlighted as the preferred method for understanding differences between species due to range size, as opposed to broad comparisons of rare and widespread species that confound a variety of factors (Gitzendanner and Soltis 2000). The detailed study of southeastern endemic plant species in relation to widespread relatives is a valuable avenue to understanding the origins of narrow endemism, and we explore this avenue here using three southeastern sunflower species.

Helianthus carnosus (Small) (the lakeside sunflower) is a perennial basal rosette species native to five counties in northeastern peninsular Florida, with the majority of the range located east of the St. Johns River (Fig. 1). This species occurs in open wet meadows and sandy wet flatwoods, as well as sandy wet roadside ditches which provide analogous conditions as availability of the former two habitats has declined under the expansion of agriculture and development in the region over the past half-century. This shift in habitat occupancy has resulted in *H. carnosus* populations being subjected to regular mowing as part of roadside maintenance, which may act to reduce growth and seed set in an already slow-growing species. This species forms linear, glabrous, near-succulent leaves from crown buds, maintaining an aboveground rosette year-round (Heiser et al. 1969). During the growing season, *H. carnosus* typically produces one to three erect nearly-leafless stems approximately 10–60 cm tall upon which solitary flower heads are borne (Fig. 1; Schilling 2006a). This growth form makes *H. carnosus* particularly susceptible to mowing, as removal of the tall flowering stems at any point during the months-long period between their initial elongation and final seed maturation will prevent seed set. While primarily flowering in late summer (June–September), there is evidence that the effect of mowing has resulted in shifts in flowering time much later in the year, with populations now flowering over a broader period between June and December (Heiser et al. 1969; C. Mason, personal observation). *H. carnosus* is a state-listed endangered species (Florida Administrative Code, Rule 5B-40.0055).

Phoebanthus tenuifolius (S.F. Blake) (the pineland false sunflower) is an erect rhizomatous perennial species native to five counties in the Florida panhandle, in and around the Apalachicola River basin (Fig. 1). This species occurs primarily on sandy soils in longleaf pine savannas, sandhills, and coastal scrub habitat, forming extensive rhizome networks from which it produces flowering stems with narrow leaves (Fig. 1). *P. tenuifolius* is winter-deciduous, senescing all aboveground tissues and re-sprouting in the spring, reaching a height of around 40–100 cm and flowering throughout the summer (Schilling 2006c). The majority of the range of *P. tenuifolius* is made up of various state and federal conservation lands, including the Apalachicola National Forest, Tate's Hell State Forest, St. Mark's National Wildlife Refuge, and a variety of smaller preserves, wildlife and water management areas, and conservation easements. *P. tenuifolius* is a state-listed threatened species (Florida Administrative Code, Rule 5B-40.0055).

By contrast to these two narrow endemics, *Helianthus radula* (Torr. & A. Gray) (the rayless sunflower) is a perennial basal rosette species that is both widespread in distribution and common within its range, ranging from South Carolina to Louisiana and far south into peninsular Florida (Fig. 1). It is thought that *H. radula* has historically occupied this widespread distribution throughout the southeastern coastal plain (Heiser et al. 1969). Like *H. carnosus*, this species forms near-succulent leaves from crown buds, maintains an aboveground rosette year-round, and sends up erect nearly-leafless stems upon which solitary flower heads are borne (Schilling 2006b). *H. radula* differs markedly, however, in leaf morphology and floral anatomy, with obtuse or orbicular leaves covered in rough trichomes and flower heads completely lacking ray florets (Fig. 1; Heiser et al. 1969). *H. radula* occupies a variety of habitats, including the longleaf pine savanna, sandhill, and coastal scrub habitats of *P. tenuifolius* as well as the wet flatwoods and open roadside habitats of *H. carnosus*. In fact, *H. radula* occurs in sympatry with both endemic species, even co-occurring in intermixed populations, though it tends to flower much later in autumn than the other species (September–November), except perhaps for mowing-induced late flowering populations of *H. carnosus* (Heiser et al. 1969; Schilling 2006b).

These three species are closely related, with *Helianthus* and *Phoebanthus* well supported as sister genera (Schilling 2001; Schilling and Panero 2002; Mandel et al. 2014), and recent phylogenetic data support *H. carnosus* and *H. radula* as likely sister species (Stephens et al. 2015). All three species are sporophytically self-incompatible, with gravity-dispersed seeds. This might make it potentially difficult for these species to colonize new locations and expand their ranges. As *H. carnosus* and *P. tenuifolius* were not described until 1902 and 1916 (Schilling 2006a; Schilling 2006c), it is unknown whether they historically occupied larger ranges before widespread deforestation and habitat alteration in the late 1800s, or whether these species are historical narrow endemics. The Apalachicola River basin and peninsular Florida are both centers of plant endemism

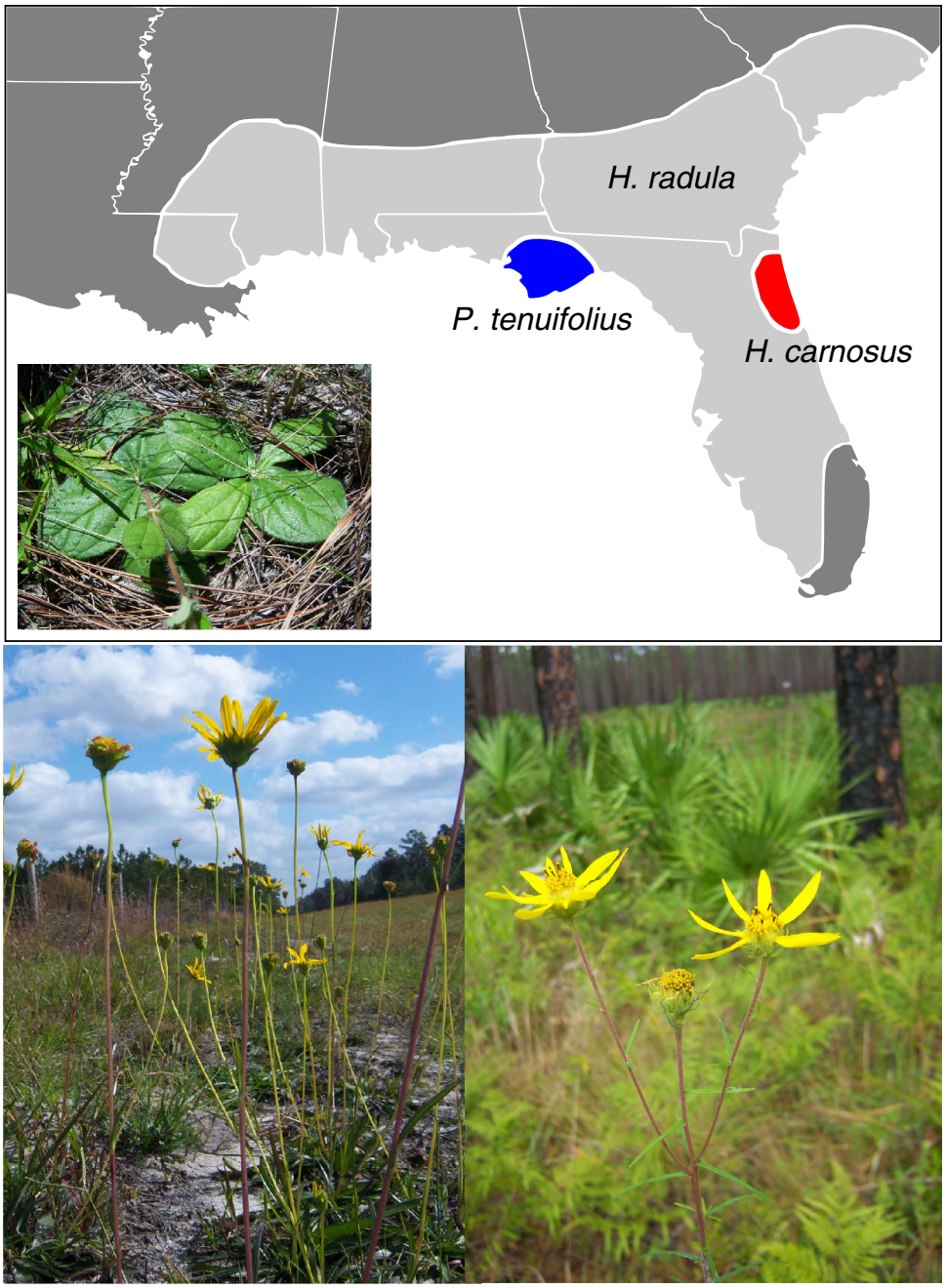

Fig. 1 Approximate geographic ranges and growth forms of study species *H. carnosus* (*bottom left*, FSR population), *P. tenuifolius* (*bottom right*, SUM population), and *H. radula* (*top inset*, ANF population). Photographs by CMM

in the southeastern United States (Estill and Cruzan 2001), so *P. tenuifolius* and *H. carnosus* serve as representatives of these two centers in relation to more broadly distributed species like *H. radula*. Here we will combine two complementary approaches to understanding the origins of narrow endemism—population genetics and environmental niche modeling. By combining these approaches, we can determine whether these narrow endemics are genetically depauperate and whether these species likely have narrow environmental requirements, and gain insight into the role each of these factors might play in the origin of narrow endemism in these species.

Materials and methods

Collection of plant and soil samples

Populations were identified for tissue and soil sampling from across the range of each species, resulting in seven

populations of *H. carnosus*, thirteen populations of *H. radula*, and eleven populations of *P. tenuifolius* sampled for this study. For each population, tissue was sampled during the growing seasons of 2010–2012, and preserved in silica desiccant. Sampled individuals were scattered spatially throughout each population at least 1 m apart to avoid sampling clonal ramets, though these species are not strongly clonal (with only *P. tenuifolius* having rhizomes, and these are typically no more than a few inches in length). Based on recommendations for population genetic studies using microsatellites (Pruett and Winker 2008; Hale et al. 2012), all populations of *H. radula* and *H. carnous* were sampled for 24 individuals, as were all populations of *P. tenuifolius* with at least 24 individuals present. Two sampled populations of *P. tenuifolius* did not meet this criterion (SJB and BLO), so all individuals present were sampled. Additionally, tissue contributed by Dr. Loren Anderson from three Florida State University herbarium records of recently collected short-lived populations of *P. tenuifolius* at the eastern, western, and northern edges of the range were included (WAK, BAY, and CAL, respectively).

For all non-herbarium populations, five soil cores were collected from throughout the spatial extent of the population. Soil samples were analyzed by A&L Eastern Laboratories (Richmond, VA) for soil organic content, phosphorus, potassium, magnesium, calcium, pH, and cation exchange capacity.

DNA extraction, loci selection, PCR, and genotyping

Total genomic DNA was extracted using sorbitol extraction, adapted from that of Štorchová et al. (2000), which was found to result in higher yields with fewer impurities for these species than a standard CTAB method (Doyle and Doyle 1987) as assessed by a NanoDrop 2000 (Thermo Scientific, Wilmington, DE).

A collection of 18 EST-SSR loci originally developed from cultivated sunflower (*Helianthus annuus* L.) sequence information were screened for transferability to the three species of interest here (Table S1). These loci have previously been used successfully in a variety of wild *Helianthus* species, including *H. porteri* (Gevaert et al. 2013), *H. niveus* ssp. *tephrodes* (Mandel et al. 2013), *H. verticillatus, H. grosseserratus,* and *H. angustifolius* (Ellis et al. 2006; Pashley et al. 2006). As the three species of interest in this study are on average more genetically distant from *H. annuus* than those in previous studies (Timme et al. 2007), fewer loci were successfully amplified for these species, with 15/18 out of the screened loci amplifying well in at least one of the three species. Seven of these loci amplified well in all three species, three loci amplified well in both *H. carnosus* and *H. radula,* one locus amplified well in both *H. carnosus* and *P. tenuifolius*, and four loci amplified in only one species. This resulted in a total of 11 scorable loci for each species (Table S1).

Genotyping was accomplished using fluorescent labeling (Schuelke 2000), as implemented by Wills et al. (2005). Polymerase chain reaction was performed using a modification of the protocol of Gevaert et al. (2013), in a total volume of 15 μL containing 22.5 ng of template DNA, 208.3 mM KCl, 41.66 mM Tris, 8.33 mM $MgCl_2$, 416.6 μM of each dNTP, 10 μM universal CAG primer labeled with either 5′ HEX, 6-FAM, or NED, 2 μM forward (CAG-tagged) primer, 10 μM reverse primer, and 2 units of *Taq* DNA polymerase. Reactions were run using a touchdown protocol, with conditions as follows: 3 min at 95 °C; 10 cycles of 30 s at 94 °C, 45 s at 65 °C, and 1 min at 72 °C, annealing temperature decreasing by 1 °C per cycle from 65 °C to 55 °C; followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C; and followed by a final extension of 10 min at 72 °C before holding at 4 °C. Products were combined with 9.0 μL of Hi-Di Formamide (Applied Biosystems, Carlsbad, CA) and 1.0 μL of a ROX-labeled size standard (GGF500R; Georgia Genomics Facility, Athens, GA). An ABI3730xl 96-capillary DNA Analyzer was used for fragment analysis, and allele sizes were determined using the software Peak Scanner v1.0 (Applied Biosystems, Foster City, CA).

Population genetic data analysis

Allelic data for each species was analyzed with MicroChecker (Van Oosterhout et al. 2004), to test for deviations from Hardy–Weinberg equilibrium (no deviations were found) and to test for the possibility of null alleles. No loci in *P. tenuifolius* exhibited evidence of null alleles, though one locus in *H. radula* (BL0002) and two loci in *H. carnosus* (BL0004, BL0007) showed possible signs of null alleles. To assess the impact this might have on further analyses, descriptive population genetic statistics were calculated using GenAlEx v. 6.5 (Peakall and Smouse 2006) for datasets both including and excluding loci suggested to contain null alleles. Removing such loci resulted in no statistically significant changes to any species-wide or population-level estimates of the mean number of alleles per locus (A), observed heterozygosity (H_o), Nei's (1978) unbiased expected heterozygosity (uH_E), or inbreeding coefficient (F_{IS}), as assessed by 95 % confidence intervals. All loci were thus included in subsequent analyses.

In addition to the descriptive population genetic statistics described above, GenAlEx was also used to calculate global and pairwise among-population F_{ST} estimates for each species using permutation analysis of molecular variance (AMOVA; Excoffier et al. 1992). A principal coordinates analysis (PCO) was also performed in

GenAlEx for each species using pairwise estimates of Nei's (1978) unbiased genetic distances among all individuals with the covariance standardized method. To assess population structure, the Bayesian clustering program STRUCTURE v 2.3.4 (Pritchard et al. 2000) was run in each of the three species using the admixture ancestry model and correlated allele frequencies. For each species, clustering was evaluated with replicate runs for numbers of clusters (K) ranging from $K = 1$ to $K = (n-1)$, where n is the number of populations sampled in that species. Initial burn-ins were set to 100,000 with 10^7 Markov Chain Monte Carlo iterations per run. The most likely number of clusters for each species was identified via the ΔK method of Evanno et al. (2005) as implemented with STRUCTURE HARVESTER (Earl and vonHoldt 2012). Cluster assignment was aligned across replicate runs with CLUMPP (Jakobsson and Rosenberg 2007). To further understand population structure in light of geographic distribution, spatial breaks in genetic relatedness were identified using Monmonier's algorithm on Nei's (1978) unbiased genetic distances with Barrier v 2.2 (Manni et al. 2004). Isolation-by-distance was examined in GenAlEx for each species using Mantel tests comparing geographic distances to genetic distances. Additionally, estimates of expected heterozygosity and F_{ST} for other members of the genus *Helianthus* were aggregrated from previously published studies for comparison with the three species examined in this study.

Environmental niche modeling

Occurrence data was aggregated for *H. carnosus* and *P. tenuifolius* from personal population observations and population records from the Florida Natural Areas Inventory and regional herbaria [including the University of Florida (FLAS), Florida State University (FSU), and the University of South Florida (USF)]. Only records that included geographic coordinates or sufficient locality detail to estimate coordinates to within 100 meters were included. This resulted in 32 occurrence localities for *H. carnosus*, spanning 1978–2012, and 67 occurrence localities for *P. tenuifolius*, spanning 1974–2012. For both species the vast majority of occurrences were reported from the 1990s and 2000s.

The geographic scope of modeling was defined as the continental United States between −78.20° and −89.07° longitude and between 24.25° and 35.82° latitude, based on recent recommendations from the literature about appropriate geographic scope to reduce bias and overfitting in niche modeling (Anderson and Raza 2010). 1-km resolution bioclimatic layers for temperature and precipitation were obtained from the WorldClim database (Hijmans et al. 2005). These climate layers are based on interpolated global climate surfaces for the years 1950–2000. The full set of 19 bioclimatic layers was reduced to six layers by removing variables that correlate strongly across the geographic scope of modeling (Table S2). This was performed to reduce redundancy of the included bioclimatic variables, inclusion of which may bias niche modeling efforts (Phillips et al. 2006; Elith et al. 2011; Milanovich et al. 2010). The final set of bioclimatic layers were thus both largely independent of each other and biologically interpretable, and included three variables describing variation in temperature and three variables describing variation in precipitation. Additionally, a 1-km resolution altitude layer was obtained from WorldClim (Hijmans et al. 2005), and a 30 m^2 resolution categorical land use layer was obtained from the 2006 National Land Cover Database (Fry et al. 2011). A 30 m^2 resolution soil type layer containing categorical designations of soil texture (i.e., mixtures of sand, silt, and clay) was aggregated by Dr. Louisa Carter Staton from county-level data obtained from the USDA Soil Survey Geographic Database (National Resources Conservation Service 2014).

Niche modeling was implemented in MaxEnt, a machine-learning approach that models species distributions with presence-only data and a set of environmental variables by estimating a species' ecological niche as a probability distribution of maximum entropy (Phillips et al. 2006). Presence-only modeling was selected for use with these charismatic endemic species given the extensive surveying and monitoring of populations that have been conducted by the Florida Natural Areas Inventory, which reasonably fulfills presence-only modeling assumptions of random or representative geographic sampling (Phillips et al. 2006; Elith et al. 2011; Yackulic et al. 2013). MaxEnt was run for each species without land use data, to generate niche models based solely on abiotic variables, as well as with land use, to generate niche models incorporating biotic and anthropogenic influences, as the land use layer includes ground cover and vegetation types, various agricultural uses, and levels of development (Fry et al. 2011). All models were run with 15 subsample replicates, 25 % random test percentage, a random seed, and 5000 iterations. Output suitability maps were converted to distributions of suitable-versus-unsuitable habitat based on the thresholding approach of Milanovich et al. (2010), generating both liberal and strict suitability thresholds based upon the minimum training presence and 10 % training presence, respectively.

Future species distributions under climate change were modeled in MaxEnt using 1-km resolution climate projections obtained from the WorldClim database. Projections used were based on downscaled global climate models of the IPCC Fourth Assessment, specifically the UKMO-HadGEM1 model for the SRES A2 emissions

scenario, as predicted for the years 2020, 2050, and 2080. The A2 scenario (which is the second-highest in both carbon emissions and global surface warming behind the A1-Fossil Intensive scenario) was selected as a conservative estimate of climate change, given that ongoing emissions are exceeding even the IPCC scenarios with highest emissions and greatest change (Le Quéré et al. 2014).

Results

Population genetic diversity

All eleven loci in each species were polymorphic in at least one population, and the mean number of alleles per locus (A) pooled across populations in each species were between four and six (Table 1). For *H. carnosus*, the mean number of alleles (A) per population ranged from 1.818 (PCR) to 3.000 (POT), with unbiased expected heterozygosity (uH_E) ranging from 0.172 (FLG) to 0.318 (WOB) (Table 1). Inbreeding coefficient (F_{IS}) ranged from −0.255 (PCR) to 0.143 (FSR), with one population (PCR) significantly below zero (Table 1). All populations had private alleles, with the most being five (in WOB). For *P. tenuifolius*, the mean number of alleles (A) per population ranged from 1.636 (BLO) to 2.818 (CAM), with unbiased expected heterozygosity (uH_E) ranging from 0.209 (TAT) to 0.358 (CAM) (Table 1). Inbreeding coefficient (F_{IS}) ranged from −0.495 (BLO) to 0.047 (BOX), with two populations (BLO and JOE) significantly below zero (Table 1). All but one population (SJB) had private alleles, with the most being four (in both ANF and CAM). For *H. radula*, the mean number of alleles (A) per population ranged from 1.727 (in both GIL and HAR) to 3.182 (DAU), with unbiased expected heterozygosity (uH_E) ranging from 0.179 (GIL) to 0.512 (DAU) (Table 1). Inbreeding coefficient (F_{IS}) ranged from −0.186 (WOB) to 0.478 (CRP), with one population significantly below zero and four populations significantly above zero (Table 1). In contrast to *H. carnosus* and *P. tenuifolius*, only six populations of *H. radula* had private alleles, with the most being four (in WOB).

Population structure and geographic distribution

Global F_{ST} estimates for *H. carnosus* and *P. tenuifolius* were 0.112 and 0.139, respectively, while *H. radula* was higher at 0.203 (Table 2). Pairwise population F_{ST} ranged from 0.008–0.226 in *H carnosus*, 0.038–0.388 in *P. tenuifolius*, and 0.010–0.486 in *H. radula* (Table 2). Despite relatively high pairwise F_{ST} values between some populations, Mantel tests did not show significant isolation-by-distance in any species (*H. carnosus*, $p = 0.120$; *P. tenuifolius*, $p = 0.110$; *H. radula*, $p = 0.950$). Graphical representation of population differentiation by PCO shows visible divergence among some populations in *H. carnosus* (e.g., FCR and WOB), though less so in *H. radula* and *P. tenuifolius* (Fig. S1). Results from the ΔK method of Evanno et al. (2005) indicate that the most likely number of genetic clusters for *P. tenuifolius* is two, while for both *H. carnosus* and *H. radula* the most likely number is four. All other numbers of clusters were far less likely, with ΔK values less than a third as large as the most likely number of clusters in each species. STRUCTURE results for *H. carnosus* indicate that three genetic clusters each have majority representation in only one population (PCR, WOB, FCR), while the remaining populations either contain a majority of the fourth cluster (POT, FSR, SOF) or a mix of clusters with none dominant (FLG) (Figs. 2, 3). Results from Barrier place the strongest geographic divisions around the WOB and PCR populations, indicating that these are most distinct from the surrounding populations. STRUCTURE results for *P. tenuifolius* split the species into nine and five populations with majority representation in each of the two genetic clusters (Figs. 2, 3), with the five populations geographically grouped in the center of the range (Fig. 3). STRUCTURE results for *H. radula* show majority representation of a single cluster in three (GIL, HAR, PEN), three (ANF, BLB, RAM), and two (MNC and WOB) populations, with the remaining five populations containing a mix of clusters with none dominant (Figs. 2, 3). Genetic clusters show rough geographic separation, with South Carolina, the Gulf Coast, and peninsular Florida having characteristic clusters with less representation outside of these regions (Fig. 3). Results from Barrier place the strongest geographic divisions in *H. radula* to the east and west of the ANF population, dividing the species into three regions: western, central, and eastern.

Soil data and environmental niche modeling

Analysis of soil samples indicates that *H. radula* has a far larger occupancy of soil conditions than *H. carnosus* or *P. tenuifolius* (Table S3). *H. carnosus* was found to occur exclusively on fine sandy loam soils, while *P. tenuifolius* occurred primarily on sandy clay soils, as well as on fine sandy loam and marly silt loam soils. By contrast, *H. radula* was found to occur on all three of these soil types, as well as loamy sand, very gravelly loamy sand, and silt loam soils. Additionally, *H. radula* was found to have far larger variation in soil phosphorus, calcium, and cation exchange capacity than the two narrow endemic species (Table S3). However, this is not a direct consequence of the larger geographic range of *H. radula*, as some of the largest differences in these soil characteristics are between geographically adjacent populations (Table S3).

Table 1 Population genetic descriptive statistics for each species

	N	*A*	H_o	uH_E	F_{IS}
Helianthus carnosus					
FCR	22	2.273 (0.237)	0.315 (0.090)	0.304 (0.065)	−0.072 (0.140)
FLG	21	2.091 (0.285)	0.177 (0.060)	0.172 (0.052)	−0.015 (0.061)
FSR	17	2.455 (0.366)	0.191 (0.069)	0.232 (0.070)	0.143 (0.100)
PCR	23	1.818 (0.263)	0.237 (0.098)	0.174 (0.065)	−0.255 (0.101)*
POT	24	3.000 (0.302)	0.310 (0.072)	0.299 (0.052)	−0.008 (0.100)
SOF	22	2.545 (0.312)	0.246 (0.041)	0.282 (0.054)	0.026 (0.082)
WOB	22	2.727 (0.273)	0.318 (0.073)	0.318 (0.048)	0.041 (0.128)
Grand mean	22.6	2.416 (0.114)	0.256 (0.028)	0.255 (0.022)	−0.009 (0.039)
Pooled species-level	151	4.455 (0.493)	0.259 (0.055)	0.284 (0.049)	0.115 (0.078)
Phoebanthus tenuifolius					
ANF	24	2.727 (0.359)	0.310 (0.095)	0.301 (0.081)	−0.057 (0.090)
BLO	5	1.636 (0.203)	0.418 (0.146)	0.298 (0.090)	−0.495 (0.227)*
BOX	21	2.636 (0.244)	0.308 (0.083)	0.288 (0.062)	0.047 (0.135)
BSP	16	2.091 (0.211)	0.413 (0.105)	0.334 (0.069)	−0.232 (0.119)
CAM	21	2.818 (0.501)	0.395 (0.092)	0.358 (0.079)	−0.176 (0.109)
CSH	20	2.091 (0.251)	0.367 (0.121)	0.314 (0.071)	−0.059 (0.220)
FRC	20	2.636 (0.432)	0.265 (0.072)	0.275 (0.063)	0.006 (0.101)
JOE	22	2.364 (0.310)	0.355 (0.085)	0.298 (0.065)	−0.202 (0.090)*
SJB	9	1.909 (0.285)	0.424 (0.123)	0.341 (0.083)	−0.360 (0.186)
SUM	23	2.273 (0.333)	0.232 (0.066)	0.215 (0.055)	−0.091 (0.063)
TAT	19	2.455 (0.366)	0.265 (0.091)	0.209 (0.058)	−0.107 (0.154)
Grand mean	18.2	2.332 (0.101)	0.341 (0.030)	0.294 (0.021)	−0.140 (0.042)*
Pooled species-level	200	5.818 (0.980)	0.326 (0.073)	0.338 (0.064)	0.131 (0.117)
Helianthus radula					
ANF	24	2.091 (0.163)	0.310 (0.062)	0.332 (0.048)	0.046 (0.119)
BLB	24	1.909 (0.091)	0.201 (0.053)	0.246 (0.048)	0.105 (0.119)
CRP	21	2.727 (0.359)	0.191 (0.057)	0.355 (0.055)	0.478 (0.103)*
DAU	23	3.182 (0.296)	0.392 (0.065)	0.512 (0.029)	0.219 (0.122)
GIL	24	1.727 (0.195)	0.173 (0.074)	0.179 (0.064)	0.158 (0.141)
HAR	22	1.727 (0.141)	0.216 (0.080)	0.235 (0.064)	0.120 (0.148)
LCR	24	2.909 (0.251)	0.276 (0.074)	0.389 (0.068)	0.237 (0.119)*
MNC	23	1.909 (0.211)	0.216 (0.064)	0.237 (0.063)	0.140 (0.116)
PAC	23	2.182 (0.163)	0.272 (0.048)	0.360 (0.048)	0.207 (0.079)*
PEN	24	2.091 (0.163)	0.153 (0.047)	0.266 (0.059)	0.402 (0.114)*
RAM	19	2.182 (0.263)	0.284 (0.069)	0.333 (0.069)	0.079 (0.106)*
RLR	21	2.818 (0.400)	0.223 (0.058)	0.287 (0.049)	0.230 (0.140)*
WOB	24	2.091 (0.368)	0.240 (0.089)	0.199 (0.071)	−0.186 (0.044)*
Grand mean	22.8	2.273 (0.077)	0.242 (0.018)	0.302 (0.017)	0.188 (0.033)*
Pooled species-level	296	4.909 (0.392)	0.239 (0.039)	0.374 (0.043)	0.365 (0.061)*

Values represent population means across all eleven loci per species, with standard errors presented in parentheses. Grand means across populations and pooled species-level means are also presented. Inbreeding coefficients significantly different from zero by 95 % confidence interval are indicated with an asterisk

N number of successfully genotyped individuals, *A* mean number of alleles per locus, H_o observed heterozygosity, uH_E Nei's unbiased mean expected heterozygosity, F_{IS} population inbreeding coefficient

Environmental niche modeling produced well-supported models for both *H. carnosus* and *P. tenuifolius*. The mean AUC for *H. carnosus* models based on current climate were 0.997 (including land use) and 0.996 (excluding land use). The mean AUC for *P. tenuifolius* models based on current climate were 0.993 (including land use) and 0.992

Table 2 Pairwise F_{ST} matrices and global F_{ST} estimates for each species calculated by analysis of molecular variance with 999 permutations

	FCR	FLG	FSR	PCR	POT	SOF	WOB
Helianthus carnosus (Global $F_{ST} = 0.112$)							
FCR	0						
FLG	0.152	0					
FSR	0.129	0.117	0				
PCR	0.091	0.136	0.159	0			
POT	0.085	0.065	0.023*	0.083	0		
SOF	0.091	0.105	0.010†	0.101	0.008†	0	
WOB	0.183	0.226	0.104	0.224	0.111	0.110	0

	ANF	BLO	BOX	BSP	CAM	CSH	FRC	JOE	SJB	SUM	TAT
Phoebanthus tenuifolius (Global $F_{ST} = 0.139$)											
ANF	0										
BLO	0.317	0									
BOX	0.190	0.192	0								
BSP	0.102	0.215	0.092*	0							
CAM	0.139	0.214	0.166	0.109	0						
CSH	0.091	0.183*	0.083	0.071	0.122	0					
FRC	0.046	0.201	0.089	0.038*	0.080	0.050*	0				
JOE	0.155	0.260	0.078	0.097	0.125	0.109	0.088	0			
SJB	0.143	0.177*	0.145	0.148	0.180	0.094*	0.124	0.162	0		
SUM	0.141	0.388	0.108	0.104	0.226	0.085	0.063	0.133	0.234	0	
TAT	0.184	0.368	0.157	0.165	0.283	0.170	0.132	0.250	0.257	0.167	0

	ANF	BLB	CRP	DAU	GIL	HAR	LCR	MNC	PAC	PEN	RAM	RLR	WOB
Helianthus radula (Global $F_{ST} = 0.203$)													
ANF	0												
BLB	0.180	0											
CRP	0.257	0.243	0										
DAU	0.140	0.192	0.199	0									
GIL	0.328	0.207	0.281	0.323	0								
HAR	0.243	0.175	0.151	0.223	0.058*	0							
LCR	0.215	0.166	0.052	0.166	0.157	0.097	0						
MNC	0.167	0.146	0.155	0.180	0.160	0.089	0.075	0					
PAC	0.135	0.158	0.177	0.118	0.160	0.078	0.079	0.079	0				
PEN	0.246	0.255	0.255	0.275	0.262	0.209	0.172	0.248	0.148	0			
RAM	0.161	0.249	0.322	0.154	0.420	0.303	0.305	0.239	0.241	0.403	0		
RLR	0.221	0.134	0.071	0.195	0.144	0.098	0.010†	0.071	0.086	0.179	0.304	0	
WOB	0.379	0.240	0.235	0.362	0.265	0.225	0.127	0.165	0.258	0.338	0.486	0.138	0

All values are significantly greater than zero at $p < 0.001$, unless noted as * $p < 0.05$ or † nonsignificant

(excluding land use). All four of these models exceeded the 95th percentile of null AUC distributions, indicating that they were significantly better than random. For both species, neither soil type nor land use had a high permutation importance, rather climate variables were found to be more explanatory (Table 4). For *H. carnosus*, the most important environmental variable was annual mean temperature, followed by mean temperature of the driest quarter (Table 4). For *P. tenuifolius*, the most important environmental variable was precipitation of the warmest month, followed by annual mean temperature (Table 4). Geographically, these models well-predicted the current geographic range of each species, with minimal suitable habitat predicted outside the current areas occupied

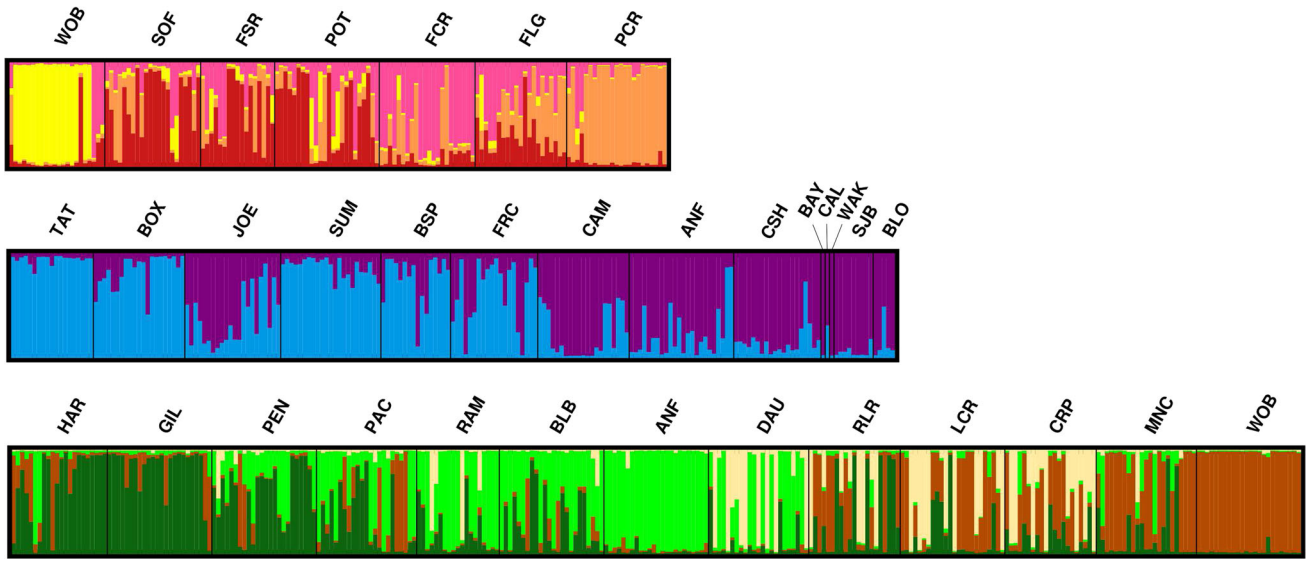

Fig. 2 STRUCTURE analysis results for *H. carnosus* (top, $K = 4$), *P. tenuifolius* (center, $K = 2$), and *H. radula* (bottom, $K = 4$). *Vertical bars* represent average cluster assignments averaged across replicate runs for each individual, with populations indicated above each plot

(Fig. 4). For *H. carnosus*, a small region of suitable habitat was predicted to the north of the current range, north of Jacksonville into Georgia (Fig. 4a), though no extant populations are known from that area. Including land use strongly reduced the prevalence and continuity of suitable habitat in both the current range and this more northern area, consistent with habitat fragmentation in the region (Fig. 4b). For *P. tenuifolius*, suitable habitat was predicted to the west of Panama City, extending almost to Choctawhatchee Bay, far further than the westernmost recorded populations (Fig. 4c). Including land use somewhat reduced the prevalence of suitable habitat, but not as severely as for *H. carnosus* (Fig. 4d). This effect was most pronounced in the eastern half of the range, in regions within the Apalachicola National Forest and Tate's Hell State Forest (Fig. 4d).

Future species distribution projections for 2020, 2050, and 2080 all show a reduction of suitable habitat to near zero, as defined by both strict and liberal thresholds (Fig. S2). These reductions in suitability appear to be driven by predicted changes in key temperature and precipitation patterns over the coming decades. Current niche models indicate high importance for both species of annual mean temperature, which is predicted to increase by an average of 2–3 °C by 2050 in both species' ranges (Fig. S3). Precipitation of the wettest month, which is of high importance for *P. tenuifolius*, is predicted to decrease range-wide by >25 mm by 2050 (Fig. S3). Mean temperature of the driest quarter, which is of high importance for *H. carnosus*, is predicted to increase ~5 °C range-wide by 2050, due to a shift in the primary season of drought, with the driest quarter moving from the current November–January to the earlier period of October–December (Fig. S3).

Discussion

Genetic factors and narrow endemism

When considering population genetic descriptive statistics across all available loci for each of the three study species, the two narrow endemics had mean within-population unbiased expected heterozygosities (uH_E) that were lower than predicted for short-lived perennials (0.55), endemics (0.42), outcrossers (0.65), and species with gravity-dispersed seeds (0.47) as compared to averages across studies using SSR markers in plants (Nybom 2004). However, the widespread *H. radula* was also lower than predicted based on these life-history traits, so low genetic diversity does not seem to be related to narrow endemism. This result is consistent with the small differences in diversity reported between pairs of rare and widespread congeners (Gitzendanner and Soltis 2000). While all three species have roughly the same mean number of alleles per locus (A, Table 1), the narrow endemics have a far higher incidence of private alleles, which is counterintuitive given the relatively shorter geographic distances among the populations of the narrow endemic species. Population inbreeding coefficients (F_{IS}) for all populations of *H. carnosus* or *P. tenuifolius* were either not significantly different from zero or significantly lower than zero, indicating that there was less inbreeding than expected (Table 1). This lack of inbreeding may be due to the combination of sporophytic self-incompatibility with generally small population sizes in these species, where limited numbers of S-alleles skew successful matings strongly toward pairings of the least related individuals (Busch et al. 2010; Charlesworth and Charlesworth 1987). This may have long-term

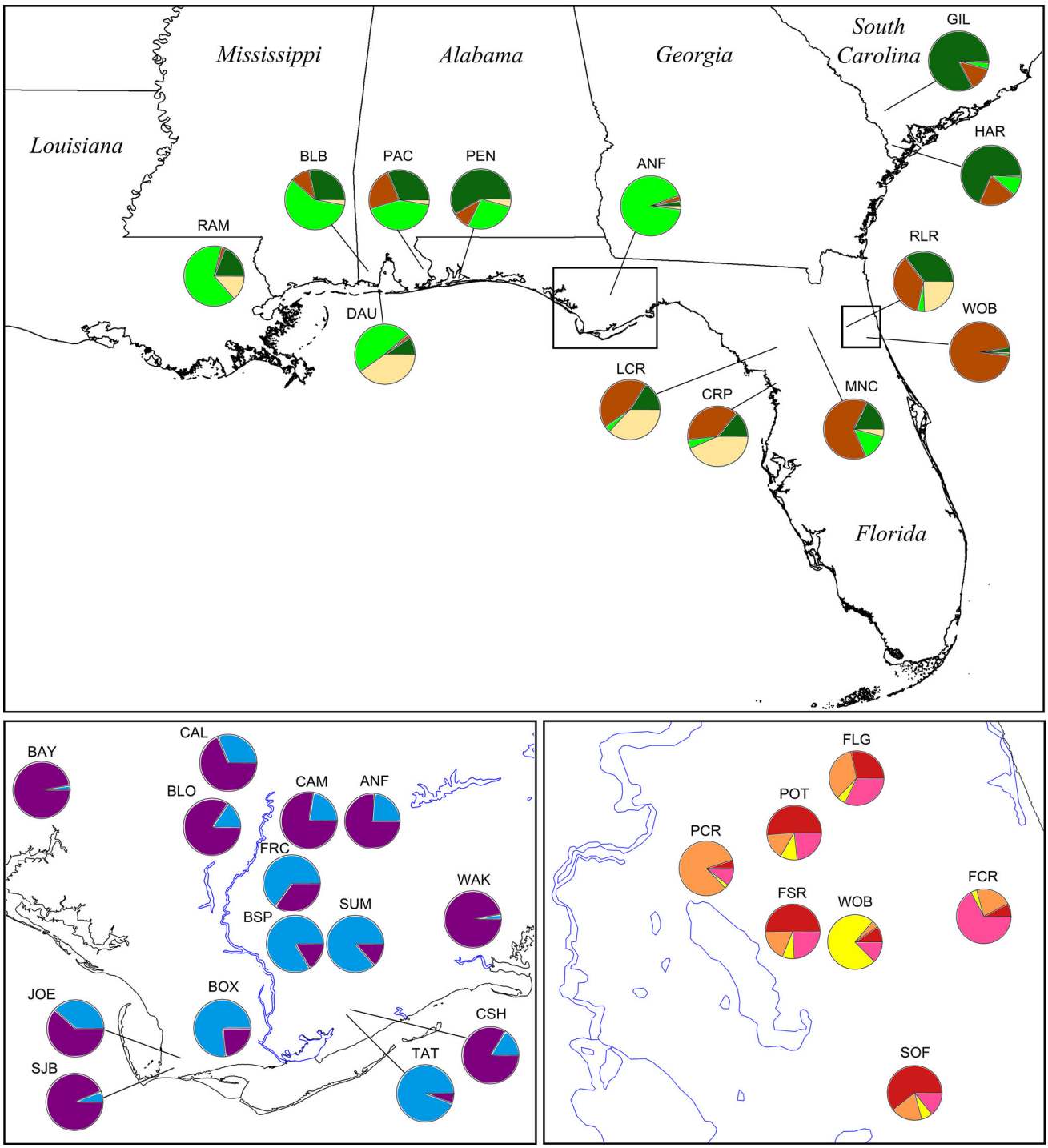

Fig. 3 Geographic distribution of sampled populations of *H. radula* (top), *P. tenuifolius* (bottom left), and *H. carnosus* (bottom right). Pie graphs represent percent STRUCTURE cluster assignment of individuals in each population. *Inset rectangles* in the top panel reflect the regions presented in the bottom two panels

conservation implications if high selection for heterozygotes in small populations leads to a lack of compatible mates in future generations, and potentially even the evolution of self-compatibility if populations do not go extinct first (Willi 2009).

Unlike the narrow endemics, four populations of *H. radula* had inbreeding coefficients significantly higher than zero, indicating higher levels of inbreeding than expected, and both the species grand mean and pooled species-level inbreeding coefficients were also significantly higher than

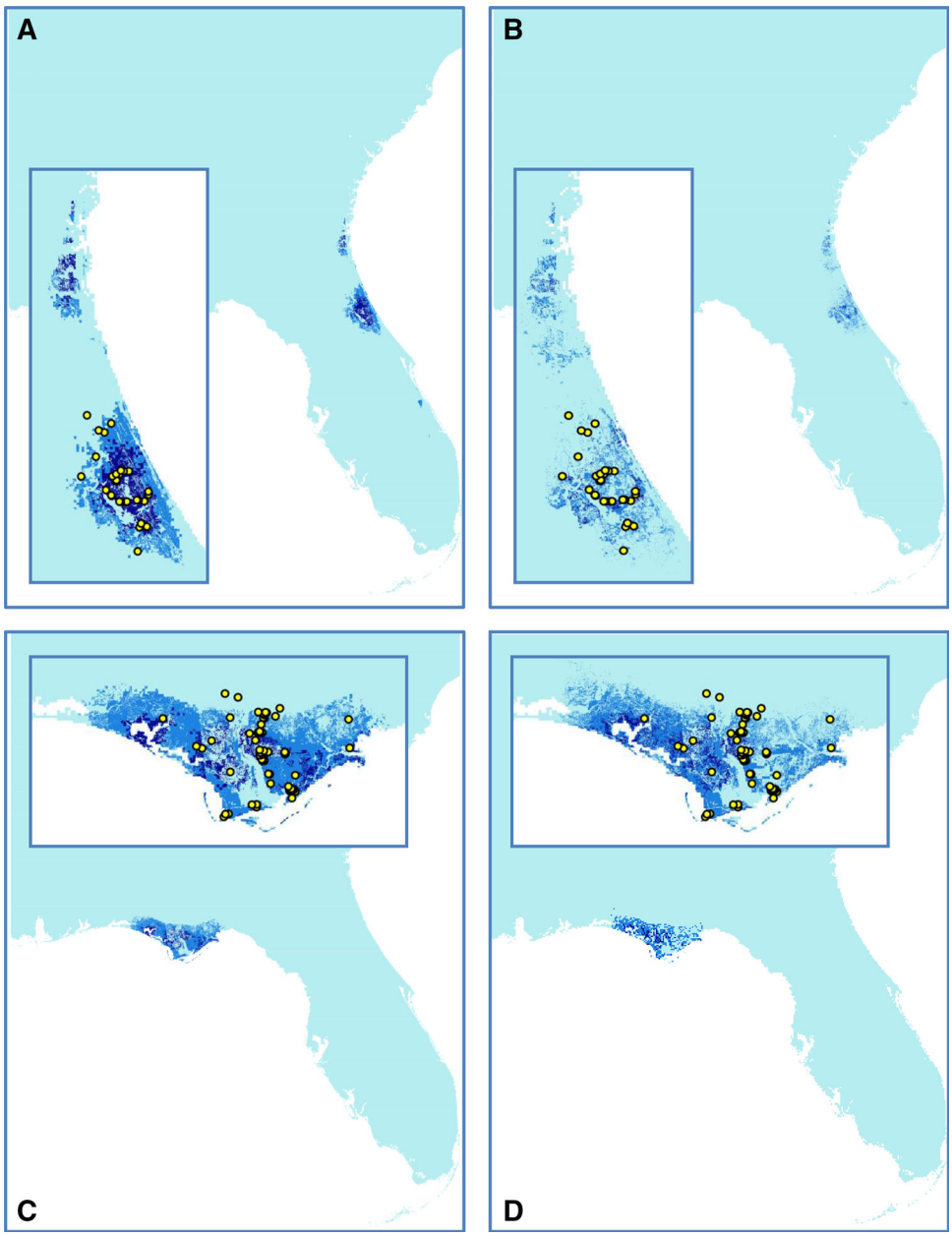

Fig. 4 Maximum entropy niche models projected as predicted distributions for two imperiled sunflowers. **a** *Helianthus carnosus* without land use, AUC = 0.997. **b** *Helianthus carnosus* with land use, AUC = 0.996. **c** *Phoebanthus tenuifolius* without land use, AUC = 0.993. **d** *Phoebanthus tenuifolius* with land use, AUC = 0.992. *Light shading* indicates a liberal threshold of suitability (minimum training presence), while *dark shading* indicates a strict threshold of suitability (fixed cumulative value 10). *Inset* panels include species occurrence points used for modeling

zero (Table 1). This high inbreeding occurs in two populations along the Gulf Coast, and two populations in western peninsular Florida. While *H. radula* is also sporophytically self-incompatible, it would seem that biparental inbreeding is more widespread in this species than in the narrow endemics. This may be explained by the fact that populations of *H. radula* are typically larger and more spatially expansive than populations of the two endemics, which is possibly due to the ability of this species to occupy a broader diversity of soil conditions than the narrow endemics and thus likely more variable microsites where it occurs (Table S3). This tendency toward spatial expansiveness in *H. radula* may increase the likelihood of observing a Wahlund effect, where subpopulation structure reduces overall heterozygosity (and thus increases the inbreeding coefficient) even if subpopulations themselves are in Hardy–Weinberg equilibrium (Wahlund 1928).

Population genetic structure (F_{ST}) of the two narrow endemics was lower than the widespread *H. radula*, despite the fact that averages across studies using SSR markers in plants do not predict significant differences in F_{ST} with geographic range size (Nybom 2004), nor do direct comparisons between rare and widespread congeners (Gitzendanner and Soltis 2000). All three species were lower in F_{ST} than predicted for short-lived perennials (0.31),

outcrossers (0.22), and species with gravity-dispersed seeds (0.34), though *H. radula* was only slightly lower than the predicted F_{ST} for outcrossers (Nybom 2004). The overall lower population genetic structure of the narrow endemics relative to *H. radula* is likely a simple function of the far larger geographic distances among populations of *H. radula*, but it is curious that there is little difference between *H. carnosus* and *P. tenuifolius* given that habitat in the range of *H. carnosus* is much more fragmented, which would be expected to give rise to higher structure. However, this fragmentation, most severe in the last half-century, may be so recent as to not yet impact the signature of F_{ST} in *H. carnosus* if gene flow was historically high. Alternatively, contemporary gene flow among populations could also be acting to reduce F_{ST} relative to *P. tenuifolius* despite less contiguous habitat, perhaps seed dispersal mediated by roadside mowing or long-distance pollination (Beekman and Ratnieks 2000; White et al. 2002; Steffan-Dewenter and Kuhn 2003).

While expected heterozygosity and population genetic structure for these three species were lower than predicted based on life history characteristics (Nybom 2004), they are in keeping with the wide range of values previously reported in species of *Helianthus*. This genus has been found to be highly variable in expected heterozygosity, ranging from very high in the annual granite outcrop narrow endemic *H. porteri* (0.62), the widespread annual *H. annuus* (0.58), and the widespread woodland and prairie perennial *H. occidentalis* (0.56), to intermediate in the narrow woodland endemic *H. verticillatus* (0.48), the widespread prairie perennial *H. grosseserratus* (0.44), the widespread woodland perennial *H. angustifolius* (0.35), and the desert narrow endemic *H. niveus* ssp. *tephrodes* (0.31), with *H. radula, P. tenuifolius*, and *H. carnosus* falling toward the lower end of this variation (0.37, 0.34, and 0.28, respectively) as examined in this study (Table 3; Ellis et al. 2006; Foré and Guttman 1999; Gevaert et al. 2013; Mandel et al. 2011, 2013). This wide variation is not clearly explained by differences in range size, life history, or other apparent characteristics. However, unlike heterozygosity, population structure is fairly similar among these diverse *Helianthus* species, ranging from 0.077 in *H. porteri* to 0.207 in *H. angustifolius*, with all other studied species to date (including those examined in this study) falling within that range (Table 3; Ellis et al. 2006; Foré and Guttman 1999; Gevaert et al. 2013; Mandel et al. 2011, 2013). Why this genus appears to buck expectations for higher than observed population structure in outcrossing annuals and short-lived perennials with gravity-dispersed seeds is unknown.

Geographic distribution of genetic variation

Spatial genetic clustering by STRUCTURE varied strongly among species. In *H. carnosus*, there is very little patterning by geography, instead populations with very similar cluster representation are found throughout the range (Fig. 3), most populations share similar cluster proportions (Fig. 2), and pair-wise F_{ST} among populations does not track with geographic distance (Table 2). The similarity of very distant populations may be due to dispersal by roadside mowing, or by a historically genetically homogenous region. Interestingly, the only outlier populations, with majority representation of only one cluster, are the three populations with the smallest census sizes (<100), indicating that these populations may be particularly genetically distinct as a result of genetic drift. This is further supported by the result that Barrier separates two of these three populations as most distinct in the landscape. In *P. tenuifolius*, there appears to be a center-of-range versus

Table 3 Species-level estimates of unbiased expected heterozygosity (uH_E) and population structure (F_{ST}) aggregated from previous studies of *Helianthus* species as well as species from this study, with total sample size (N) and number of loci (L) listed for each

Species	N	L	uH_E	F_{ST}	Range size/life history
H. annuus	96	22	0.58 (0.02)	n/a	Widespread annual
H. angustifolius	38	19	0.35 (0.07)	0.207	Widespread perennial
H. carnosus	151	11	0.28 (0.05)	0.112	Narrow endemic perennial
H. grosseserratus	56	18	0.44 (0.05)	n/a	Widespread perennial
H. niveus ssp. tephrodes	119	22	0.31 (0.05)	0.143	Narrow endemic annual/perennial
H. porteri	200	18	0.62 (0.05)	0.077	Narrow endemic annual
H. radula	296	11	0.37 (0.04)	0.203	Widespread perennial
H. verticillatus	71	19	0.48 (0.07)	0.118	Narrow endemic perennial
P. tenuifolius	200	11	0.34 (0.06)	0.139	Narrow endemic perennial

For all species, estimates are derived from varying subsets of the same 22 microsatellite loci used in Ellis et al. (2006), with uH_E recalculated as pooled species means across loci ± standard error and F_{ST} as calculated by AMOVA in each study. Sources: *H. annuus* (Mandel et al. 2011); *H. verticillatus, H. angustifolius*, *H. grosseserratus* (Ellis et al. 2006); *H. porteri* (Gevaert et al. 2013); *H. niveus ssp. tephrodes* (Mandel et al. 2013); *H. carnosus*, *H. radula*, *P. tenuifolius* (this study)

edge-of-range pattern dividing the two genetic clusters (Fig. 3), though interestingly the Apalachicola River does not appear to represent a barrier to gene flow, based on the distribution of clustering and pair-wise F_{ST} among populations (Table 2). In *H. radula*, genetic clustering is distributed primarily by geography (Fig. 3), with quite high pairwise F_{ST} values between regions (Table 2). While there appear to be three major regions by STRUCTURE, the results of Barrier suggest that the Apalachicola River basin represents an additional unique area of spatial genetic variation.

From a phylogeographic perspective, the split between Gulf Coast versus Florida and South Carolina seen in *H. radula* fits with genetic divisions seen in many other plant and animal species, and this pattern is typically attributed to fluctuating sea level and an insular history of populations in Florida through the Pliocene and Pleistocene (Soltis et al. 2006). Additionally, the high level of botanical endemism around the Apalachicola River basin has long been interpreted as evidence of large climatic glacial refugia in the region, and the same reasoning has been applied to sites in peninsular Florida (Soltis et al. 2006; Estill et al. 2001). Given the frost-intolerance of all three study species, one interpretation is that *H. carnosus* and *P. tenuifolius* simply never left these glacial refugia, while *H. radula* expanded into the largely frost-free regions of the southeastern coastal plain. Why one species would escape these refugia while the others would not is unknown, but the conclusion that the narrow endemics are not genetically depauperate suggests environmental requirements may play a larger role that genetic factors.

Environmental requirements and historical narrow endemism

Niche modeling results indicate that these species are likely historical narrow endemics, with specialized environmental requirements that limit their distributions. This interpretation is supported by the lack of predicted suitable habitat outside of the current ranges of *H. carnosus* and *P. tenuifolius*, which indicates that specific combinations of environmental variables that well explain the distribution of these species are not found elsewhere. Likewise, the lack of importance of land use suggests that the distributions of these species are not driven primarily by deforestation or habitat degradation outside of their current ranges relative to within. This further supports the interpretation of these species as historical narrow endemics. Interestingly, both *H. carnosus* and *P. tenuifolius* occupy far fewer soil types and narrower ranges of soil nutrient characteristics like soil phosphorus, calcium, and cation exchange capacity than does *H. radula*. This suggests that the widespread *H. radula* is tolerant of a wider range of soil conditions, and this plasticity may contribute to its larger relative distribution. However, while edaphic factors are known to drive endemism in several sunflower species (e.g., *H. porteri* on granite outcrops, *H. longifolius* on sandstone outcrops, and *H. exilis* on serpentine soils), soil traits do not likely explain range limits in either *H. carnosus* or *P. tenuifolius*, as soils with analogous textures and nutrient profiles exist outside their respective ranges and niche modeling finds that soil type has little importance to these species' distributions.

Niche modeling efforts should always be interpreted with caution, as a best possible estimate of the fundamental niche as defined by the included environmental parameters (Araújo and Peterson 2012). Climate has long been supported as a major determinant of species distributions, though more difficult to measure biotic and dispersal factors also play an important role in most cases. However, unlike biotic interactions or dispersal of species, which are known to change through time, only inherited physiological tolerances of species to environmental factors are expected to be conserved over the time scales implicit in niche modeling, especially the time scales of future projections (Araújo and Peterson 2012). The potential role of biotic interactions in limiting the distributions of these species seems small, as both *H. carnosus* and *P. tenuifolius* are not known to have specialized pollinators (mostly generalist bees), dispersers (both species have gravity-dispersed seeds), or pathogens that are specific to these narrow endemics and not their sympatric widespread relatives (Heiser et al. 1969). However, dispersal factors like gravity-dispersed seeds and self-incompatibility likely limit the ability of these species to expand their ranges. Of course, all of these biotic and dispersal characteristics are shared with the widespread *H. radula*, as well as many other widespread sunflower species in the southeast (Heiser et al. 1969), so abiotic factors remain the most likely candidates to explain the distributions of these species.

Niche modeling supports that the ranges of *H. carnosus* and *P. tenuifolius* are defined largely by temperature and precipitation (Table 4). For both species, annual mean temperature is highly explanatory, along with precipitation as defined by either the amount in the wettest month of the year, or the quarter in which the least precipitation falls (Table 4). Precipitation is particularly interesting as the timing of high or low rainfall in both species is related to the timing of reproduction. In the range of *P. tenuifolius*, the wettest month of the year occurs during mid-summer flowering, while outside the range to the north, east, and west the amount of precipitation is lower during this key phenological period (Fig. S3). In the range of *H. carnosus*, the driest quarter of the year occurs from November to January, while the inland boundary aligns with the transition of the driest quarter earlier in the year (October–

Table 4 Percent permutation importance of environmental variables in MaxEnt models including and excluding land use for *Helianthus carnosus* (AUC = 0.997 and 0.996, respectively) and *Phoebanthus tenuifolius* (AUC = 0.993 and 0.992, respectively), averaged over fifteen replicate runs for each model

	Helianthus carnosus		*Phoebanthus tenuifolius*	
	With land use	Without land use	With land use	Without land use
Altitude	0.0	0.0	1.1	0.5
Soil type	1.4	1.6	2.0	4.9
Annual mean temperature	**59.3**	**59.7**	**19.1**	**14.5**
Mean diurnal temperature range	0.3	0.2	2.1	2.3
Maximum temperature of the warmest month	0.8	1.1	0.2	0.1
Annual precipitation	0.9	1.0	6.6	8.2
Precipitation of the wettest month	11.6	5.6	**64.5**	**46.4**
Mean temperature of the driest quarter	**25.6**	**30.7**	3.7	8.2
Land use	0.2	–	0.7	–

Percent permutation importance indicates the relative dependence of the model on each variable, independent of the path MaxEnt used to arrive at the maximum entropy solution. Percent permutation importance is assessed by randomly permuting the values of each variable among training points, and measuring the decrease in training AUC. The two most important environmental variables for each model are shown in bold

December), where autumn drought would overlap more with seed-set in *H. carnosus* (Fig. S3). The mechanisms underlying the importance of annual mean temperature are less clear, though this coarse environmental variable is strongly correlated ($R^2 > 0.50$) with multiple other climate variables across the study region, with warmer sites for instance having lower temperature seasonality, lower precipitation during the driest month and quarter, and higher precipitation in the warmest quarter (Table S2). Niche modeling alone cannot tease apart which of these highly correlated variables might be the most biologically important in determining distributions, for instance buffered temperatures limiting the impact of winter frost, increases in the occurrence of drought during the driest portions of the year, or increased water availability during the growing season. Regardless of the mechanism, annual mean temperature clearly captures a dimension of environmental variation that is highly explanatory for these species.

Future projections indicate climate change will severely reduce the suitability of *H. carnosus* and *P. tenuifolius* habitat (Fig. S2). This appears to be explained by the most important temperature and precipitation factors that define range limits under current conditions changing in ways that make areas within the current ranges more similar to areas outside the current ranges. For *P. tenuifolius*, precipitation during flowering declines while temperatures increase, and for *H. carnosus* temperatures increase while the timing of the driest period moves earlier in the year (Fig. S3). These interacting changes in temperature and precipitation across the study region result in no future regions that are climatically analogous to that in the current ranges. That being said, future projections of niche models have major caveats, principal among them being that they assume fixed species environmental requirements through time (e.g., no plasticity or adaptation), that the environmental variables that define range limits currently will continue to be limiting in the future, and of course that climate projections are accurate (Sax et al. 2013). Lack of suitability is not synonymous with short-term extinction, as there is likely a large geographic range outside the realized niche where species can grow and survive, but not establish self-sustaining populations, often termed the "tolerance niche" (Sax et al. 2013). For *H. carnosus* and *P. tenuifolius*, there is some evidence that these species can grow and survive in regions outside their current ranges (e.g., outdoor experimental gardens in Athens, GA for *P. tenuifolius* but not *H. carnosus*; Bok Tower Gardens in Lake Wales, FL for *H. carnosus*), but no long-term self-sustaining populations are known outside the current ranges. While the current ranges may describe the current realized distribution of these species, and niche modeling can only approximate the fundamental niche, the data suggests that the fundamental niches of these species are not large and climate change is likely to reduce the ability of these species to maintain self-sustaining populations in the wild.

Recommendations for conservation under climate change

If there is indeed a primarily environmental origin of narrow endemism in *H. carnosus* and *P. tenuifolius*, either the identified variables or factors indirectly related to them, then these species are at risk from changing climate in their

small ranges (Warren 2012). Species that already occupy warm environments have been found to be especially at risk, as these species are likely near their thermal maximum and unlikely to be able to evolve tolerances to increasing temperatures (Araújo et al. 2013). The extinction risks posed by climate change have been acknowledged and incorporated into management plans for another endangered sunflower, *Helianthus paradoxus*, with high importance placed on mitigating long-term precipitation decline, which is a likely problem for both *P. tenuifolius* and *H. carnosus* as well (Povilitis and Suckling 2010).

As there is no projected suitable habitat outside the current ranges of these species, in situ conservation efforts are likely the most practical course, coupled with germplasm conservation in seedbanks and botanical gardens. Given the relative geographic homogeneity and low F_{ST} in *H. carnosus* and likely drift in multiple populations, admixture of seed sources might be a good idea for any planned reintroduction efforts. Admixture has been shown to increase the success of colonizing populations through increased genetic variation and likelihood of matches between genotypes and suitable new habitat in the short term, and adaptive responses in the long term (Rius and Darling 2014). Selection is unlikely to counteract genetic drift when population sizes are small, especially under 100 individuals, and populations dipping toward this size should be of particular concern to managers (Lacy 1987). This is especially true for self-incompatible species like these. In particular, ill-timed roadside mowing that reduces seed set and population size poses the most easily mitigated threat to *H. carnosus*, and should be a high priority for conservation managers. For *P. tenuifolius,* monitoring known populations for decline and local extinctions would be a valuable management priority, with possible reintroductions from similar genetic sources as appropriate. Hopefully this study will allow for better informed monitoring and selection of germplasm for conservation strategies, and the study of these narrow endemics will prove useful to our understanding of the origins of narrow endemism in the southeastern United States.

Data Accessibility

EST-SSR genotypes and other data used in this study are available in the Supporting Information. It should be noted that precise locality data are withheld for conservation reasons, as populations of *H. carnosus* and *P. tenuifolius* were identified primarily from herbarium records with normally redacted locality information and Florida Natural Areas Inventory records that are not available to the public.

Acknowledgments Financial support for this research was provided by a Society for the Study of Evolution Rosemary Grant Award to CMM, a Florida Native Plant Society Conservation Grant to CMM, a University of Georgia Innovative and Interdisciplinary Research Grant to CMM, a University of Georgia Department of Plant Biology Palfrey Research Grant to CMM, a Plant Biology Graduate Student Association Research Assistance Award to CMM, and National Science Foundation Grant IOS 1122842 to LAD and DBI 0820451 to JMB. The authors thank Eric Goolsby and Elise Wygant for assistance with field collections, Kirsten Scofield, Charmaine Woody, and Kristi Haisler for assistance with DNA extraction, Charlotte Carrigan Quigley for assistance with PCR and genotyping protocols, and the Donovan lab group for helpful comments on earlier versions of this manuscript. The authors also thank the Florida State Park Service, the Florida Department of Agriculture and Consumer Services, the Florida Department of Transportation, the Florida Fish and Wildlife Commission, the Flagler Estates Road and Water Control District, Morningside Nature Center, Crystal River Preserve, Apalachicola National Forest, Tate's Hell State Forest, St. Joseph's Bay State Buffer Preserve, and Box-R Wildlife Management Area for assistance with collection permits for both *H. carnosus* and *P. tenuifolius*. In addition, the authors thank Dr. Louisa Carter Staton for aggregated soil layers and helpful advice on niche modeling, as well as Dr. Loren Anderson for tissue samples of *P. tenuifolius* from herbarium records at Florida State University.

References

Anderson RP, Raza A (2010) The effect of the extent of the study region on GIS models of species geographic distributions and estimates of niche evolution: preliminary tests with montane rodents (genus Nephelomys) in Venezuela. J Biogeogr 37:1378–1393

Araújo MB, Peterson AT (2012) Uses and misuses of bioclimatic envelope modeling. Ecology 93:1527–1539

Araújo MB, Ferri-Yanez F, Bozinovic F, Marquet PA, Valladares F, Chown SL (2013) Heat freezes niche evolution. Ecol Lett 16:1206–1219

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Harvard

Beekman M, Ratnieks FLW (2000) Long-range foraging by the honey-bee, *Apis mellifera* L. Funct Ecol 14:490–496

Busch JW, Joly S, Schoen DJ (2010) Does mate limitation in self-incompatible species promote the evolution of selfing? The case of Leavenworthia alabamica. Evolution 64:1657–1670

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Earl D, vonHoldt B (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ (2011) A statistical explanation of MaxEnt for ecologists. Divers Distrib 17:43–57

Ellis JR, Pashley CH, Burke JM, McCauley DE (2006) High genetic diversity in a rare and endangered sunflower as compared to a common congener. Mol Ecol 15:2345–2355

Estill JC, Cruzan MB (2001) Phytogeography of rare plant species endemic to the southeastern United States. Castanea 66:3–23

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes:

application to human mitochondrial DNA restriction data. Genetics 131:479–491
Foré SA, Guttman SI (1999) Genetic structure of *Helianthus occidentalis* (Asteraceae) in a preserve with fragmented habitat. Am J Bot 86:988–995
Fry J, Xian G, Jin S, Dewitz J, Homer C, Yang L, Barnes C, Herold N, Wickham J (2011) Completion of the 2006 national land cover database for the conterminous United States. Photogramm Eng Remote Sens 77:858–864
Gevaert SD, Mandel JR, Burke JM, Donovan LA (2013) High genetic diversity and low population structure in Porter's sunflower (*Helianthus porteri*). J Hered 104:407–415
Gitzendanner MA, Soltis PS (2000) Patterns of genetic variation in rare and widespread plant congeners. Am J Bot 87:783–792
Hale ML, Burg TM, Steeves TE (2012) Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS One 7:e45170
Hamrick JL, Godt MJW (1996) Conservation genetics of endemic plant species. In: Conservation genetics (pp 281–304). Springer, New York
Heiser CBJ, Smith DM, Clevenger SB, Martin WCJ (1969) The North American sunflowers: *Helianthus*. Mem Torrey Bot Club 22:1–218
Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978
Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806
Kruckeberg AR, Rabinowitz D (1985) Biological aspects of endemism in higher plants. Annu Rev Ecol Syst 16:447–479
Lacy RC (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conserv Biol 1:143–158
Le Quéré C, Peters GP, Andres RJ, Andrew RM, Boden TA, Ciais P, Friedlingstein P, Houghton RA, Marland G, Moriarty R, Sitch S, Tans P, Arneth A, Arvanitis A, Bakker DCE, Bopp L, Canadell JG, Chini LP, Doney SC, Harper A, Harris I, House JI, Jain AK, Jones SD, Kato E, Keeling RF, Klein Goldewijk K, Körtzinger A, Koven C, Lefèvre N, Maignan F, Omar A, Ono T, Park GH, Pfeil B, Poulter B, Raupach MR, Regnier P, Rödenbeck C, Saito S, Schwinger J, Segschneider J, Stocker BD, Takahashi T, Tilbrook B, van Heuven S, Viovy N, Wanninkhof R, Wiltshire A, Zaehle S (2014) Global carbon budget 2013. Earth Syst Sci Data 6:235–263
Mandel JR, Dechaine JM, Marek LF, Burke JM (2011) Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annuus* L. Theor Appl Genet 123:693–704
Mandel J, Milton E, Donovan L, Knapp S, Burke J (2013) Genetic diversity and population structure in the rare Algodones sunflower (Helianthus niveus ssp. tephrodes). Conserv Genet 14:31–40
Mandel JR, Dikow RB, Funk VA, Masalia RR, Staton SE, Kozik A, Michelmore RW, Rieseberg LH, Burke JM (2014) A target enrichment method for gathering phylogenetic information from hundreds of loci: an example from the compositae. Appl Plant Sci 2:1300085
Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Hum Biol 76:173–190
Milanovich JR, Peterman WE, Nibbelink NP, Maerz JC (2010) Projected loss of a salamander diversity hotspot as a consequence of projected global climate change. PLoS One 5:e12189
Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/
Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590
Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155
Pashley CH, Ellis JR, McCauley DE, Burke JM (2006) EST databases as a source for molecular markers: lessons from *Helianthus*. J Hered 97:381–388
Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295
Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecol Model 190:231–259
Povilitis A, Suckling K (2010) Addressing climate change threats to endangered species in US recovery plans. Conserv Biol 24:372–376
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
Pruett CL, Winker K (2008) The effects of sample size on population genetic diversity estimates in song sparrows *Melospiza melodia*. J Avian Biol 39:252–256
Rius M, Darling JA (2014) How important is intraspecific genetic admixture to the success of colonising populations? Trends Ecol Evol 29:233–242
Sax DF, Early R, Bellemare J (2013) Niche syndromes, species extinction risks, and management under climate change. Trends Ecol Evol 28:517–523
Schilling EE (2001) Phylogeny of Helianthus and related genera. OCL-Ol Corps Gras Lipides 8:22–25
Schilling EE (2006a) *Helianthus carnosus*. In: Flora of North America Editorial Committee (ed) 1993+. Flora of North America North of Mexico. 16+ vols, vol 21. Flora of North America, New York, p 156
Schilling EE (2006b) *Helianthus radula*. In: Flora of North America Editorial Committee (ed) 1993+. Flora of North America North of Mexico. 16+ vols, vol 21. Flora of North America, New York, p 155
Schilling EE (2006c) *Phoebanthus tenuifolius*. In: Flora of North America Editorial Committee (ed) 1993+. Flora of North America North of Mexico. 16+ vols, vol 21. Flora of North America, New York, p 114
Schilling EE, Panero JL (2002) A revised classification of subtribe Helianthinae (Asteraceae: Heliantheae). I. Basal lineages. Bot J Linn Soc 140:65–76
Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234
Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS (2006) Comparative phylogeography of unglaciated eastern North America. Mol Ecol 15:4261–4293
Sorrie BA, Weakley AS (2001) Coastal plain vascular plant endemics: phytogeographic patterns. Castanea 66:50–82
Stebbins GL Jr (1942) The genetic approach to problems of rare and endemic species. Madroño 6:241–258
Steffan-Dewenter I, Kuhn A (2003) Honeybee foraging in differentially structured landscapes. Proc: Biol Sci 270:569–575
Stephens JD, Rogers WL, Mason CM, Donovan LA, Malmberg RL (2015) Species tree estimation of diploid *Helianthus* (Asteraceae) using target enrichment. Am J Bot (in press)
Štorchová H, Hrdličková R, Chrtek J Jr, Tetera M, Fitze D, Fehrer J (2000) An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. Taxon 49:79–84

Timme RE, Simpson BB, Linder CR (2007) High-resolution phylogeny for *Helianthus* (Asteraceae) using the 18 s–26 s ribosomal DNA external transcribed spacer. Am J Bot 94:1837–1852

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Wahlund S (1928) The combination of populations and the appearance of correlation examined from the standpoint of the study of heredity. Hereditas 11:65–106

Warren DL (2012) In defense of 'niche modeling'. Trends Ecol Evol 27:497–500

White GM, Boshier DH, Powell W (2002) Increased pollen flow counteracts fragmentation in a tropical dry forest: an example from *Swietenia humilis* Zuccarini. Proc Natl Acad Sci 99:2038–2042

Willi Y (2009) Evolution towards self-compatibility when mates are limited. J Evol Biol 22:1967–1973

Wills D, Hester M, Liu A, Burke J (2005) Chloroplast SSR polymorphisms in the compositae and the mode of organellar inheritance in *Helianthus annuus*. Theor Appl Genet 110:941–947

Yackulic CB, Chandler R, Zipkin EF, Royle JA, Nichols JD, Campbell Grant EH, Veran S (2013) Presence-only modelling using MAXENT: when can we trust the inferences? Methods Ecol Evol 4:236–243