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Lavanya Challagundla

Lisa E. Wallace
*Old Dominion University*, lewallac@odu.edu



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# PATTERNS OF GENETIC DIVERGENCE ACROSS GEOGRAPHICALLY VARIABLE POPULATIONS OF *XANTHISMA GRACILE* (ASTERACEAE)

Lavanya Challagundla* and Lisa E. Wallace[1],†

*University of Mississippi Medical Center, Department of Data Science, John D. Bower School of Population Health, Jackson, Mississippi 39216, USA; and †Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529, USA

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*Premise of research*. Numerous biotic and abiotic factors can contribute to local selection and lead to geographic structure and genetic divergence between populations. The southwestern United States contains many distinctive plant communities, ranging from woodlands to desert scrub, that are shaped by species adapting to local variation in elevation, precipitation, seasonality, and soils. Given this variation, species occurring across diverse habitats are expected to harbor high genetic diversity and exhibit significant genetic differences associated with environmental variation.

*Methodology*. Here, we studied the genetic divergence of populations of *Xanthisma gracile* (Asteraceae) across Arizona using amplified fragment length polymorphisms and evaluated associations between genetic structure, geographic distance between populations, and variation in climatic factors. This species occurs in desert grasslands at low altitudes as well as in open pine forests at intermediate altitudes and exhibits phenotypic variation in plant height, leaf shape and pubescence, and floral traits.

*Pivotal results*. We detected significant genetic structure across populations and found that a population from arid central Arizona is much more genetically distant than samples from northern and southern Arizona that occur in more mesic habitats. We also detected evidence for selection on numerous loci associated with variation in temperature and precipitation.

*Conclusions*. Major changes have occurred across the Southwest since the Last Glacial Maximum, and genetic divergence in *X. gracile* across Arizona likely reflects selection for survival in climatically diverse habitats.

*Keywords:* amplified fragment length polymorphism (AFLP), population genetic structure, *Xanthisma gracile*, climatic variation, genetic diversity.

## Introduction

Genetic variation allows for population survival and reproduction in new and changing environments (Barrett and Kohn 1991; Hamrick et al. 1991; Holsinger and Gottlieb 1991; Huenekke 1991). When local adaptation resulting from natural selection is stronger than gene flow or genetic drift, it can affect patterns of genetic differentiation in accordance with habitat variables (Wright 1951). As a result of local adaptation, species with wide distributions or occurring in heterogeneous habitats may be expected to have increased levels of genotypic and phenotypic variation compared with more restricted species or those in homogeneous habitats (Bradshaw 1984; Kawecki and Ebert 2004; Knight and Miller 2004; Bonin et al. 2006; Raabová et al. 2007; Kronholm et al. 2012). Additionally, large populations are more susceptible to the effects of local selection than those with a limited distribution (Leimu and Fischer 2008). As populations adapt to novel environments, ecotypes that are suited to the new conditions even if they are not necessarily reproductively isolated from each other may be formed (Clausen et al. 1948; Gibson and Pollard 1988). Thus, characterization of a species' genetic diversity and divergence patterns across geographic space can provide insight into understanding the distributional responses of species to changes in climate and other abiotic and biotic factors (Petit et al. 2003; Vandewoestijne et al. 2008; Kunin et al. 2009; Sepulveda-Villet and Stepien 2012). Species distributed in heterogeneous environments may be expected to exhibit high genetic diversity and associations between the degrees of population divergence and environmental heterogeneity (Ortego et al. 2012). One of the impacts of different selection pressures resulting from environmental heterogeneity is the driving of evolutionary divergence and speciation (Doebeli and Dieckmann 2003; Harter et al. 2015).

The southwestern United States underwent substantial change in climate and plant community structure in response to numerous glacial cycles throughout the Pleistocene. During the Last Glacial Maximum (LGM), the climate of the American Southwest was much cooler and wetter because of changes in the air currents that brought the westerlies south of their present location (Toggweiler et al. 2006). Arid lands were much more restricted

[1] Author for correspondence; email: lewallac@odu.edu.

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in distribution, and mixed conifer forests, chaparral, and grasslands were quite common in the Southwest (Betancourt 2004). Thus, species occupying deserts of the Southwest may have either survived in an arid refugium and subsequently migrated after the LGM or occupied forests and grasslands during the LGM and subsequently adapted to arid conditions. Empirical data testing these alternative hypotheses suggest multiple periods of evolutionary diversification during the Pleistocene and earlier in the Southwest. For example, Wood et al. (2013) found genetic splits in several faunal species of the Mojave and Sonoran Deserts that occurred before the major climatic changes of the Pleistocene, although some species appeared to have expanded their populations more recently in the Pleistocene. Likewise, Smith et al. (2011) identified strong divergence in Joshua trees (*Yucca brevifolia* Engelm.) and expansion into the Sonoran Desert around 200,000 years ago, with the species reaching its current distribution around 50,000 years ago. Lira-Noriega et al. (2015) found phylogeographic structure in *Phoradendron californicum* Nutt., a hemiparasitic plant in the Sonoran Desert and Baja California that occurred throughout the Pliocene and Pleistocene and was associated with geoclimatic events and changes in host distributions. Although these studies indicate temporal climatic heterogeneity in this region (Betancourt 2004; Toggweiler et al. 2006) and varied organismal responses (Smith et al. 2011; Wood et al. 2013; Lira-Noreiga et al. 2015) to these changes, they all support the expectation that species occurring in the American Southwest will have significant population genetic differentiation associated with climatic variation.

In this study, we examined population genetic diversity and structure in *Xanthisma gracile* (Nutt.) D. R. Morgan and R. L. Hartman (Asteraceae), which is a common species across the American Southwest (Hartman 2006). We examined genetic diversity at amplified fragment length polymorphism (AFLP) loci in populations across Arizona and tested the following hypotheses: (1) *X. gracile* maintains high genetic diversity because it is self-incompatible and is able to grow in variable habitats and (2) if populations are adapted to local habitat conditions, then they will be genetically divergent. We predicted that variation in climatic variables involving temperature and precipitation would be associated with the observed genetic polymorphisms among populations. Because AFLPs reflect variation at regions across a genome, they can be useful in identifying genomic regions that underlie adaptive population divergence (e.g., Wang et al. 2012; Yang et al. 2016). Thus, we tested for the presence of AFLP loci that exhibited both a strong degree of population divergence and a strong association with divergent climatic variables as evidence suggesting selection by climatic factors for different genotypes. Alternatively, in the absence of the local selection of populations, we expected that gene flow would more likely exhibit a pattern consistent with isolation by distance, which could be driven by constraints on pollinator and/or seed dispersal.

## Material and Methods

### Study System

*Xanthisma gracile*, also known as the yellow spiny daisy or slender goldenweed, is an annual self-incompatible species that is widely distributed in the American Southwest, including in Arizona, California, Colorado, Nevada, New Mexico, Texas, and Utah (USDA NRCS 2020), as well as in northern Mexico (Jackson 1960, 1962). Although chromosomally and morphologically divergent populations have been recognized within this species and occasionally as distinct taxa (Jackson 1965; Jackson and Crovello 1971), we (Challagundla and Wallace 2016) did not find evidence for genetically distinct groups that would suggest separate species. Thus, we treat all samples collected for this study as *X. gracile*. Dominant plant communities in the Southwest include grasslands, desert scrub, evergreen or pinyon-juniper woodlands, ponderosa pine and mixed conifer forests, and riparian wetlands (Bahre 1991). Within Arizona, *X. gracile* is a member of at least three plant communities, semidesert grasslands, desert scrub, and pinyon-juniper woodlands. These areas differ in climate, elevation, and community type (fig. 1). In southern Arizona, grasslands occur at elevations of 3000–5500 ft and are characterized by the presence of many shrubs. By contrast, central Arizona has arid desert scrub and includes much of the Sonoran Desert, which is characterized by upland sites and the presence of a wide variety of succulents. Small portions of the Mojave Desert occur in northwestern Arizona, and the Chihuahua Desert is in southeastern Arizona. Pinyon-juniper woodlands, common in northern Arizona, occur at elevations of 4000–7000 ft, and the density of the woodlands increases with elevation. Across these habitats of Arizona, *X. gracile* exhibits phenotypic variation that likely reflects resource availability and adaptation to differing habitat conditions (Matos 1979).

### Sampling and AFLP Data Collection

Individuals ($n = 243$) used in genetic analysis originated from 16 extant populations in Arizona. We focused on Arizona because there is variation in climatic conditions across the state (fig. 1) and previous studies identified differentiation in *X. gracile* populations across Arizona (Jackson 1960, 1973; Matos 1979). In each population, we sampled seeds and leaf tissue. Seeds were maintained in dry conditions at 4°C until germination, and leaf tissue was preserved at −80°C. We obtained samples from three geographic regions: eight populations from the north (woodland region), seven populations from the south (grassland), and one central population (desert scrub; fig. 1). GPS coordinates were determined at the time of sampling. Voucher specimens from each population are deposited at the Mississippi State University Herbarium (see table 1 for accession numbers).

We extracted genomic DNA from frozen leaf tissue or 10-d-old seedlings using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA) or a modified CTAB DNA extraction protocol (Doyle and Doyle 1987). After extraction, DNA was dissolved in 100 μL of TE buffer and run on a 1.5% agarose gel with ethidium bromide to check for quality. We performed AFLP analysis using a protocol modified from Vos et al. (1995) and incorporating the recommendations made by Trybush et al. (2006). Individual genomic DNA was digested in 30-μL reactions incubated at 37°C for 1 h in a thermal cycler, followed immediately by ligation of the linkers. Restriction digest enzymes and reagents used per 30-μL reaction were five units of EcoRI (New England BioLabs, Ipswich, MA), five units of MseI (New England BioLabs, Ipswich, MA), 1X NEBuffer 2, 0.0005 μg of BSA, and 5 μL of

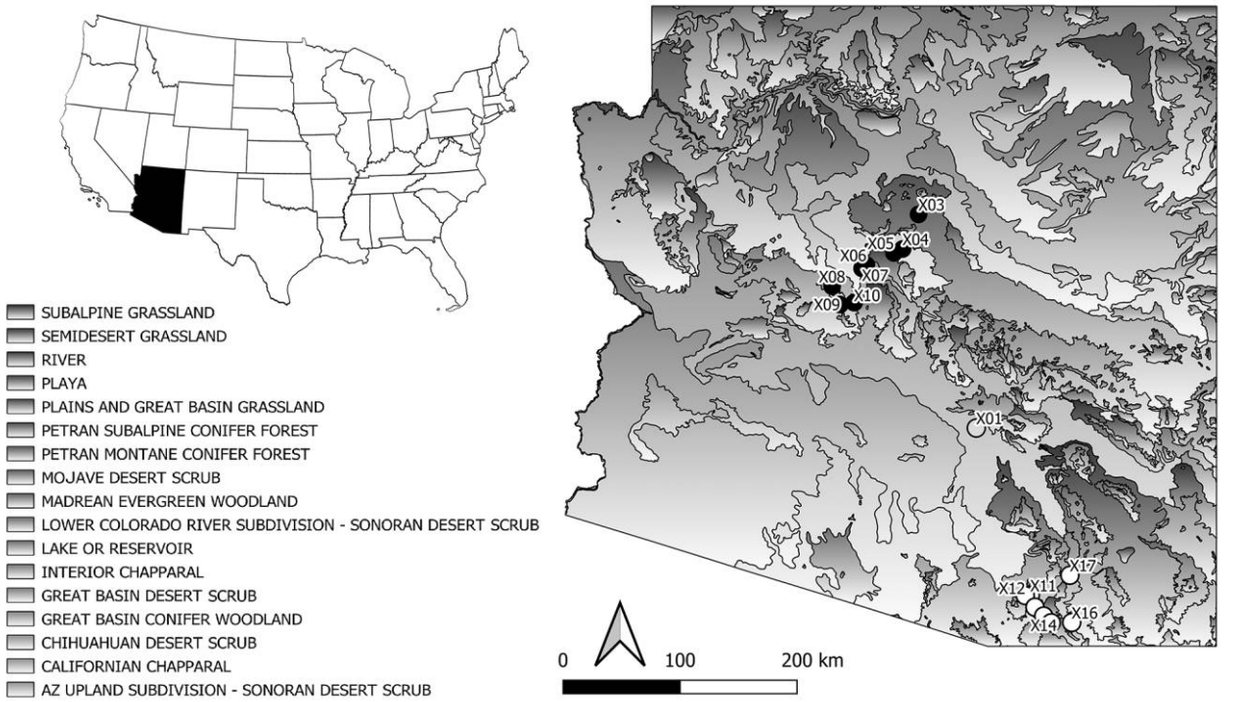


**Fig. 1** Locations of sampled populations of *Xanthisma gracile* overlaid on elevational variation across the study area. The central population is indicated by a gray circle, the northern populations by black circles, and the southern populations by white circles.

**Table 1**

**Population Names, GPS Coordinates, Sample Sizes, and Estimates of Genetic Diversity for Samples of *Xanthisma gracile* from Central, Northern, and Southern Areas of Arizona**

| Population | Lat. | Long. | *n* | %*P* | DW | *H* | *I* | Voucher[a] |
|---|---|---|---|---|---|---|---|---|
| Central: | | | | | | | | |
| X_01 | 33.26482 | −111.176 | 13 | 47.1 | .364 | .126 | .201 | LC1 |
| North: | | | | | | | | |
| X_03 | 35.1562 | −111.687 | 14 | 16.3 | .049 | .029 | .052 | LC3 |
| X_04 | 34.8498 | −111.828 | 23 | 53.2 | .166 | .094 | .164 | LC4 |
| X_05 | 34.81525 | −111.905 | 19 | 43.8 | .143 | .084 | .144 | LC5 |
| X_06 | 34.70784 | −112.149 | 11 | 13.1 | .037 | .032 | .052 | LC6 |
| X_07 | 34.67791 | −112.187 | 13 | 20.2 | .060 | .039 | .068 | LC7 |
| X_08 | 34.51982 | −112.452 | 21 | 55.3 | .189 | .092 | .164 | LC8 |
| X_09 | 34.35759 | −112.383 | 14 | 18.2 | .071 | .032 | .057 | LC9 |
| X_10 | 34.37462 | −112.258 | 19 | 44.4 | .141 | .083 | .145 | LC10 |
| North total | NA | NA | 133 | 91.5 | .107 | .074 | .147 | NA |
| South: | | | | | | | | |
| X_11 | 31.7841 | −110.697 | 20 | 48.4 | .192 | .096 | .163 | LC11 |
| X_12 | 31.78249 | −110.743 | 13 | 2.1 | .003 | .007 | .011 | LC12 |
| X_13 | 31.67921 | −110.656 | 15 | 17.2 | .041 | .029 | .051 | LC13 |
| X_14 | 31.60082 | −110.578 | 15 | 14.8 | .027 | .027 | .047 | LC14 |
| X_15 | 31.54105 | −110.512 | 12 | 13.8 | .058 | .045 | .069 | LC15 |
| X_16 | 31.54361 | −110.330 | 10 | 10.6 | .033 | .028 | .045 | LC16 |
| X_17 | 31.96148 | −110.347 | 12 | 5.3 | .009 | .013 | .022 | LC17 |
| South total | NA | NA | 97 | 60.2 | .052 | .045 | .092 | NA |
| Mean of all populations | NA | NA | 15.25 | 26.5 | .099 | .054 | .091 | NA |

Note. %*P* = percentage of polymorphic loci; DW = rarity index; *H* = Nei's gene diversity; *I* = Shannon index of diversity; NA = not applicable.

[a] Voucher specimens are deposited at the Mississippi State University Herbarium.

genomic DNA. Eco AFLP linkers were annealed in a thermal cycler by being heated to 65°C for 10 min and cooled to room temperature using 10 μL of 100-μM Eco Linker 1 (5′-CTC GTA GAC TGC CC) and 10 μL of 100-μM Eco Linker 2 (5′-AAT TGG TAC GCA GTC TAC). Mse AFLP linkers were also prepared using the above procedure using Mse Linker 1 (5′-GAC GAT GAG TCC TGA G) and Mse Linker 2 (5′-TAC TCA GGA CTC AT). Ligation of Eco and Mse linkers was conducted in 40-μL reactions including 0.1 μL of Eco and 1 μL of Mse linker (as annealed above), 0.15 μL of T4 DNA ligase enzyme and 4 μL of 10X T4 DNA ligase reaction buffer (New England BioLabs, Ipswich, MA), 30 μL of digested DNA, and 4.75 μL of sterile water. Ligation reactions were incubated in a thermal cycler at 37°C for 4 h, followed by storage at −80°C. Preselective amplifications were conducted in 10-μL reactions using 0.5 μM each of Eco+A (5′-GAC TGC GTA CCA ATT CA) and Mse+C (5′-GAT GAG TCC TGA GTA AC) primers, 1 mM of dNTP, 1X GoTaq Flexi Buffer (Promega, Madison, WI), 0.25 mM of $MgCl_2$ (Promega, Madison, WI), 2.5 μL of ligated DNA, and 0.1 μL of GoTaq DNA polymerase (5 U/μL, Promega, Madison, WI). Preselective amplifications consisted of an initial denaturing step for 5 min at 65°C, 30 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C. We diluted preselective amplification products 1:20 with sterile water before conducting selective amplifications with a single Mse primer and three fluorescent-labeled Eco primers per reaction. Selective amplifications were conducted in a 10-μL volume consisting of 0.5 μM of each Mse-CAG and 0.07 μM of each selective primer, Eco-ACT FAM, Eco-ACC NED, and Eco-AGG VIC, 0.4 mM of dNTP, 1X LongAmp Buffer (New England Biolabs, Ipswich, MA), 1 μL of diluted preselective amplification product, and 0.5 μL of LongAmp Taq DNA polymerase (5 U/μL; New England Biolabs, Ipswich, MA). The thermal cycler program included an initial denaturing step for 15 min at 95°C, 13 cycles of 30 s at 94°C, 1 min at 65°C, and 1 min at 72°C (reducing annealing temperature by 0.7°C per cycle), 25 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, and it finished with 10 min at 72°C. Each sample was diluted 1:10 with sterile water, and then 1 μL was mixed with LIZ-600 size standard (Life Technologies, Carlsbad, CA) before electrophoresis at the DNA Lab at Arizona State University. Peaks were visualized using GeneMarker (Softgenetics, State College, PA), and polymorphic bands were scored as present (1) or absent (0). Only bands in size ranges of 75–600 bp were used in data analyses. Four samples were consistently run on all plates to test for the repeatability of banding patterns.

### Data Analysis

The genetic diversity of populations was quantified by determining the percentage of polymorphic loci (%*P*), Nei's (1978) gene diversity (*H*), and Shannon index (*I*) using GenAlEx version 6.5 (Peakall and Smouse 2006, 2012). To estimate the frequency of rare bands, which accumulate over time and indicate old divergence (Schönswetter and Tribsch 2005), the rarity index (DW) was calculated using the R script AFLPdat (Ehrich 2006) in R version 2.15.0 (R Core Team 2012). Population genetic differentiation was assessed by analysis of molecular variance (AMOVA; Excoffier et al. 1992) using GenAlEx version 6.5 (Peakall and Smouse 2006, 2012). This analysis was conducted considering three hierarchical levels designated as regions (north pinyon-juniper woodlands, central desert, southern grasslands), populations within each region, and individuals within populations. We also calculated pairwise genetic distance ($F_{ST}$) between all populations in Arlequin version 3.5 (Excoffier and Lischer 2010). The significance of $F_{ST}$ values was assessed using a permutation procedure with 1000 iterations.

We conducted a Bayesian analysis of genetic structure using the software STRUCTURE version 2.3.4 (Pritchard 2000; Falush et al. 2007). The number of genetically distinct groups tested (*K*) ranged from 1 to 11, which was determined to be a suitable range of possible *K* after initial testing from 1 to 17 groups. We ran STRUCTURE using an admixture model with independent allele frequencies and population origin as prior information in the CIPRES Science Gateway (Miller et al. 2010). For each *K*, we conducted 10 runs with 100,000 generations after a burn-in period of 50,000 runs. Output files were processed through STRUCTURE HARVESTER (Earl and vonHoldt 2012) using the method of Evanno et al. (2005) to determine the largest $\Delta K$ or the most likely number of clusters. We averaged results across duplicate runs of the chosen *K* using CLUMPP (Jakobsson and Rosenberg 2007).

For each sampled population, we extracted data for 19 climatic variables from the WorldClim data set (Fick and Hijmans 2017) using 30-s layers and the point sampling tool in QGIS (QGIS Development Team 2020). A multivariate principal component analysis (PCA) was conducted to determine whether populations are separated by these climatic variables. Pairwise correlations of the climatic variables were calculated in SPSS version 27 (IBM 2020) using Pearson's coefficient. Variables that were positively correlated at >0.7 were considered redundant, and a single variable of the pair was retained for further analysis. This resulted in seven variables (mean diurnal range, temperature annual range, mean temperature in the wettest and coldest quarters, precipitation seasonality, and precipitation in the warmest and coldest quarters) that were used in linear stepwise regression analyses with measures of genetic diversity (%*P*, DW, *H*, and *I*) as dependent variables. A regression analysis was conducted for each genetic parameter using SPSS version 27 (IBM 2020).

We tested for strict isolation by distance using a Mantel test of population genetic versus geographic distances and for a correlation between genetic and environmental distances based on the climatic variables. Matrices of $F_{ST}$ and geographic distance (Euclidean distance) were calculated for all populations using GenAlEx (Peakall and Smouse 2006, 2012). A Euclidean distance matrix based on the seven retained climatic variables was created using PASSAGE 2 (Rosenberg and Anderson 2011). Mantel tests were performed in PASSAGE 2 (Rosenberg and Anderson 2011) with 999 random permutations to test for significance in a two-tailed test. A *P* value of 0.05 was used to assess the significance of the correlation. Mantel tests were conducted between genetic and geographic distance, genetic and environmental distance, and geographic and environmental distance. We also conducted partial Mantel tests between genetic distance and environmental distance while controlling for geographic distance.

We assessed evidence for selection on AFLP loci using Bayescan 2.1 (Foll and Gaggiotti 2008) and SamBada version 0.8.3 (Stucki et al. 2017). Loci for which the presence of a band was less than 3% or greater than 97% were removed before running

analyses, as these can influence the number of positively identified loci. In Bayescan, we used the default chain parameters and prior odds of 10. To determine loci exhibiting significant $F_{ST}$ and therefore considered to be under selection, a false discovery rate (FDR) of 5% was used. For the SamBada analysis, we used the same genetic data set, considered the seven climatic variables that were used in the regression, and selected the best models after Bonferroni correction at a $P$ value of 0.01.

## Results

A total of 754 polymorphic loci from three primer combinations were scored in 243 individuals. The repeatability of the bands for the multiple runs ranged from 96% to 98.5%. The percentage of polymorphic loci within a population ranged from 2.12% to 55.31%, with an average of 26.49% (table 1). Population X_01, from the central desert, exhibited the highest gene diversity ($H = 0.126$), followed by X_11 in the southern grasslands ($H = 0.096$). The southern region contained the population (X_12) with the lowest gene diversity ($H = 0.007$). Shannon's index of diversity exhibited similar trends across populations, and Nei's diversity and Shannon's index were highly correlated for this data set ($r = 0.99$). On the basis of the rarity index, population X_01 had a nearly twofold higher frequency of rare alleles (DW = 0.364) than any other population in the north or south region (DW range, 0.003–0.192; table 1). At the regional level, the northern populations exhibited higher diversity than the southern populations, but this was still lower than the diversity in the central population (table 1).

The AMOVA revealed that variation among regions ($\phi_{RT} = 0.073$, $P < 0.01$; table 2) is greater than among populations within regions ($\phi_{PR} = 0.045$, $P < 0.01$), although most of the variation occurs within populations ($\phi_{PT} = 0.115$, $P < 0.01$). Much of the difference among regions is due to the extreme divergence of the central population. Considering mean pairwise population $F_{ST}$, populations of the northern and southern regions were more closely related ($F_{ST} = 0.173$) than the central and northern populations ($F_{ST} = 0.315$) or the central and southern populations ($F_{ST} = 0.308$).

On the basis of $\Delta K$, the most likely number of genetic clusters is two (fig. 2). However, the results suggest admixture in several populations, especially in the central and northern regions. A total of 154 individuals (63%) are assigned to a single cluster with a probability of 0.90 or greater. The remaining individuals have varying degrees of assignment to the two clusters and are considered admixed.

The populations sampled across Arizona occur in distinct vegetation types and experience differences in precipitation and temperature. The mean elevation for the central, northern, and southern populations sampled is 771, 1711, and 1476 m. Correspondingly, the sampled areas are distinct in mean temperature (central, 20°C; northern, 13°C; southern, 16°C) and mean annual precipitation (central, 400 mm; northern, 533 mm; southern, 478 mm). On the basis of the full set of bioclimatic variables, the PCA supports the distinction among populations in environmental space (fig. 3). The central population is separated from the northern and southern populations along PC1, which largely reflects a difference in temperature, as the top three variables contributing to dispersion along this axis are annual mean temperature and mean temperature of the coldest and driest quarters. The northern and southern populations are separated along PC2 and reflect differences in precipitation (i.e., wettest month and quarter) as well as temperature seasonality. The first two PCs explain 87.5% of the observed variation in climatic variables among the sampled populations.

We did find significant associations between climatic variables and all genetic diversity measures in regression analyses. For %$P$, $H$, and $I$, precipitation in the warmest quarter was the sole significant variable in the regression analyses, whereas the model for DW included two significant variables, precipitation in the warmest quarter and temperature annual range (table 3). The $r^2$ values for each regression ranged from 0.370 to 0.613 (table 3).

Genetic distances were not significantly correlated with geographic distances ($r = -0.04$, $P = 0.27$), but they were significantly correlated with environmental distances ($r = 0.351$, $P = 0.01$). As expected, geographic and environmental distances were positively correlated ($r = 0.443$, $P < 0.001$). Thus, when controlling for geographic distance, the correlation between genetic and environmental distance increased slightly ($r = 0.41$, $P = 0.01$).

Tests of selection using Bayescan revealed seven loci with significant $F_{ST}$ when an FDR of 5% was considered (fig. 4). SamBada identified 11 loci that may be under selection, including five that were also identified by Bayescan. Among the 11 loci identified in SamBada, there was significant association with variation in three climatic variables, mean temperature in the wettest quarter (one locus), mean temperature in the coldest quarter (three loci), and precipitation in the wettest quarter (eight loci).

## Discussion

The distribution of genetic variation within and among populations of plants reflects life-history traits of species (Hamrick and Godt 1989, 1996), historical factors, and habitat preferences

**Table 2**

**Analysis of Molecular Variance for Populations of *Xanthisma gracile***

| Source | df | SS | MS | Estimated variance | Variation (%) |
|---|---|---|---|---|---|
| Among regions | 2 | 340.24 | 170.12 | 1.98 | 7 |
| Among populations | 13 | 532.04 | 40.93 | 1.11 | 4 |
| Within populations | 228 | 5448.20 | 23.90 | 23.90 | 89 |
| Total | 243 | 6320.48 | | 26.99 | 100 |

Note. The $P$ values for the following $\phi$ estimates are based on 9999 permutations: $\phi_{RT} = 0.073$, $P < 0.01$; $\phi_{PR} = 0.045$, $P < 0.01$; $\phi_{PT} = 0.115$, $P < 0.01$. The subscripts are defined as follows: P = population; R = region; T = total. MS = mean squared deviation; SS = sum of squares.

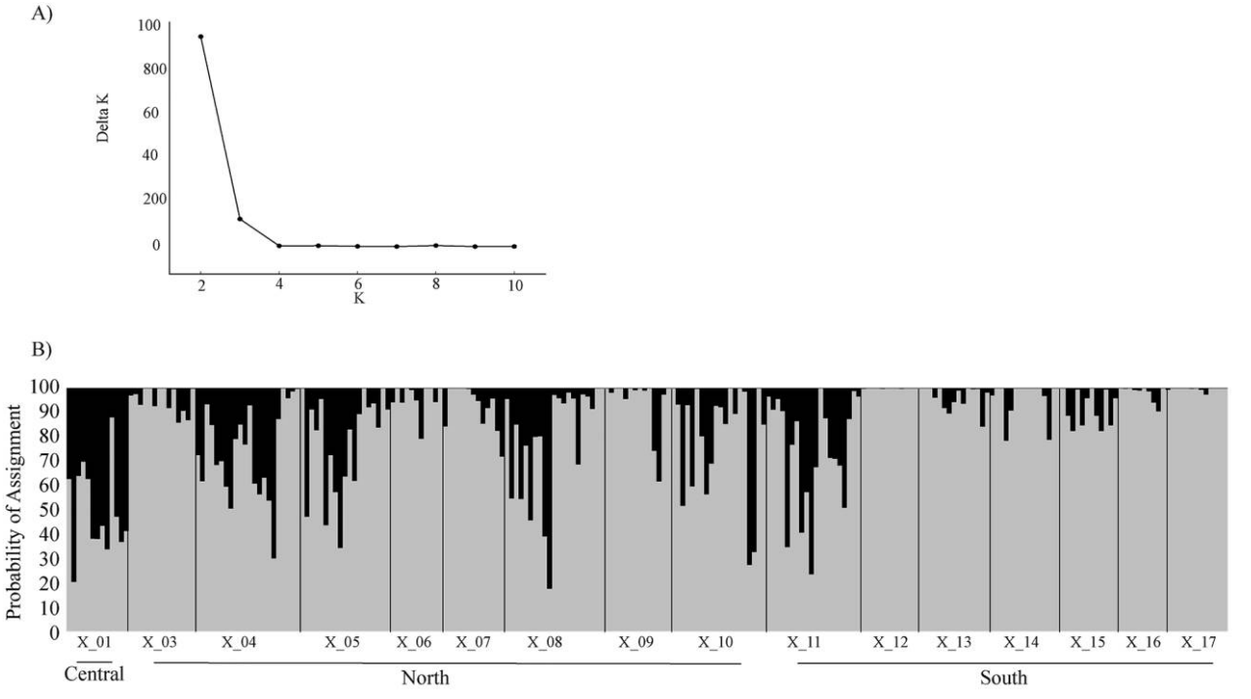


**Fig. 2** Clustering of sampled *Xanthisma gracile* individuals in a STRUCTURE analysis. *A*, According to the method of Evanno et al. (2005), a *K* value of two was selected as the most likely number of clusters. *B*, Probability of assignment to each of the two clusters for each individual. Population names are indicated below the *X*-axis and correspond to those shown in figure 1 and table 1.

(Vellend et al. 2000) that strongly influence gene flow. Because it is an annual self-incompatible species, we expected the genetic diversity of *Xanthisma gracile* to be high and comparable to that of species with similar life-history traits. Nybom (2004) found that estimates of population genetic diversity that are based on variation at dominant markers (e.g., random amplified polymorphic DNA [RAPD], AFLPs, intersimple sequence repeats [ISSRs]) were very similar and suggested that they may be directly compared. Thus, we consider genetic diversity in plants with life-history traits similar to those of *X. gracile* as well as other species of Asteraceae in evaluating support for our hypothesis. Nybom (2004) reported a mean value for $H$ as 0.13 for annual species, 0.21 and 0.22 for species with regional and widespread distributions, respectively, and 0.27 for outcrossing species. The mean genetic diversity for all surveyed populations of *X. gracile* is considerably less ($H = 0.054$) than mean values for species with similar life-history traits, suggesting that this species may be genetically depauperate. Only the central population (X_01) has population diversity comparable to estimates from other studies. Krauss (2000) suggested that outcrossing species generally have >75% polymorphic loci, whereas selfing or clonal species have <50%, but other studies suggest that very often locus polymorphism is <10% (Haldimann et al. 2003; Ramakrishna et al. 2004). The *X. gracile* populations varied widely in locus polymorphism from less than 10% to more than 50%, more in line with selfing or clonal species. Within the Asteraceae, population genetic diversity varies widely among species and likely reflects the diverse life-history strategies of the species. For example, Tremetsberger et al. (2003) reported only 24% polymorphic loci and gene diversity of 0.056 for *Hypochaeris acaulis* (Remy) Britton, a long-lived perennial in the Andes, whereas Nielsen et al. (2003) reported more than 75% polymorphic loci among populations of *Scalesia* Arn. species, which are endemic to the Galápagos Islands. Because dominant markers generally have greater potential for genotyping errors and homoplasy compared with microsatellites or single-nucleotide polymorphisms, use of this marker type may have contributed to the lower than expected population genetic diversity observed for *X. gracile*. Additionally, because annual species are frequently early-successional species, metapopulation dynamics may drive genetic diversity, and consequently, genetic drift could limit variation at any given site because of founder effects (Slatkin 1977; Pannell and Charlesworth 2000). The higher diversity of the central population may reflect an enhanced need for genetic diversity in a more stressful habitat (Markert et al. 2010).

We found a moderate level of population differentiation in *X. gracile* ($\phi_{ST} = 0.115$), slightly lower than values reported for other outcrossing species based on either RAPD markers (mean $\phi_{ST} = 0.27$) or microsatellites ($F_{ST} = 0.22$; Nybom 2004). In widely distributed species, the interaction between gene flow and exposure to varied selection pressures creates geographic structure. Limited gene flow combined with large distances between populations should increase genetic differentiation among populations (Slatkin 1977; Hutchison and Templeton 1999). Metapopulation dynamics are also expected to contribute to greater population differentiation (Wade and McCauley 1988). Within its geographic range, *X. gracile* occurs in a variety of habitats, and populations experience variation in climatic variables (figs. 1, 3) that could be expected to limit successful gene flow between them. Subsequent divergence of populations

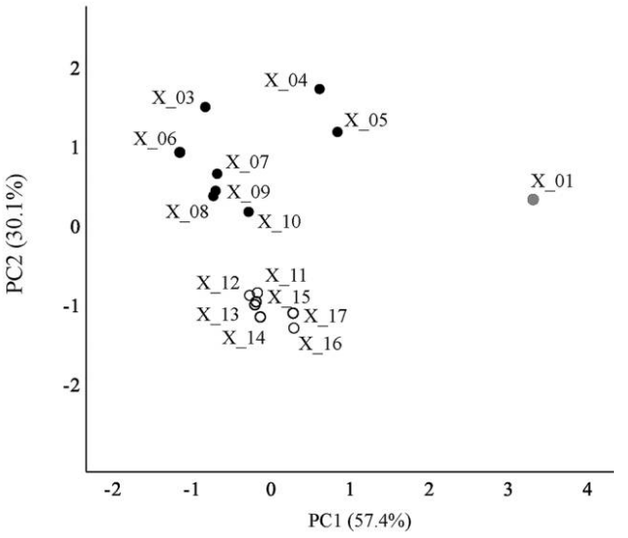


**Fig. 3** Distribution of sampled populations of *Xanthisma gracile* along two dimensions in a principal component analysis (PCA) based on 19 climatic variables. The first two dimensions explain 87.5% of the variation, with PC1 explaining 57.4% and PC2 30.1% of the observed variation among sample sites. The central population is indicated by a gray circle, the northern populations by black circles, and the southern populations by white circles.

could occur as a result of genetic drift in isolation or as a result of natural selection for certain phenotypic or physiological traits. The effect of geographic variation has been studied previously in a cytotype of *X. gracile* across four geographically isolated populations (Matos 1979). On the basis of the limited sampling and geographic range, the study concluded that both morphological and genetic divergence had taken place in those four isolated populations so that they could adapt to the different selection pressures imposed by the environment. It is unlikely that these differences reflect phenotypic plasticity because the growth and survival of two types of *X. gracile* were found to vary in response to variation in habitat conditions in both field and common-garden experiments (Monson and Szarek 1979, 1981). In those studies, plants from desert populations grown in a greenhouse had higher photosynthetic rates, entered a reproductive state faster, and allocated more biomass to reproductive structures compared with plants from mesic populations grown under the same conditions. Thus, variable selection pressures may contribute to the maintenance of genetic variation and enable this species to adapt to a broad spectrum of habitats, including the very arid conditions in central Arizona (Monson and Szarek 1979).

It is clear in this data set that populations of *X. gracile* exhibit genetic divergence, particularly the central population occupying arid habitats. From the analysis of diversity statistics (table 1), the central population exhibited higher diversity when compared with the northern and southern populations. The higher frequency of rare bands (DW) in population X_01 suggests that it has a long history of separation from other populations or strong localized selection. Central Arizona contains xeric habitats with upland desert scrub as the primary vegetation. The central region experiences higher mean temperature (>20°C) in comparison with the northern (13°C) and southern (16°C) areas of Arizona. The central region also experiences lower annual precipitation (~400 mm) than the other areas we examined in Arizona (>450 mm). Populations in extreme environments can diverge rapidly because of strong localized selection (Grant et al. 2017). We suggest that the evolution of *X. gracile* across Arizona is driven by selection from temperature and water stress given the significant results we found in this study between genetic variation and climatic factors involving temperature and precipitation. This study provides evidence for substantial differentiation of a population in central Arizona and, to a lesser degree, that the northern and southern populations are also diverging. Although only a single central population was surveyed in this study, data from Challagundla (2013) suggest that this is not simply an outlier population but represents a wider pattern of population divergence in xeric habitats compared with more mesic areas in other parts of the species range.

Extensive changes in climate and vegetation since the LGM have resulted in lasting changes to the genetic structure of plants and animals in the Northern Hemisphere (Hewitt 1996, 2001; Rebernig et al. 2010; Allen et al. 2012; Slovák et al. 2012). During the wetter and cooler pluvial period, the desert vegetation, as suggested by paleoclimatic and vegetation data, was strongly restricted to the lower Colorado River basin and the plains of the Sonoran and southern Chihuahua Deserts (Van Devender and Spaulding 1979; Van Devender 1990; Thompson and Anderson 2000; Hunter et al. 2001). Aridification began at the end of the LGM (McClaran and Van Devender 1995; Musgrove et al. 2001; Holmgren et al. 2007). Progressive drying resulted in

**Table 3**

**Significant Climatic Predictors of Four Measures of Genetic Diversity across *Xanthisma gracile* Populations in Arizona Identified Using Stepwise Regression Analyses**

| Dependent variable | Significant predictor | $\beta$ | $t$ | $r^2$ | $F$ | $P$ |
|---|---|---|---|---|---|---|
| $\%P$ | Precipitation in warmest quarter | −.609 | −2.870 | .370 | 8.238 | .012 |
| DW | Precipitation in warmest quarter, temperature annual range | −1.138, −.676 | −4.436, −2.634 | .613 | 10.311 | .002 |
| $H$ | Precipitation in warmest quarter | −.643 | −3.141 | .413 | 9.866 | .007 |
| $I$ | Precipitation in warmest quarter | −.644 | −3.148 | .414 | 9.908 | .007 |

Note. A single variable was retained in each analysis. Nonsignificant variables included annual mean temperature, annual precipitation, and precipitation in the wettest and driest months for percentage of polymorphic loci ($\%P$) and annual mean temperature, temperature seasonality, annual precipitation, and precipitation in the driest month for the remaining genetic diversity variables. DW = rarity index; $H$ = Nei's gene diversity; $I$ = Shannon index of diversity.

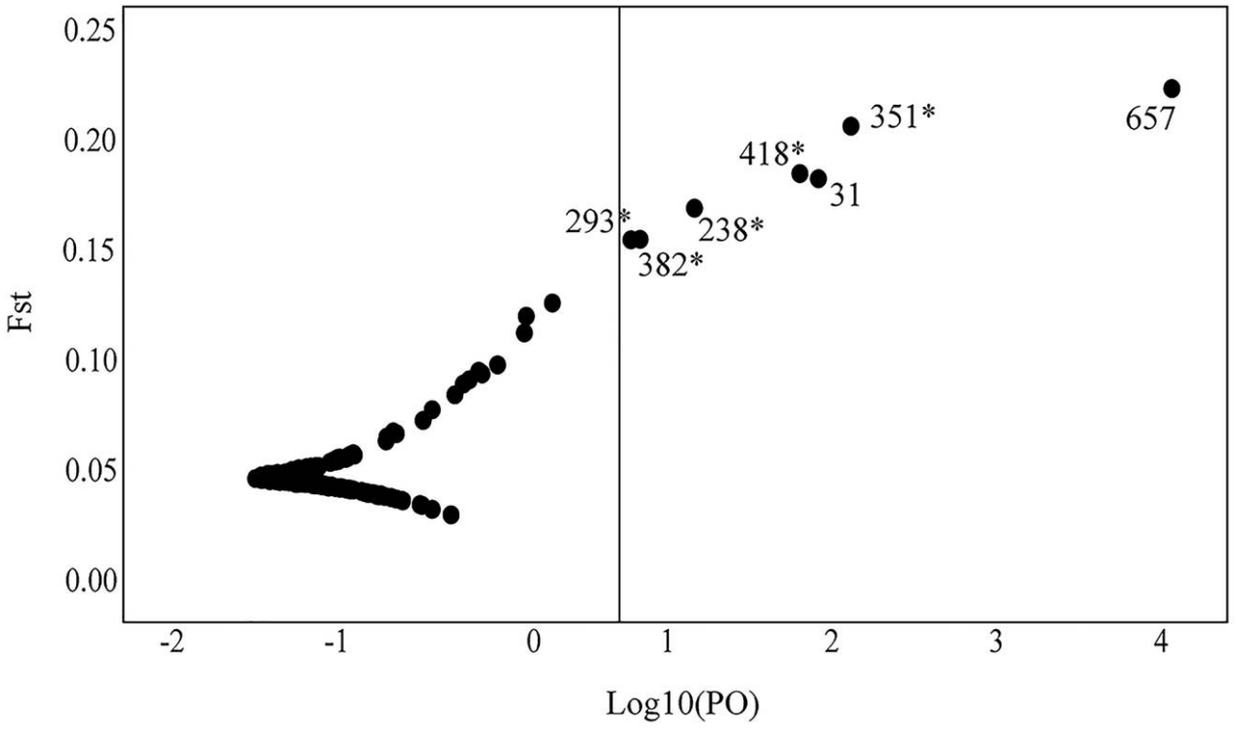


**Fig. 4** Plot of $\log_{10}$ probability against pairwise genetic distance ($F_{ST}$) values for all amplified fragment length polymorphism loci considered in the analysis of loci under selection conducted in Bayescan. The critical value at a false discovery rate of 5% is indicated by the vertical line. The seven loci identified as significant outliers are labeled. Loci also identified in SamBada as divergent are indicated by an asterisk. PO = posterior odds score.

the conversion of historical contiguous pinyon-juniper woodlands (Van Devender 1977) in the Southwest to semidesert grassland and eventually the desert scrub present today (Neilson 1986). Given these changes, populations of *X. gracile* in central and southern Arizona likely would have originated from those adapted to the climate of woodland habitats, which are found in northern Arizona today. These areas have lower mean annual temperature and higher levels of precipitation. Thus, selective pressure exerted by increasing temperature and lowering precipitation is predicted to underlie the observed differentiation of the central and southern populations from conspecific populations in northern areas of Arizona. Gene flow between differentially adapted populations is not expected to produce viable intermediates, which would serve to further reinforce the population divergence of *X. gracile* in Arizona. Although gene flow between northern and southern populations due to seed dispersal is not expected to be strong (Anderson 1992) because of the distance between them, they may retain similar diversity and diverge much more slowly because of more similar climatic conditions.

The AFLP data set suggests possible evidence of selection at the molecular level and that these outlier loci are associated with variation in climatic factors. We identified multiple loci in two independent analyses suggestive of the effects of selection or linkage to loci under selection. Five loci identified in both analyses are linked to variation in climatic factors involving precipitation and/or temperature. These results appear to be driven by the distinction of the central population because a comparison involving only the northern and southern populations resulted in no loci identified by Bayescan and three loci associated with mean temperature of the wettest quarter and precipitation of the warmest quarter identified by SamBada. These results suggest evolution associated with localized selection across Arizona. While we do not know the nature of the genomic regions containing the identified AFLP loci, these results still provide evidence of the potential for selection to have increased genetic divergence among populations of *X. gracile*, thereby supporting previous field and greenhouse studies that have also identified prominent differences in fitness across the geographical distribution of this species (Matos 1979; Monson and Szarek 1981).

*Xanthisma gracile* also occurs in the Mojave and Chihuahua Deserts of the Southwest. Thus, an alternative phylogeographic hypothesis is that extant desert lineages of *X. gracile* existed before the LGM, having originated from a common desert form whose habitat shrank during the Pleistocene changes that brought more widespread mesic conditions to the Southwest. Unfortunately, a lack of samples from other deserts of the Southwest precludes a definitive conclusion on this alternative origin. Phylogeographic studies of taxa spanning the deserts of the Southwest are not common, but some studies have found substantial divergence within populations across Arizona (Merrill et al. 2005) and between the Mojave and Sonoran Deserts (Fehlberg and Ranker 2009; Graham et al. 2013; Wood et al. 2013), suggesting that this area is likely to carry interesting stories of organismal divergence associated with Pleistocene climatic cycles.

### *Conclusions*

*Xanthisma gracile* populations occur in climatically diverse regions of the Southwest, including pinyon-juniper woodlands, semidesert grasslands, and desert scrub. We found genetic differentiation between populations in desert scrub and other habitats. Local habitat selection pressures are predicted to have contributed to the observed genetic differentiation, and we expect that populations will continue to adapt to a changing climate. These results support a scenario of the early divergence of populations in central Arizona from other areas, perhaps from a woodland form.

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