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PROGRAM

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Assessing Genetic Diversity of Gila Monsters in Nevada

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Dustin A. Wood[1] **and Amy G. Vandergast**[1]

[1] U.S. Geological Survey, Western Ecological Research Center. San Diego Field Station. 4165 Spruance Road, Suite 200. San Diego, CA 92101. Email: dawood@usgs.gov; avandergast@usgs.gov

Executive Summary
Understanding a species' population structure and contemporary genetic diversity can reveal environmental factors, both natural and anthropogenic, that affect genetic connectivity of populations and can help to inform conservation and management of the species. The Gila monster is a large venomous lizard native to the southwestern United States and northern Mexico. Given its reclusive behavior and patchy distribution throughout its range, the Gila monster is a notoriously difficult species to study and much remains to be understood regarding genetic population units, diversity, and landscape connectivity. We used restriction site-associated DNA sequencing to develop a genomic dataset for the Gila monsters. We sampled at the northern periphery of their range, with a focus on populations within the state of Nevada, and examined genetic structure, contemporary patterns of genetic diversity, and associations between genetic distance and landscape factors. Our results revealed evidence of moderate population structure throughout Nevada and Utah that partitioned populations into three regional clusters. The strong isolation by distance among populations suggests that historical genetic connectivity likely occurred via a stepping-stone pattern of movement and gene flow. In the two robustly sampled focal sites, genetic diversity and effective population size estimates were similar to a protected population from the core of the species distribution. Finally, we found that genetic differentiation in this part of the species' range appears strongly associated with habitat suitability, habitat fragmentation by presumed barriers to movement (highways and rivers) and clines in climate variables, particularly minimum temperature and annual temperature range. Whether genetic differentiation and climate associations may be of adaptive significance in these peripheral populations warrants further investigation with broader sampling of the species range.

Introduction

Habitat loss and fragmentation are among the largest threats to native populations. These impacts can significantly reduce species persistence and population resilience to future environmental change. Habitat fragmentation acts to reduce a species' ability to disperse across the landscape, altering natural movement patterns and landscape use, that can lead to increased risk of population decline, loss of genetic diversity, and extinction for native populations (Frankham 2005, Fischer and Lindenmayer 2007). Genetic diversity and population size are positively linked to population persistence and represent essential components for species genetic management and recovery programs (Frankham and Ralls 1998). Therefore, identifying populations that need to be prioritized for conservation requires, in part, understanding the species current genetic diversity and population structure (Petit et al. 1998). Furthermore, understanding how environmental factors, both natural and anthropogenic, affect genetic connectivity can assist in identifying important corridors for movement and gene flow and informing restoration efforts (e.g. Epps et al. 2007, Hagerty et al. 2011, Vandergast et al. 2013, Shryock, 2016).

Rapid urban expansion throughout the Mojave Desert has steadily increased over the last century (Hughson 2009). Within the state of Nevada, rapid growth has resulted in substantial loss of habitat and connectivity in the region (Leu et al. 2008, Webb et al. 2009). The Vegas Valley is the largest metropolitan area in the state and is home to the three largest cities within Clark County: Las Vegas, Henderson and North Las Vegas. Over the span of three decades population growth in Clark County tripled in size from 700,000 in 1990 to over 2 million in 2020 (US Census Bureau, 2020 Demographic profile data). As human population growth and urbanization are expected to continue to increase in the region, understanding how species move through this landscape can inform efforts to mitigate impacts to habitat connectivity.

The Gila monster is a large venomous lizard native to the southwestern United States and northern Mexico (Beck, 2005). Within the U.S., Gila monsters reach their northern most range limit in Nevada and southwestern Utah. Gila monsters in Nevada primarily occur in Clark County, with only small portions of their range extending into Nye and Lincoln counties. Despite inhabiting arid environments, these lizards do not tolerate high temperatures and can spend a majority (> 95 percent) of their time seeking refuge in underground shelters (Lowe et al. 1986, Beck 2005), making them among the rarest and most secretive animals in Nevada. Due to this elusiveness, too little is known about population size, structure, and genetic connectivity of populations to evaluate the long-term viability of the Gila monster throughout Nevada.

Here we estimate patterns of genetic diversity of Gila Monsters at the northern part of their range within the state of Nevada, using single nucleotide polymorphisms (SNPs). Our results can be used to inform future conservation management decisions. Our three major objectives were as follows: (1) describe overall genetic structure among sites sampled across Nevada and Utah, (2) describe contemporary patterns of genetic diversity and estimate effective population size for two focal sites in Clark County, Nevada, and (3) test for associations between genetic distance and landscape factors identified in species distribution models across Clark County, Nevada to determine features that may promote or impede genetic connectivity.

Methods and Materials

Genomic DNA Collection and NGS Library Preparation

Tissue and blood samples from Gila Monsters were provided to the Western Ecological Research Center (WERC) San Diego Field Station (SDFS) by the Nevada Department of Wildlife (NDOW) and other project collaborators. Opportunistic samples were collected throughout Nevada and Utah and were grouped into 22 geographic sites. Focused survey efforts were also used to collect robust samples at two sites (Site 16 and Site 20) within Clark County, Nevada to address genetic diversity objectives. The number of samples obtained per site ranged from 1 to 35 (Fig. 1, Table S1). We extracted genomic DNA at the San Diego Conservation Genetics Laboratory with Gentra Puregene Kits (Qiagen, Valencia, California) according to manufacturer's protocol with minor modifications, including cell lysis in the presence of Proteinase K and dithiothreitol (DTT), an overnight DNA precipitation step, and a 20-minute centrifugation step at 21,194g and 4° C.

We followed the double-digest restriction-associated DNA (ddRAD) sequencing protocol developed in Peterson et al.(2012) for Next Generation Sequencing (NGS) library preparation, with some modifications. We digested genomic DNAs using 20 units each of the restriction enzymes *Sbf*I and *Msp*I (New England Biolabs, U.S.A.) and used Agencourt AMPure beads (Beckman Coulter, Danvers, Massachusetts) to purify the digestions prior to ligating uniquely bar-coded adapters with T4 ligase (New England Biolabs). We quantified all ligation products on the Qubit fluorometer, pooled across 12 index groups in equimolar concentrations, and then size selected fragments between 415 and 515 base pairs (bp) using a Pippin Prep size fractionator (Sage Science, Beverly, Mass.). We amplified the recovered fragments from each pool using 5–10 ng of the recovered DNA, Phusion High-Fidelty *Taq* (New England Biolabs), and Illumina's primers. Polymerase chain reaction (PCR) products were then cleaned with Agencourt AMpure beads (Beckman Coulter) and quantified using the Qubit fluorometer (Life Technologies) and Bioanalyzer (Agilent Technologies, U.S.A.). SNP libraries were sent out for sequencing to the Genomics and Cell Characterization Core Facility (Eugene, Oregon) and Medgenome, Inc (Foster City, California).

Bioinformatics and Data Analysis

We filtered and selected datasets using the STACKS v.2.60 (Catchen et al. 2013) bioinformatics pipeline. We used the process_radtag program to clean and filter raw reads following default settings. We chose 25 samples from across the range with depth of coverage at least 50X to conduct initial parameter testing following the *R80* protocol detailed in Rochette & Catchen (2017). This involved examining a series of *de novo* RAD locus assemblies that used a range of values for the mismatch distance between loci within an individual (M), the number of mismatches between loci in the catalogue (n) from 1 to 8 (fixing $n = M$), and a range of values for the minimum stack depth (m = 3-5). The final set of parameters chosen for analyses (m = 3, M = 2, n = 3) was based on the total number of polymorphic loci shared by samples and how the distribution of SNPs per locus was affected. We ran the denovo_map.pl program within STACKS to build loci and call SNPs for each locus. All datasets were then generated using the *populations* program within STACKS, retaining only loci present in at least 80 percent of individuals and retaining SNPs after applying a minimum minor allele count of 3.

Genetic Structure

We evaluated population genetic structure with both parametric (model assumptions used) and non-parametric (no model assumptions) approaches. Our parametric approach involved the maximum-likelihood method of ADMIXTURE (Alexander et al. 2009) to estimate the most likely number of genetic clusters (K) given the data assuming Hardy-Weinberg Equilibrium (HWE) within clusters and absence of linkage disequilibrium among markers. We performed 10 replicate analyses to evaluate up to 6 genetic clusters. To assess the best value of K, we performed 10-fold cross-validation and determined the K values with the lowest cross validation error and examined the individual assignment plots. We also used Principal Component Analysis (PCA), a multivariate ordination method that evaluates the optimal number of genetic clusters and because it is a non-parametric approach does not require the assumption of Hardy-Weinberg Equilibrium (HWE) or unlinked markers. We performed PCA in R using adegent v. 2.1.5 (Jombart and Ahmed 2011). All population structure analyses were conducted on two different datasets, referred to as the 'rangewide' and 'focal'. To create the 'rangewide' dataset, we randomly selected 15 samples from Site 16 given the larger sample sizes at this site relative to all other sites; all other sites were included without further reduction. To further evaluate genetic structure between the two focal sites, Site 17 and Site 20, we created a reduced dataset that contained only individuals from these two sites with equal representation (n=17), and we refer to this as the 'focal' dataset.

Population differentiation was also estimated using the fixation index (F_{ST}) among sites that had at least three sampled individuals. Fixation indices were calculated in R using StAMPP v. 1.6.3 (Pembleton et al. 2013). This package calculates pairwise F_{ST} values along with confidence intervals and P-values (using 10,000 bootstraps) according to the methods described in Weir and Cockerham (1984).

Genetic Diversity

We used summary statistics from STACKS v. 2.60 to estimate genetic diversity within the two focal sites (Site 16 and Site 20) within Clark County, Nevada. Summary statistics included the following: number of private alleles (P), mean observed heterozygosity (Hobs), mean expected heterozygosity (Hexp), and nucleotide diversity (π). We estimated contemporary effective population size (Ne) using the linkage disequilibrium method (Waples and Do 2008) within the program NeEstimator v. 2.1 (Do et al. 2014) to obtain Ne values. Given that Gila monsters exhibit high site-fidelity (Beck and Jennings 2003), mating behavior is most likely non-random. Therefore, we estimated Ne using both random and monogamy mating systems. We calculated 95% confidence intervals for point estimates using the jackknife-across-samples method (Jones et al. 2016) and screened out rare alleles using a critical cut-off value (P_{crit}) of 0.05.

Landscape analyses

Landscape factors often shape genetic differentiation beyond geographic distance alone (Wang and Bradburd 2014). We used a multivariate approach, Multiple Regression of Matrices (MRM), to examine associations between pairwise genetic differentiation among individuals and

habitat and climate differences. MRM involves a multiple regression of a response matrix on multiple explanatory matrices, where each matrix contains distances or similarities between all pairwise combinations of units (here individuals and their collection locations, see Lichstein 2007). We tested for associations among genetic distance, habitat cost and least cost path distances (defined using a species distribution model provided by K. Nussear), roads and rivers as barriers, and differences in climate variables. The study area was limited to Clark County plus a 20 km buffer and the 78 individuals within this buffer (Fig. 1).

We calculated Nei's genetic distances (Nei 1972) among all pairs of individuals from the full dataset using StAMPP v. 1.6.2 with the stamppNeisD function in R (Pembleton et al. 2013). Euclidean distances, habitat cost and least cost paths among individuals were calculated in ArcMap v. 10.4.1 using the Landscape Genetics Toolbox (Etherington 2011). We created two barrier matrices, one for major highways and one for major rivers that extended from Lake Mead south along the Colorado River through Lake Mohave (Fig. 1). Pairs of locations across barriers were coded as 1 (isolated) or 0 (not isolated). Bioclimatic variables (30s, ~1km^2 resolution) were averaged across years from 1970-2000 (https://worldclim.org/data/worldclim21.html; accessed June 2021). After initial examination of cross-correlations among all 19 variables, we chose eight climate variables to include in testing: mean diurnal temperature range, isothermality, maximum temperature of the warmest month, minimum temperature of the coldest month, annual range in temperature, precipitation of the driest month, precipitation of the wettest month and seasonality in precipitation (Pearson's $r \leq 0.9$). Values were extracted for each collection point in ArcGIS v. 10.4.1, and Euclidean distance matrices for individual climate variables were calculated in Primer v.7.0.13 (Clarke and Gorley 2015) after normalization. Mantel tests for matrix correlations (Mantel 1967) and Multiple Regression of Matrices (MRM) models were performed in the R package ecodist v. 2.0.7 (Goslee and Urban 2007). MRM models were selected using stepwise elimination of variables that were found to be significantly positively correlated with genetic distance using single Mantel tests. Significance of tests was assessed with 1000 permutations.

Results and Evidence of Results

Data Quality

A total of 93 samples were sequenced, but 4 individuals had greater than 35% missing data and were removed prior to any downstream analysis. The final genomic dataset included a total of 89 individuals, with 79 individuals from Nevada (76 from Clark County; 3 from Lincoln County) and 10 individuals from Utah (Fig. 1; Table S1). The average coverage per sample was 85.5x (range 24-110x) and missing data across all loci was 8.4%. The full dataset consisted of 2004 loci with a total of 2585 polymorphic SNPs. For most analyses, we further restricted the data set to only a single random SNP per RAD locus to avoid linkage disequilibrium (Andrews et al. 2016) for a total of 2004 SNPs. Raw data are accessible as Short Sequence Read Archive (NCBI submission link provided upon acceptance). Additional summary statistics per individuals are found in Supplemental Table 1. Genotype calls and datasets are available in Dryad (https://doi.org/10.5061/dryad.w3r2280ss) and sampling locality information are available in Zenodo (https://zenodo.org/record/6342440#.Yil3SRPMLUI; note access currently restricted to protect species).

Genetic Structure

Admixture analyses using the rangewide dataset supported three genetic clusters across Nevada and Utah, and grouped samples into Utah, Central Nevada, and Southern Nevada regional clusters throughout the sampled range (Fig. 2a). Assignment probabilities among some individuals were admixed between the Utah and Central Nevada clusters, with individual posterior probability of assignment increasing to the Utah cluster as latitude increased. PCA analysis of the rangewide dataset revealed structuring among three main groups, similar to the Admixture results (Fig. 2b). The first principal component (PC) axis (7.46%) separated northern latitude samples within Utah and northern Nevada from central and southern latitude samples within Nevada, and the second PC axis (5.27%) separated central Nevada samples and southern Nevada samples. Several individuals from mid-latitudes were structured intermediate between these three groups (Fig. 2b), these individuals also had admixed posterior assignment probabilities in the Admixture analysis.

We further evaluated genetic structure between the Site 16 and Site 20 using the focal dataset. Admixture analysis marginally supported two genetic clusters (K = 2 cross validation 0.4688; K = 1 cross validation 0.4693). At K=2, most samples were assigned to their respective sites with 0.9 assignment probability (Fig. 3), but a few Site 16 samples had probability assignments well below 0.7. PCA analysis separated samples from the two sites along the first PC axis (12%), with differences among individuals within sites revealed along the second axis (6%). Global F_{ST} among the sites sampled based on the rangewide dataset was 0.098. Genetic differentiation as measured by the fixation index (F_{ST}) was 0.078 (0.071 - 0.086) between the focal sites (Site 16 and Site 20). Pairwise F_{ST} estimates between all sites with at least 4 samples were significant and ranged from 0.014 to 0.182 (Table 1).

Genetic Diversity

We found similar levels of genetic diversity among focal sites, although estimates were slightly higher at Site 20. Private alleles were also 1.8 times higher at Site 20 than Site 16. Effective population size (N_e)estimates at Site 16 were ≤ 70, with 95% confidence intervals ranging from 20 to 167 (Table 2). N_e point estimates at Site 20 ranged between 134 – 278, but the upper confidence interval included infinity regardless of the mating system assumed., suggesting that not enough information was available in the sample. To evaluate whether the low sample size at Site 20 resulted in infinite upper confidence intervals, we reduced the number of samples to Site 16 to the same number of individuals (n=17) as Site 20. This analysis recovered higher point estimates at Site 16 (N_e = 171 – 345) when the dataset was reduced, but both estimates included an infinite upper CI, indicating that there was not enough information to obtain a reliable estimate when sites were reduced to only 17 individuals.

Private alleles among the regional clusters ranged from 11 (North Nevada admixed sites) to 59 (Southern Nevada cluster), with equal numbers within the Utah and Central Nevada clusters (21 and 24, respectively). All other genetic diversity estimates were highest within the Southern Nevada cluster and second highest within the northern Nevada admixed group.

Genetic Habitat and Climate Associations

There were strong signals of genetic isolation by Euclidean and habitat cost distances at the individual level, with the strongest correlation with least cost path distance through suitable habitat (Table 3; Fig. 4). In addition, both habitat barriers (highways and the Colorado River) and all tested climate variables were significantly correlated with genetic distance when examined individually (Table 3; Fig S1). The final multiple regression model retained habitat least cost path distance (LCPdist), minimum temperature of the coldest month (Tmin), annual temperature range (Tar), highways and the river as significant variables associated with genetic differentiation across Clark County (Table 3; Figs. 4-6). These results suggest that climate, particularly temperature, and habitat connectivity are important factors associated with genetic differentiation in the Gila Monster.

Discussion

Genomic data were collected from the most northern periphery of the range of Gila monsters to provide insight into patterns of connectivity, genetic diversity and size among populations that inhabit this region, with a particular focus on Clark County, Nevada. We developed a robust genomic dataset with over 2000 nuclear SNP markers to assess genetic patterns. Our results revealed evidence of moderate population structure throughout Utah and Nevada that partitioned populations into three regional clusters with isolation by distance. Genetic diversity metrics were highest in the southern Nevada cluster and in an admixed region in northern Nevada. We found that genetic differentiation in the Clark County portion of the species' range appears strongly associated with suitable habitat, habitat fragmentation by presumed barriers to movement (highways and rivers), and clines in climate variables, particularly temperature (minimum temperature of the coldest month and annual temperature range). Below we detail these findings and discuss their implications for management.

Regional Population Structure

Knowledge of the evolutionary history and the distribution of genetic variation across a species' range can inform conservation and management. Genetic data can reveal patterns of population connectivity, unravel evolutionary histories, and help to determine appropriate geographic boundaries for management of unique genetic lineages and populations of high diversity (Waples and Gaggiotti 2006; Carroll et al. 2010; Doak et al. 2015). While Gila monsters are a conspicuous component of the desert southwest, their notoriously secretive nature make them a difficult species to study (Beck 2005), and genetic investigations involving this species are limited. Douglas et al. (2010) used nuclear and mitochondrial genes to study the evolutionary history and diversification of the Beaded lizard (*Heloderma horridum*) and Gila monster (*Heloderma suspectum*). Diversification of *Heloderma horridum* lineages (the sister lineage to Gila Monsters) began during the Miocene (9.7 million years ago, mya), at the time when North American deserts had become drier and more subtropical. However, Gila monster (*H. suspectum*) diversification was much more recent, estimated to have occurred during the Pleistocene (2 mya). Douglas et al. (2010) also recovered low levels of genetic diversity across the range of the Gila monster and lacked any phylogenetic signal to support monophyletic clades

consistent with subspecific taxonomy. Taken together, regional population structure across the range of Gila Monsters was presumed unlikely.

Our study focused on the northern periphery of the Gila monster range and results from genetic clustering analysis (Admixture and PCA) support three regional groups throughout Utah and Nevada. The Utah cluster was composed of lizards that inhabit the extreme southwestern region of Utah, while lizards across Nevada were grouped into the two remaining clusters. The Central Nevada cluster was restricted to lizards sampled from just east of Las Vegas (Site 16 and Site 13) while the Southern Nevada cluster comprised a broader range of sites (Sites 20-22) in southern Clark County. Nonetheless, the most notable pattern was the broad range of admixture (mixed genetic ancestry) among individuals sampled throughout central Clark County that suggests widespread historical connectivity across this region. This is particularly true for lizards centered around the Vegas Valley and northeastern regions of Nevada (northern Clark and Lincoln counties), where admixture between and among sites in this region was so extensive that little distinction can be made among them. Strong isolation by distance among populations suggests that historical genetic connectivity likely occurred via a stepping-stone pattern of movement and gene flow. Combined with the current understanding of limited above ground activity, limited home range size and short movement distances (see Geinger et al. 2021), genetic connectivity was likely a result of continued occupancy and limited individual movements over generations in areas of suitable habitat, similar to that described in Mojave desert tortoises (Dutcher et al. 2020; Hagerty et al. 2011). Movement and dispersal in Gila monsters may also be sex-biased. Males have been reported to move farther and have larger home range sizes than co-occurring females (Kwiatkowski et al. 2008). Movement and genetic connectivity have likely been reduced by loss and fragmentation due to recent urbanization, and other development. Highways, for example, are significantly associated with greater genetic differentiation among individuals across Clark County, even after accounting for other factors such as distance through habitat and differences in climatic conditions throughout the range.

Where our sampling density was the highest (focal sites: Site 16 and Site 20), most samples were assigned to their respective sites with high assignment probability. However, a few individuals from Site 16 had admixed assignment probabilities providing some signal of recent genetic connectivity. Historically, based on patterns of habitat suitability derived from the SDM (K. Nussear), a wide band of habitat likely connected these areas directly through Spring Valley, Las Vegas, Paradise and Henderson. The majority of this area is now developed. Remaining open space with suitable habitat between these two sites is constrained to a relatively narrow corridor south of the city, that extends along the Calico and Blue Diamond hills southeast to North McCullough and Sloan Canyon Wilderness Areas. This region could be of importance for continued occupancy surveys and maintenance or enhancement of habitat to support Gila monsters to retain gene flow. This corridor is bisected by Highway 160 and Interstate Highway 15, a major highway route along the Vegas Valley corridor. These roads likely represent barriers to connectivity in this region. There is limited published information on urbanization and road impacts on Gila monsters. One study in the Phoenix area found that Gila monsters readily crossed narrow roads, and did not detectably alter movement rates or home range size in more highly urbanized study sites (Kwiatkowski et al. 2008). However, this same study reported female-skewed sex ratios in urbanized areas and suggested that higher mortality rates associated

with higher movement rates in males could contribute to this pattern. Another study of road kill near Saguaro National park found that Gila monster road mortalities were correlated with traffic density (Paredes 2017). Existing culverts or other undercrossing structures and fencing along major roads and highways between the Site 16 and Site 20 could be assessed to better understand if existing structures are useful or could be enhanced to support regional connectivity.

Genetic Diversity

Effective population size (N_e) is an important parameter for conservation management because it provides a way to quantify the amount of change in finite populations caused by genetic drift (chance loss of alleles through time) and inbreeding (Frankham et al., 2017). Thus, effective population size provides a measure of the ability of populations to maintain genetic diversity over future generations. The contemporary effective population size estimates at Site 16 were ≤ 70, with 95% confidence intervals ranging from 20 to 167. These estimates fall within the recommended range (N_e = 50 – 100) to avoid short-term inbreeding effects (Franklin 1980, Frankham et al. 2014), and are consistent with previous estimates of effective size (95% CI ranged from 78-137) from a protected population located in the core of the species range at Saguaro National Park, Arizona (Farrar et al. 2017). Although effective size estimates at Site 20 resulted in confidence intervals that included an infinite upper bound, this site exhibited higher estimates of genetic diversity than Site 16 using other diversity indices (heterozygosity, nucleotide diversity, private alleles; Table 2) which suggests that effective population size is likely similar, if not larger, to the estimates we obtained at Site 16. The lack of an upper bound in the confidence interval for Site 20 suggests that the sample size (N = 17) was too small (see Waples and Do, 2010), a deficit that could be improved with additional sampling. Overall, our results highlight the value of continued population monitoring across the state to better understand population sizes and densities and how they are affected by landscape connectivity.

Climate Associations

Protecting populations on the periphery of a species range, where environmental conditions potentially leverage more influence on population dynamics than in central portions of the range, may be beneficial to safeguard evolutionary processes that are likely to generate future evolutionary diversity (Lesica and Allendor 1995). Our landscape genomic results suggest that climate, in particularly temperature, was an important factor associated with genetic differentiation in Gila monsters in Nevada. The association of genetic diversity with climate is consistent with what is known of the narrow thermal tolerances of Gila monsters. Much of their time is spent seeking refuge in underground shelters to escape heat and potentially prevent high rates of water loss (Beck 1990; Beck and Jennings 2003). Previous work on thermal tolerances of Gila monsters has emphasized the role of high temperature extremes and heat avoidance (Bogert and Martin del Camp 1956, Beck 1990, Gienger 2003, 2009, Gienger et al. 2013). Our results suggest that temperature range and minimum temperature may also drive aspects of genomic differentiation and possibly regional adaptation linked to genetic clusters in our study area. In particular, sites comprising the southern NV cluster appear to have lower annual temperature ranges and higher minimum temperatures than those in northern Nevada and Utah (Figs. 5 and 6). Overall, the annual temperature range (Tar) differed among sites by a maximum of 5° C (Tar

range = 34.8 – 39.8° C), and minimum temperature of the coldest month (Tmin) differed among sites by a maximum of 6.7° C (Tmin range = -2.5 – 4.2° C). Whether these genetic differences and climate associations may be of adaptive significant warrants further investigation with broader sampling in other portions of the species' range.

Management Implications

The work here is intended to inform developing management plans for this species, specifically by providing baseline genomic metrics for future monitoring of population status and guiding effective mitigation measures to maintain species viability. In the two most robustly sampled focal locations, genetic diversity and effective population size estimates are generally similar to those in the protected area of Saguaro National Park. However, sample numbers are currently too low to estimate local effective population sizes in sampling sites in northeastern Clark County. A better understanding of the abundance and density of Gila monsters throughout Clark County could help identify additional robust population centers. Survey efforts could be focused using habitat suitability and evidence of past genetic connectivity and admixture. This could be particularly relevant in northeastern Clark County, through the region encompassing the Moapa Valley, Valley of Fire, Muddy Mountains and Gale Hills, and west of Interstate Highway 15 through the Dry Lake Range (sites 9, 10, 12, and 14). This region is still relatively free from urban development and appears to be a region of high historical admixture between the northernmost Utah cluster and the Nevada genetic clusters. Additional blood collection and genetic sampling efforts (approx. 20+ individuals per population center) could provide more robust estimates of N_e in these locations. Evidence presented here that roads are associated with greater genetic divergence, together with previous studies of road mortalities and links between sex ratio and urban development, support efforts to identify corridors and structures for highway avoidance and crossings. If future translocation efforts are deemed warranted by managers, information provided here on the three major genetic clusters, could be used to provide appropriate source sites within the same regional genetic cluster and with similar climatic conditions.

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Tables

Table 1. Pairwise fixation index (Fst) among sites with at least three samples. Fst = fixation index calculated using StAMPP v.1.6.3 (Pembleton et al., 2013); Lower and Upper = lower and upper confidence intervals of the fixation index; P values = p-value using 10,000 bootstraps, significance assessed with Bonferroni correction of alpha value ≥ 0.00179 (28 tests).

Pairwise site comparison	Fst	Lower CI	Upper CI	P value
Site6 vs Site5	0.059	0.039	0.079	0.0000
Site6 vs Site10	0.091	0.073	0.108	0.0000
Site6 vs Site12	0.102	0.085	0.120	0.0000
Site6 vs Site16	0.153	0.137	0.169	0.0000
Site6 vs Site20	0.108	0.095	0.122	0.0000
Site6 vs Site21	0.108	0.090	0.127	0.0000
Site6 vs Site22	0.152	0.133	0.171	0.0000
Site5 vs Site10	0.108	0.087	0.130	0.0000
Site5 vs Site12	0.139	0.118	0.160	0.0000
Site5 vs Site16	0.169	0.152	0.187	0.0000
Site5 vs Site20	0.137	0.122	0.153	0.0000
Site5 vs Site21	0.144	0.125	0.164	0.0000
Site5 vs Site22	0.182	0.162	0.203	0.0000
Site10 vs Site12	0.026	0.011	0.042	0.0001
Site10 vs Site16	0.092	0.080	0.104	0.0000
Site10 vs Site20	0.079	0.068	0.091	0.0000
Site10 vs Site21	0.066	0.049	0.083	0.0000
Site10 vs Site22	0.129	0.111	0.147	0.0000
Site12 vs Site16	0.058	0.047	0.069	0.0000
Site12 vs Site20	0.051	0.041	0.061	0.0000
Site12 vs Site21	0.046	0.031	0.062	0.0000
Site12 vs Site22	0.097	0.082	0.113	0.0000
Site16 vs Site20	0.078	0.071	0.086	0.0000
Site16 vs Site21	0.086	0.073	0.098	0.0000
Site16 vs Site22	0.138	0.125	0.152	0.0000
Site20 vs Site21	0.014	0.006	0.022	0.0004
Site20 vs Site22	0.072	0.062	0.083	0.0000
Site21 vs Site22	0.053	0.040	0.067	0.0000

Table 2. Diversity statistics for the two focal sites (Site 16 and Site 20). N = number of individuals, P = private alleles, Hobs = mean observed heterozygosity, Hexp = mean expected heterozygosity, π = mean nucleotide diversity, and Ne = effective population size estimate using random and monogamy mating systems. Jackknife on loci was used to estimate upper and lower confidence intervals, 'Inf' indicates an estimated confidence interval of infinity.

Sites	**N**	**P**	**Hobs**	**Hexp**	$\boldsymbol{\pi}$	**Ne (random)**	**Ne (monogamy)**
Site 16	30	198	0.292	0.252	0.257	34 (20 - 83)	70 (41 - 167)
Site 20	17	292	0.315	0.275	0.275	134 (18.4 - Inf)	278 (38 - Inf)

Regional Clusters	**N**	**P**	**Hobs**	**Hexp**	$\boldsymbol{\pi}$
Utah (Blue)	10	21	0.265	0.234	0.248
North NV (Admixed)	15	11	0.279	0.257	0.267
Central-NV (Yellow)	35	24	0.270	0.237	0.241
Southern-NV (Green)	29	59	0.287	0.259	0.264

Table 3: Mantel test and Multiple Regression of Distance Matrices for individual pairwise genetic distances across the Clark County study area.

Mantel Test			
Among Individuals		**Mantel R**	**p-value**
Nei's GD	Euclidean Dist	0.684	0.001
	Habitat Least Cost Path Dist	0.694	0.001
	Habitat Cost Dist	0.662	0.001
	Tmax	0.384	0.001
	Tmin	0.150	0.005
	Tdr	0.448	0.001
	Tar	0.556	0.001
	PrecipW	0.135	0.015
	PrecipD	0.247	0.001
	PrecipS	0.341	0.001
	Highways	0.606	0.001
	Lake&River	0.303	0.001

Multiple Regression of Matrices			
Full Range	**Variable**	**Coef.**	**p-value**
Model : GD~LCPDist +Tmin+Tar+Hwys+'Lake&River'			
	LCPDist	0.380	0.001
	Tmin	0.128	0.002
	Tar	0.130	0.004
	Highways	0.192	0.015
	Lake&River	0.202	0.020
		R^2 = 0.517	**0.001**

Figures

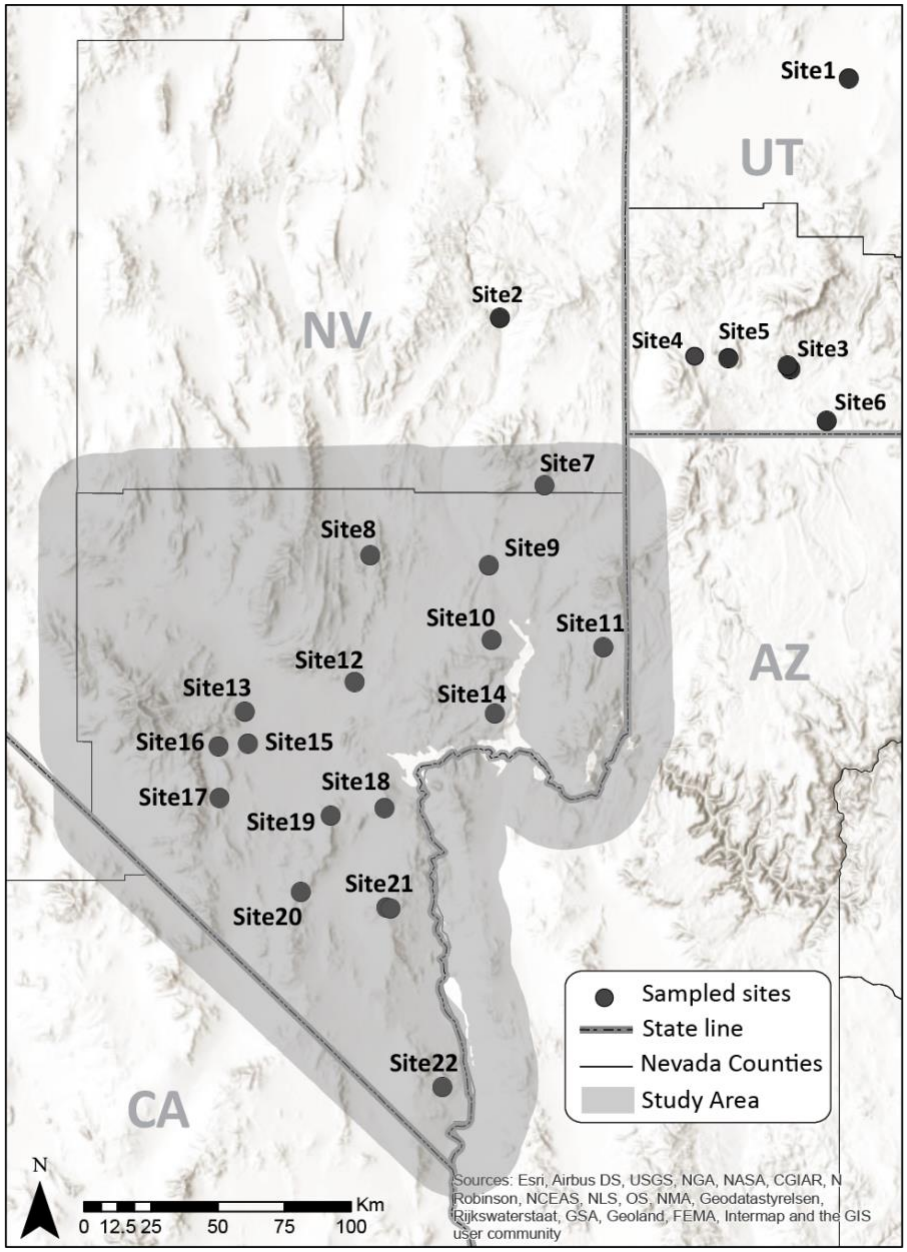

Figure 1. Map of sites where genetic samples were collected across Nevada and Utah. The grey polygon outlines the study area used in the landscape analyses, which encompassed all of Clark County, Nevada plus a 20 km buffer. More detailed site information is provided in Tables S1.

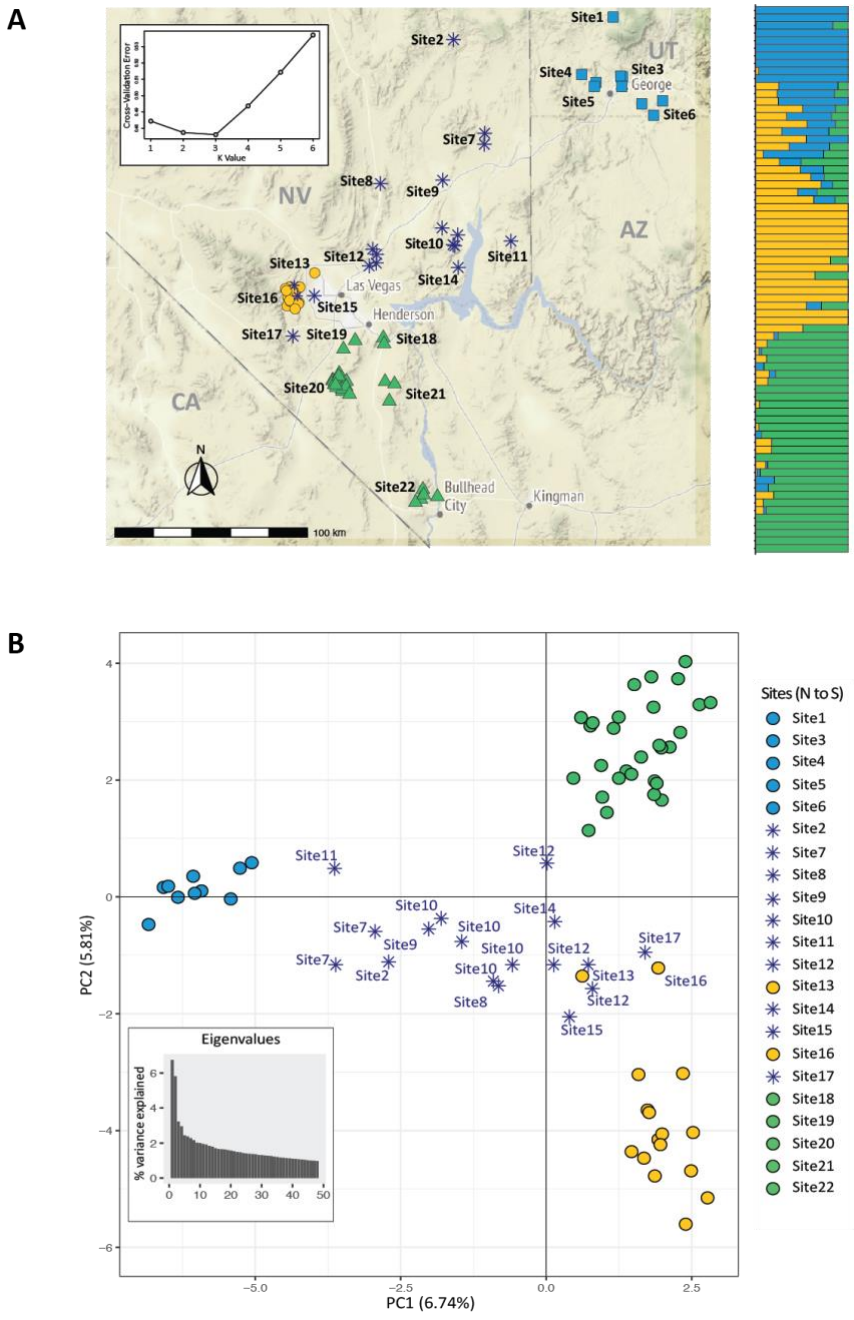

Figure 2. Genetic structure of Gila monsters throughout Nevada and Utah (A) Map of Admixture assignment plot at K = 3 with individuals arranged by collection location from north to south. Inset on map: plot of cross validation errors from Admixture at values of K ranging from 1 to 6. The lowest error was found at K = 3. (B) PCA of eigenvectors 1 versus 2 using the rangewide dataset show similar clustering recovered in Admixture. Blue, yellow, and green shapes correspond to the Utah, Central-NV and Southern-NV regional clusters; samples on the map with asterisks indicate admixed group of individuals in northern Nevada found primarily in both Admixture and PCA.

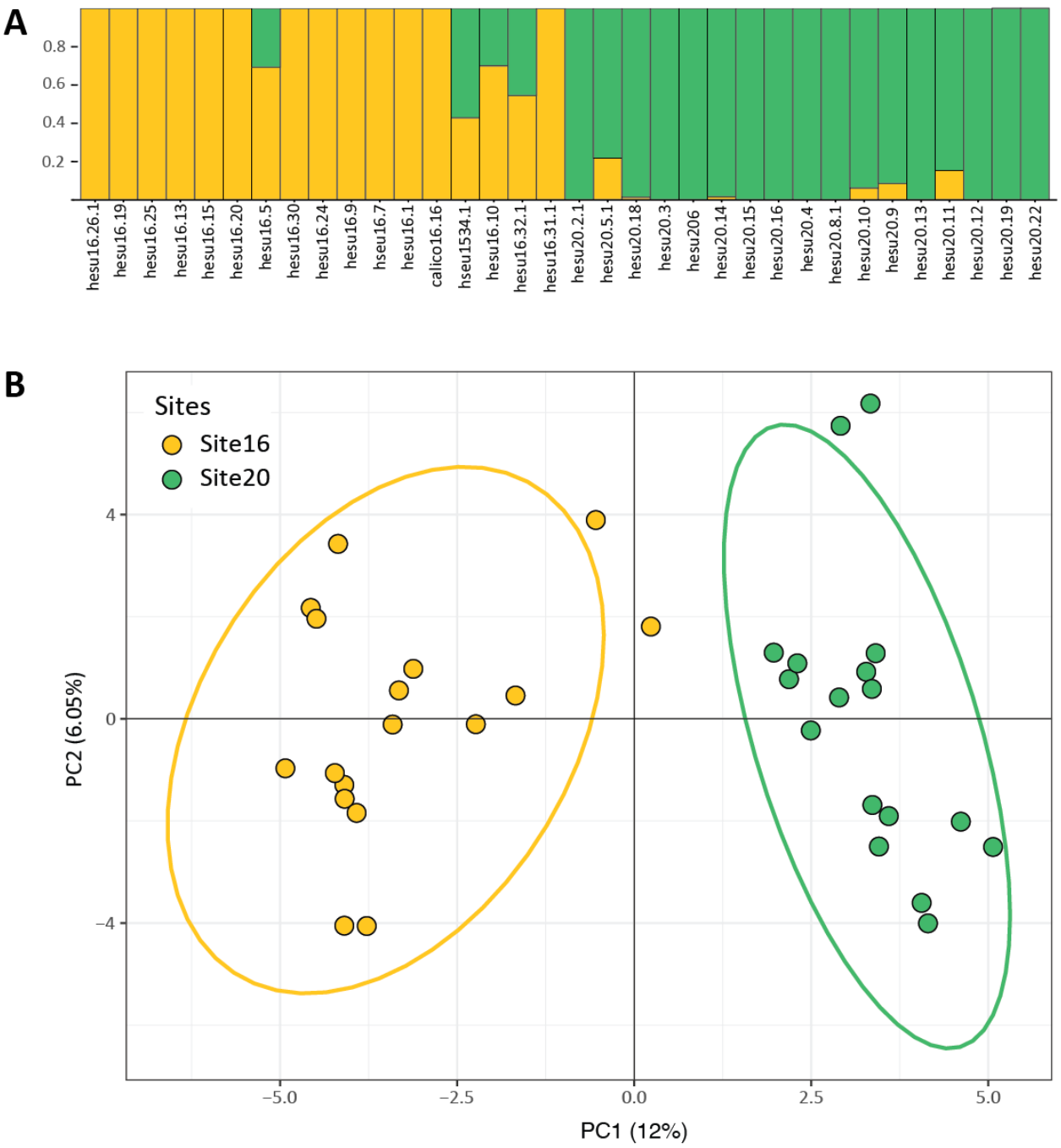

Figure 3. Genetic structure between the focal sites (Site 16 ans Site 20) using (A) individual assignment plot at K = 2 and (B) PCA analysis of eigenvectors 1 versus 2.

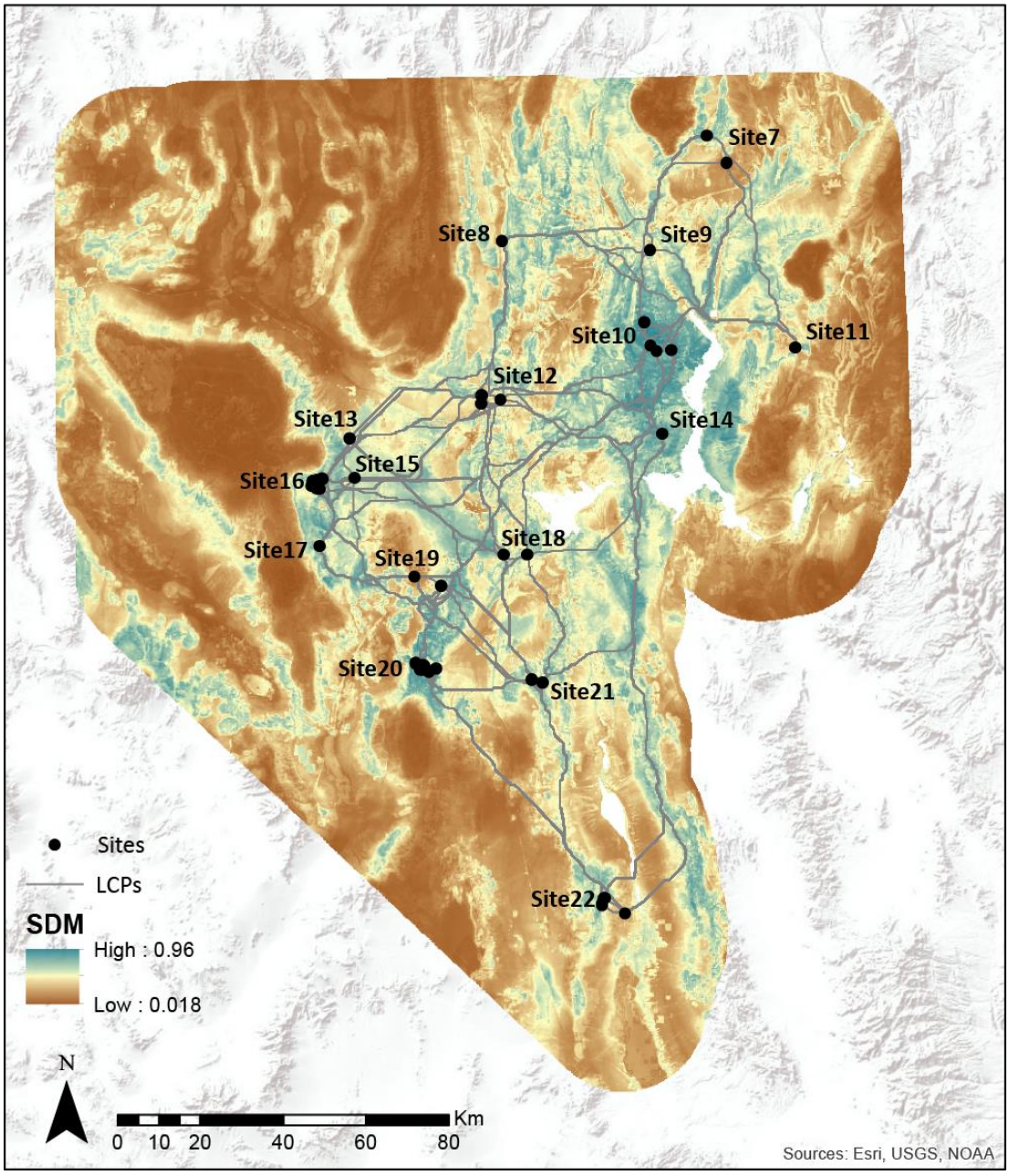

Figure 4. Least cost paths among sites using habitat cost (defined using a species distribution model provided by K. Nussear) within the Clark County study area. The SDM ranges from 0 to 1 with higher values associated with a higher probability of presence. To define movement "costs" between locations and least cost path distances, we used the inverse of the SDM values.

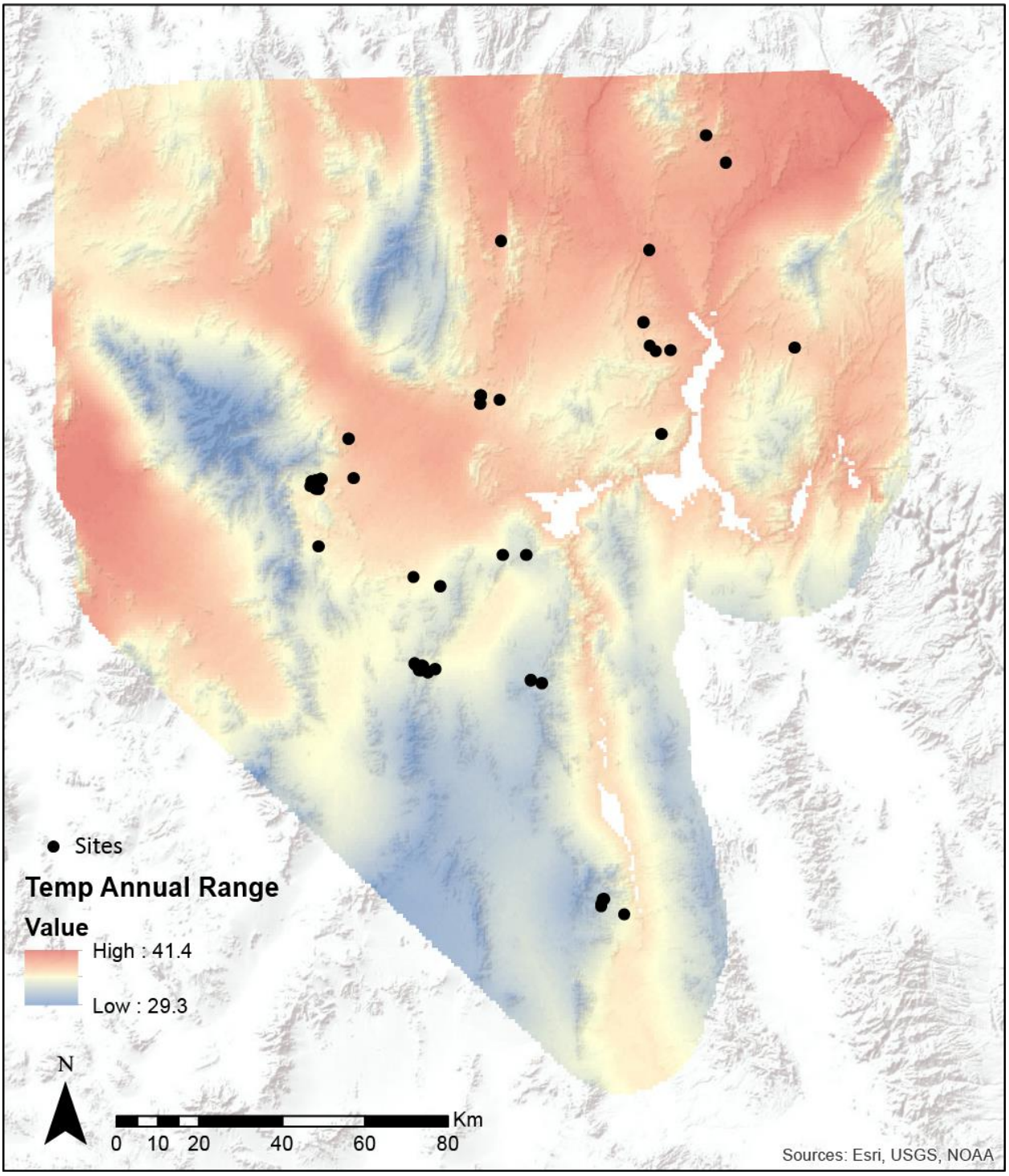

Figure 5. Map of annual temperature range (Tar) associated with genetic differentiation across Clark County study area. Values depict the annual range in °C between high and low temperatures averaged across the 30-year period between 1970 and 2000 at a 1 km^2 grid cell size.

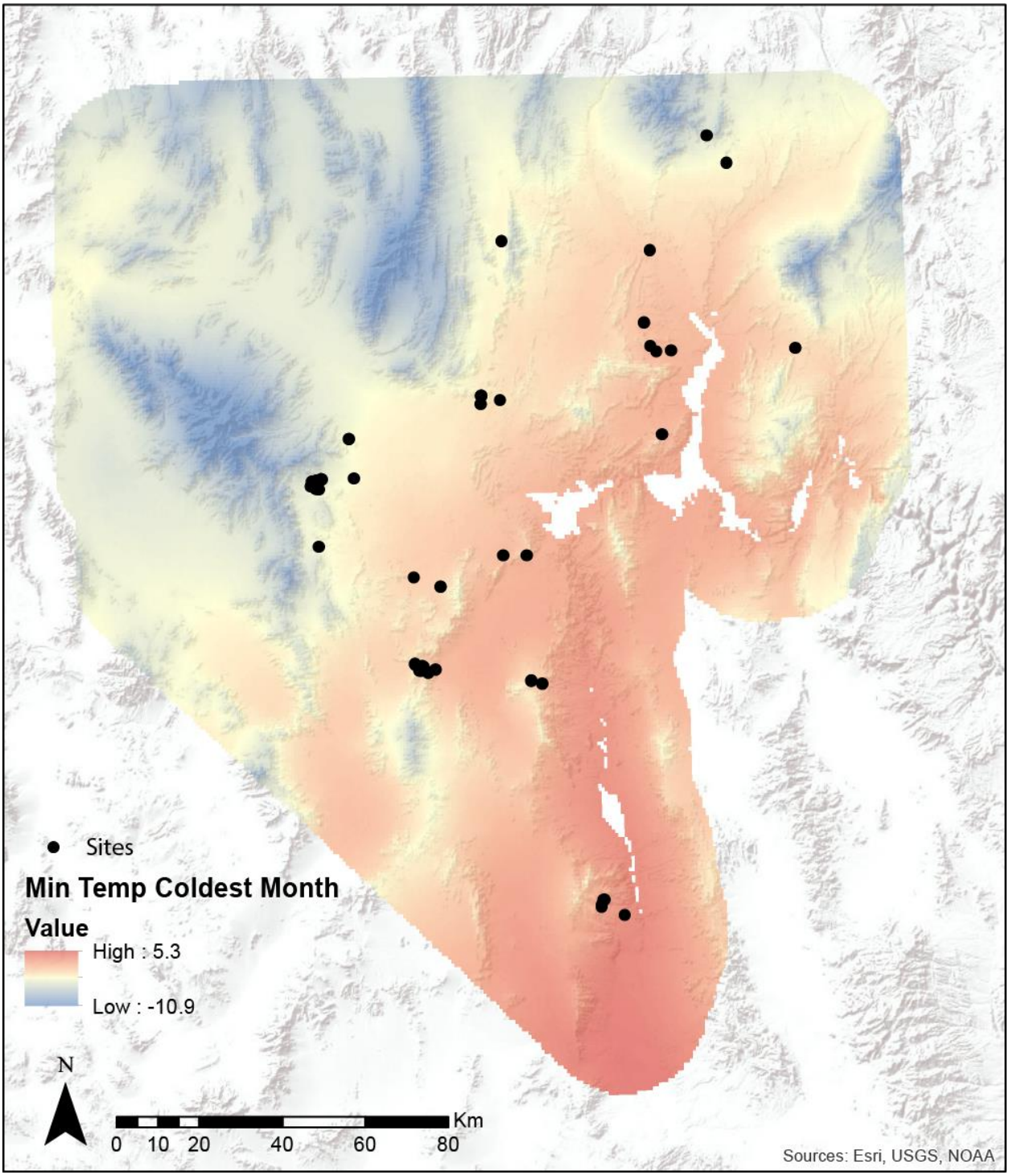

Figure 6. Map of minimum temperature of the coldest month (Tmin) associated with genetic differentiation across Clark County study area. Values depict the coldest monthly temperature in ℃ averaged across the 30-year period between 1970 and 2000 at a 1 km^2 grid cell size.

Supplemental Figures

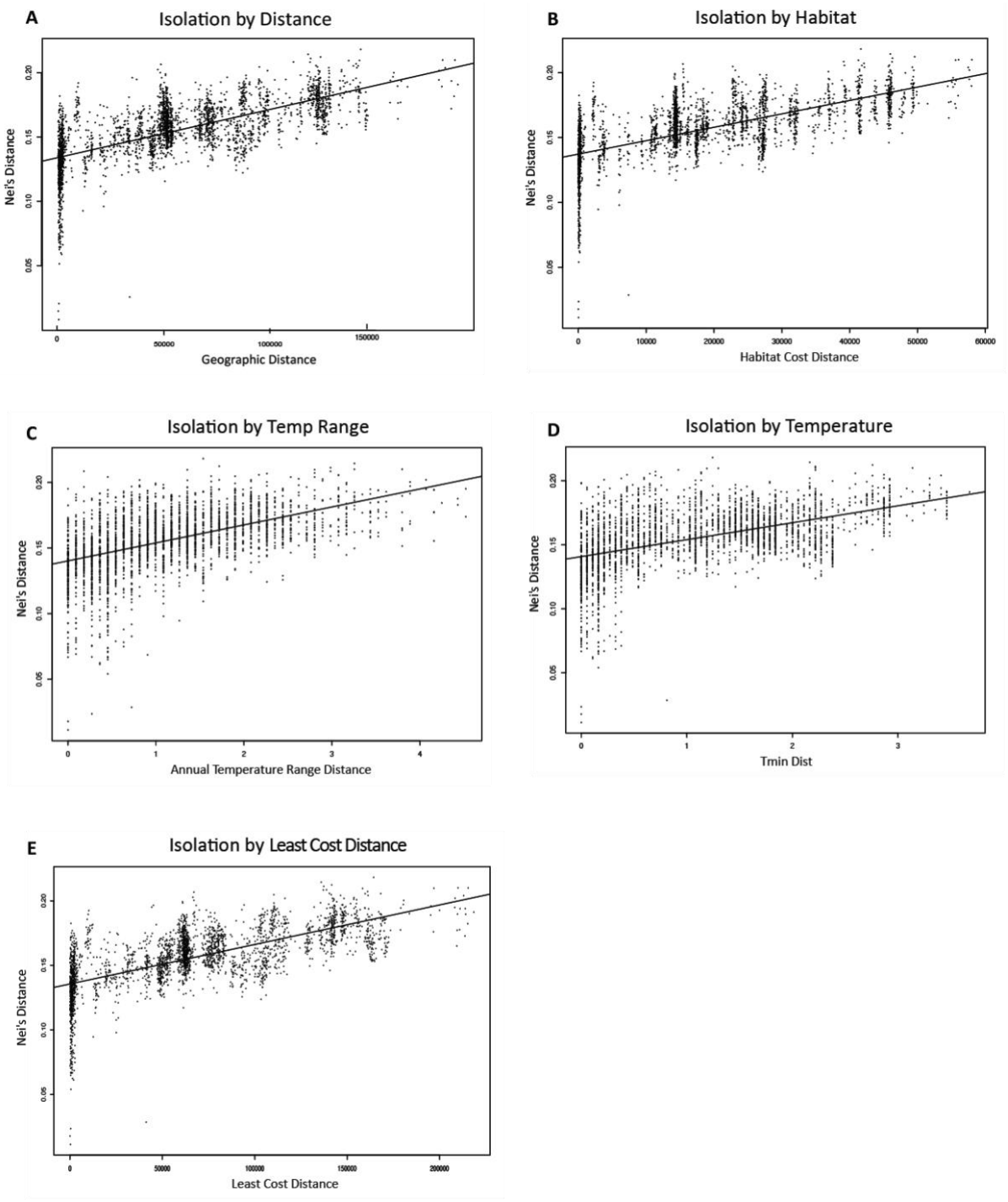

Figure S1. Significantly correlated habitat and climate variables with of genetic isolation by Euclidean distances at the individual level (A) geographic, (B) habitat cost, annual temperature range, (D) minimum temperature of the coldest month, and (E) least cost distances.

Table S1: Summary of Gila Monster sample and genetic information collected for individuals sequenced in Nevada and Utah. Information includes: Sample, unique sample name; State, State where sample was collected; County, county where the site occurs; Locality, collection site; Date, date of tissue collection; Sex, M=Male, F=Female, U=Unknown; Filter, whether sample 'Passed' or 'Failed' bioinformatic filtering; n_loci, the number of loci recovered; mean_cov, mean coverage per sample; missing, frequency of missing data. or all measurements an 'NA' indicates that no measurements were available.

Sample	State	County	Locality	Date	Sex	Filter	n_loci	mean_cov	missing
hesu12.1	NV	Clark	Site 12	9-Jul-18	U	passed	38154	97.726	0.02
hesu12.2	NV	Clark	Site 12	30-Apr-14	U	passed	28402	97.559	0.07
hesu12.3	NV	Clark	Site 12	17-Apr-17	U	passed	30803	98.294	0.06
hesu12.4	NV	Clark	Site 12	28-Apr-14	U	passed	21005	51.33	0.31
hesu8.1	NV	Clark	Site 8	12-May-13	U	passed	33472	99.81	0.01
hesu17.1	NV	Clark	Site 17	24-May-18	M	passed	39256	63.377	0.03
hesu18	NV	Clark	Site 18	20-Apr-16	U	passed	32829	94.242	0.03
hesu19.1	NV	Clark	Site 19	1-Jun-09	M	passed	23439	75.658	0.28
hesu16.1	NV	Clark	Site 16	21-Jun-19	U	passed	28867	89.514	0.06
hesu16.10	NV	Clark	Site 16	10-May-17	M	passed	32440	107.266	0.03
hesu16.11	NV	Clark	Site 16	8-May-19	U	passed	35385	105.979	0.03
hesu16.12	NV	Clark	Site 16	1-Jun-17	U	passed	30403	93.375	0.06
hesu16.13	NV	Clark	Site 16	16-May-18	U	passed	28494	99.669	0.08
hesu16.14	NV	Clark	Site 16	23-May-16	U	passed	32572	104.51	0.03
hesu16.15	NV	Clark	Site 16	21-May-18	U	passed	34229	100.617	0.05
hesu16.16	NV	Clark	Site 16	20-May-19	U	passed	38801	85.23	0.03
hesu16.17	NV	Clark	Site 16	22-May-18	U	passed	32515	93.759	0.04
hesu16.18	NV	Clark	Site 16	26-Mar-19	F	passed	29802	79.64	0.06
hesu16.19	NV	Clark	Site 16	29-Sep-19	U	passed	31557	93.216	0.06
hesu16.20	NV	Clark	Site 16	21-May-19	F	passed	40942	89.462	0.03
hesu16.21	NV	Clark	Site 16	1-Jun-19	U	passed	30320	84.326	0.07
hesu16.23	NV	Clark	Site 16	4-May-18	M	passed	39755	101.942	0.03
hesu16.24	NV	Clark	Site 16	19-Jun-19	F	passed	28015	98.475	0.06

Sample	State	County	Locality	Date	Sex	Filter	n_loci	mean_cov	missing
hesu16.25	NV	Clark	Site 16	6-Apr-20	F	passed	32048	84.491	0.06
hesu16.26.1	NV	Clark	Site 16	9-Apr-20	F	passed	29281	103.689	0.04
hesu16.27	NV	Clark	Site 16	20-Apr-20	F	passed	27023	77.186	0.13
hesu16.28.1	NV	Clark	Site 16	29-Apr-20	U	failed	10293	35.944	NA
hesu16.29.1	NV	Clark	Site 16	10-May-20	U	passed	30937	88.949	0.06
hesu16.3	NV	Clark	Site 16	21-May-15	U	passed	34693	97.539	0.02
hesu16.30	NV	Clark	Site 16	14-May-20	U	passed	28695	74.196	0.07
hesu16.31.1	NV	Clark	Site 16	2-Jun-20	F	passed	28984	97.961	0.04
hesu16.32.1	NV	Clark	Site 16	17-May-20	U	passed	33691	68.595	0.08
hesu16.33	NV	Clark	Site 16	4-Apr-18	M	passed	28224	103.005	0.09
hesu16.34.1	NV	Clark	Site 16	19-May-20	U	passed	23657	67.301	0.20
hesu16.35	NV	Clark	Site 16	1-Sep-17	U	passed	34055	101.417	0.02
hesu16.4	NV	Clark	Site 16	1-Jul-19	U	passed	32775	106.405	0.02
hesu16.5	NV	Clark	Site 16	18-May-19	F	passed	34949	109.926	0.01
hesu16.6	NV	Clark	Site 16	13-May-16	U	passed	40666	82.387	0.03
hesu16.7	NV	Clark	Site 16	22-May-16	U	passed	32096	53.931	0.05
hesu16.8	NV	Clark	Site 16	2-Aug-16	U	passed	37144	102.528	0.02
hesu16.9	NV	Clark	Site 16	5-Apr-17	F	passed	33229	88.794	0.04
hesu21.1	NV	Clark	Site 21	8-May-19	U	passed	37991	53.926	0.03
hesu21.2	NV	Clark	Site 21	9-May-19	U	passed	35760	60.846	0.02
hesu21.3	NV	Clark	Site 21	3-Apr-16	U	passed	37140	74.901	0.34
hesu14.1	NV	Clark	Site 14	15-May-19	F	passed	19156	84.993	0.08
hesu11.1	NV	Clark	Site 11	22-May-94	U	passed	37074	102.433	0.01
hesu19.2	NV	Clark	Site 19	13-Jun-16	U	passed	21782	34.98	0.03
hesu20.1	NV	Clark	Site 20	3-Jun-19	U	passed	36101	100.939	0.03
hesu20.17	NV	Clark	Site 20	6-Jul-13	U	failed	16975	115.351	NA
hesu13.1	NV	Clark	Site 13	9-Jun-20	U	passed	29153	71.004	0.04
hesu20.10	NV	Clark	Site 20	5-May-17	U	passed	38119	90.456	0.02
hesu20.11	NV	Clark	Site 20	20-May-18	M	passed	32090	89.957	0.04

Sample	State	County	Locality	Date	Sex	Filter	n_loci	mean_cov	missing
hesu20.12	NV	Clark	Site 20	20-May-15	U	passed	31743	88.513	0.02
hesu20.13	NV	Clark	Site 20	20-May-15	U	passed	34447	105.575	0.03
hesu20.14	NV	Clark	Site 20	31-May-15	U	passed	39017	77.729	0.04
hesu20.15	NV	Clark	Site 20	31-May-15	U	passed	32046	91.304	0.06
hesu20.16	NV	Clark	Site 20	31-May-15	U	passed	35232	102.192	0.05
hesu20.18	NV	Clark	Site 20	12-Apr-13	M	passed	30285	104.064	0.05
hesu20.19	NV	Clark	Site 20	2-Jul-19	U	passed	40679	68.294	0.03
hesu20.2.1	NV	Clark	Site 20	22-Apr-13	U	passed	35394	72.59	0.02
hesu20.22	NV	Clark	Site 20	12-May-18	F	passed	39176	73.241	0.15
hesu20.3	NV	Clark	Site 20	30-Apr-13	M	passed	31631	94.687	0.03
hesu20.4	NV	Clark	Site 20	1-May-13	M	passed	33952	88.863	0.15
hesu20.5.1	NV	Clark	Site 20	29-Apr-13	F	passed	26031	73.095	0.02
hesu20.6	NV	Clark	Site 20	11-Jun-13	U	passed	34499	90.963	0.02
hesu20.7	NV	Clark	Site 20	16-Sep-14	M	failed	1227	375.297	NA
hesu20.8	NV	Clark	Site 20	23-Aug-13	U	passed	25424	98.944	0.02
hesu20.9	NV	Clark	Site 20	6-Oct-14	U	passed	34316	103.31	0.05
hesu9.1	NV	Clark	Site 9	11-Apr-18	U	passed	37032	99.924	0.03
hesu15.1	NV	Clark	Site 15	24-Jul-09	U	passed	39567	76.281	0.01
hesu10.1	NV	Clark	Site 10	5-Jun-16	U	passed	35538	23.677	0.28
hesu10.2	NV	Clark	Site 10	25-May-20	M	passed	40772	74.799	0.03
hesu10.3	NV	Clark	Site 10	25-Jul-12	U	passed	28097	57.865	0.04
hesu10.4	NV	Clark	Site 10	12-Oct-20	F	passed	50304	57.822	0.25
hesu22.1	NV	Clark	Site 22	22-May-08	U	passed	33983	100.342	0.03
hesu22.2	NV	Clark	Site 22	30-May-09	U	passed	37304	105.406	0.26
hesu22.3	NV	Clark	Site 22	24-Apr-15	U	passed	24157	75.553	0.06
hesu22.4	NV	Clark	Site 22	21-May-15	M	passed	30372	87.683	0.01
hesu22.5	NV	Clark	Site 22	7-May-20	F	passed	27631	68.177	0.01
hesu7.1	NV	Lincoln	Site 7	23-Apr-19	U	passed	36612	110.541	0.23
hesu7.2	NV	Lincoln	Site 7	20-Jul-20	F	passed	29568	105.64	0.06

Sample	State	County	Locality	Date	Sex	Filter	n_loci	mean_cov	missing
hesu2.1	NV	Lincoln	Site 2	13-May-17	U	passed	35461	57.794	0.09
hesu1.1	UT	Iron	Site 1	10-May-20	M	passed	32395	93.014	0.05
hesu5.2	UT	Washington	Site 5	17-May-20	U	passed	32449	65.727	0.05
hesu5.3	UT	Washington	Site 5	17-May-20	M	passed	40411	70.838	0.12
hesu5.1	UT	Washington	Site 5	1-May-20	F	passed	31470	79.303	0.07
hesu4.1	UT	Washington	Site 4	10-May-20	F	passed	31140	80.698	0.06
hesu3.1	UT	Washington	Site 3	1-May-20	M	passed	33483	85.971	0.03
hesu3.2	UT	Washington	Site 3		U	passed	40471	85.683	0.04
hesu24.1	UT	Washington	local unknown	22-May-20	M	failed	25005	59.227	NA
hesu6.1	UT	Washington	Site 6	22-May-20	F	passed	30964	84.89	0.02
hesu6.2	UT	Washington	Site 6	24-Apr-21	M	passed	44308	77.129	0.04
hesu6.3	UT	Washington	Site 6	24-Apr-21	F	passed	49665	70.095	0.05