

Elusive does not always equal rare: genetic assessment of a protected Gila monster (*Heloderma suspectum*) population in Saguaro National Park, Arizona

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Abstract. Population genetic baselines for species perceived to be at-risk are crucial for monitoring population trends and making well-informed management decisions. We characterized the genetic status of a population of Gila monsters (*Heloderma suspectum*), a large venomous lizard native to deserts of the southwestern United States and northern Mexico, by sampling 100 individuals in Sonoran Desert upland habitat at Saguaro National Park, Arizona, USA. We used 18 microsatellite markers, along with 1195 bp of sequence data from the mitochondrial DNA 12S locus, to examine genetic diversity, estimate effective population size, and assess demographic history. Despite suburban development adjacent to the study area, we observed high genetic diversity with uninhibited gene flow within this protected population. We estimated effective population size (N_e) for the total sample area (80 km²) using the linkage disequilibrium method in NeEstimator to be 94 individuals (95% confidence interval: 80.7-111.2). In 2011, we used capture-recapture methods to estimate that 80 adult Gila monsters (95% CI = 37-225) inhabited the area along the 14-km transect that we surveyed most frequently; probability of detecting resident Gila monsters during surveys was <0.01, highlighting the challenges of studying the species. Despite being considered an elusive and thus potentially rare species, these data reveal that in this protected environment the population appears healthy and robust. The results provide an important genetic baseline for future studies and monitoring, and exemplify the success of protective population measures in National Parks and under Arizona state laws.

Keywords: effective population size, genetic baseline, Helodermatidae, microsatellites, mitochondrial DNA, panmixia, Sonoran Desert, STRUCTURE.

Introduction

By assessing the diversity of neutral genetic markers in a stable population, a population genetic “baseline” can be established which may be especially important for elusive species that are challenging to monitor with other methods. This baseline can then provide a metric to evaluate population status and risks to population viability in species believed to be threatened (Martínez-Cruz et al., 2007). These metrics are extremely valuable for informing future management decisions or evaluating the success of current ones (Frankham et al., 2010). Genetic

analyses of threatened species have informed successful re-introductions (Garcia-Moreno et al., 1996; Rhodes et al., 2001) and translocation projects (Gutierrez-Espeleta et al., 2000; Grativol et al., 2001), helped define management units (i.e. sub-species boundaries; O’Ryan et al., 1994; Gutierrez-Espeleta et al., 2000; Haig et al., 2001), and even led to the definition of new species (Helgen et al., 2013; Rasoloarison et al., 2013). However, when conservation decisions are made without baseline genetic information, unanticipated consequences can occur, such as using hybrids as a genetic source for re-introduction (Garcia-Moreno et al., 1996), decreasing overall genetic diversity during captive breeding (Robichaux et al., 1997; Friar et al., 2000), or erasing local adaptation through outbreeding depression (O’Ryan et al., 1994; Marshall and Spalton, 2000). Population genetic baseline data not only shed light on the studied population, but provide a point of comparison for other populations of the species

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(Culver et al., 2000; Grativol et al., 2001) and future studies (Carson et al., 2014). Nonetheless, genetic assessments have not been completed for many threatened species, especially elusive species that are difficult to sample in the wild.

The Gila monster (*Heloderma suspectum*), a large venomous lizard native to the southwestern United States and northern Mexico (Beck, 2005), is one such species that can benefit from the establishment of a population genetic baseline. Within its range, this lizard inhabits rocky outcrops and crevices (Beck, 2005), where it spends up to 92 percent of its time underground (Lowe et al., 1986; Beck, 2005). During its above-ground activity, Gila monsters have been observed to have home ranges that average 48.1 hectares (Beck, 2005), varying from 6–68 hectares (Sullivan et al., 2004). Males generally have larger home ranges than females (Beck, 2005). Despite large home ranges, encounters with these lizards are very uncommon; encounter rate for targeted searches of Gila monsters at a Sonoran Desert site was 30.3 person-hours per individual sighting (Flesch et al., 2010).

Due to its elusive nature, little is known about the actual population size, structure, and genetic status of these animals in the wild. The total population size of Gila monsters is estimated by the International Union for Conservation of Nature (IUCN) to be in the hundreds-of-thousands (Hammerson et al., 2007), and population studies across the southwest USA (including southern Arizona) have estimated densities of 3.8–7.5 individuals per square kilometer (data from reports and unpublished manuscripts referenced in Beck, 2005). All of these studies use capture-recapture methods, and no genetic estimates of population structure or effective population size have been conducted to date.

Threats from human development place the Gila monster at risk for habitat loss and fragmentation; impacts already observed in portions of the USA range outside of Arizona

(Beck, 2005). At our study site in Saguaro National Park-Rincon Mountain District, Arizona located in the Sonoran Desert east of the growing Tucson metropolitan area, continual suburban construction and road development since the 1990s may pose a threat to Gila monsters in their natural desert habitat. As a large slow-moving reptile, the Gila monster is at risk for road mortality as traffic increases with development (Beck, 2005). This risk is greatest for dispersing individuals, such as juveniles or mate-seeking males (Bonnet et al., 1999). Further habitat modification due to invasive species, shifts in vegetative communities, or climate change also may exacerbate the effects of habitat fragmentation in reptiles by reducing available microhabitats (Eyre et al., 2009; Sirios et al., 2014). In addition, poaching for the illegal pet trade continues to threaten the genetic diversity of native populations (Beck, 2005). The Gila monster has been listed as “Near Threatened” by the IUCN (Hammerson et al., 2007) and is protected from hunting and collection by Arizona State Law (AGFD, 2012). Populations in New Mexico, Nevada, Utah, and California are less robust and have greater legal protections than those in Arizona (Beck, 2005).

We aim to establish a population genetic baseline for Gila monsters, using the protected population at Saguaro National Park-Rincon Mountain District in southern Arizona as a comparison point for future sampling and studies of Gila monsters across their range. Using 18 microsatellite markers recently developed for this genus (Feltoon et al., 2007; Hess et al., 2013) and mitochondrial DNA sequences, we aim to a) assess genetic diversity and structure within the population, b) estimate effective population size, and c) examine potential threats of habitat fragmentation, inbreeding, or other detriments to the long-term genetic viability of this population. We also seek to provide greater insight into the biology and genetic status of these elusive animals.

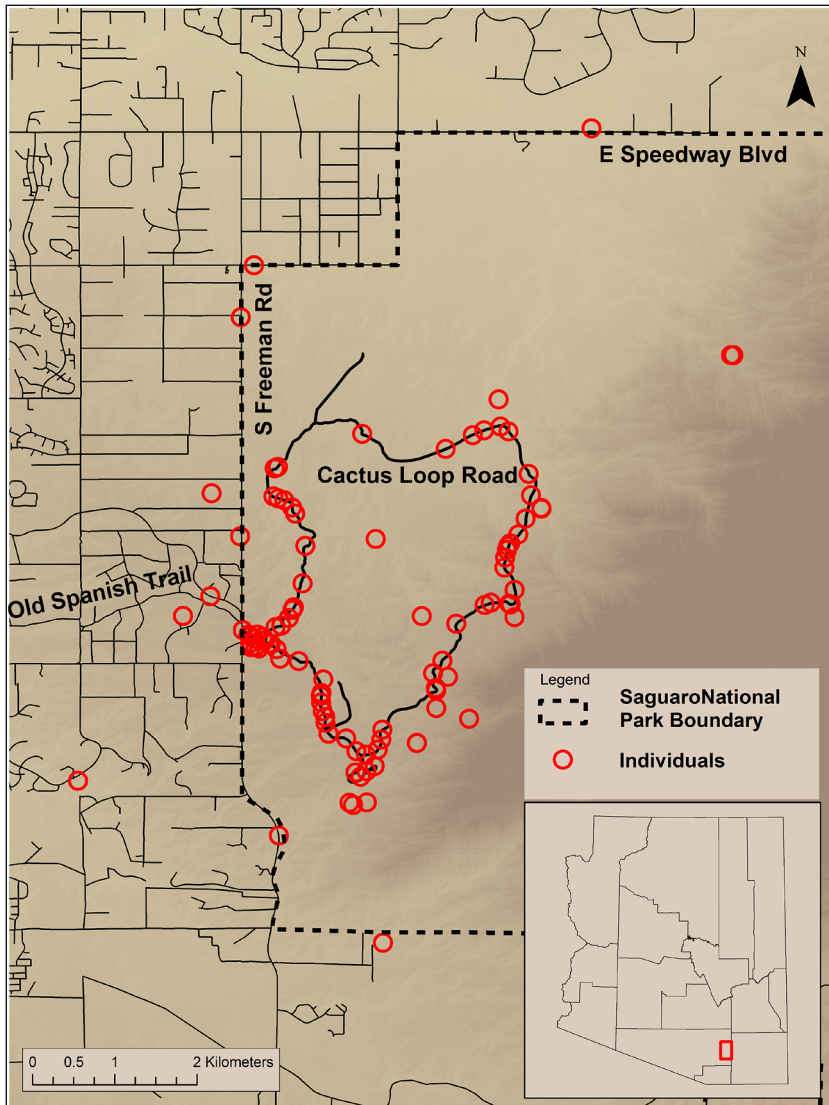


Figure 1. Map of sampling area in Saguaro National Park-Rincon Mountain District, Arizona. Each placemaker represents an individual sample collected. The approximately 14-km Cactus loop road where sampling was conducted is the heart-shaped loop central to the figure. This figure is published in colour in the online version.

Methods

Sample collection

We collected biological samples, for subsequent DNA extraction, from wild Gila monsters in and around the urban boundary of Saguaro National Park-Rincon Mountain District between 2010 and 2012 covering an area of approximately 81 km² within the park (fig. 1). We encountered sampled animals during focused surveys hiking around the desert areas of the park, driving on the 14-km “Cactus loop road” within the park boundary, and during radio telemetry tracking. Park staff and volunteers also provided samples opportunistically. We obtained samples via buccal swabs

(Whatman OmniSwab, WB100035; Whatman Inc., USA), as tissue from dead animals, or using whole blood from the caudal vein. We stored all samples in lysis buffer (Goldberg et al., 2003). We conducted field sampling under IACUC protocol 09-062 and permits from the United States Department of the Interior (permit number SAGU-2009-SCI-0004) and Arizona Game and Fish Department (license #SP559682) and subsequent renewals of these permits.

Abundance estimation

We marked all Gila monsters that we encountered with passive integrated transponders (PIT tags; AVID Identifica-

tion Systems, Inc. Norco, CA). Consistent sampling effort throughout late-summer 2011 along the 13-km loop road and connected spurs from the visitor's center and to a picnic area, a total paved road distance of 14 km that served as our primary sampling transect in the approximately 80 km² Sonoran desert study area, generated evening field captures (a subset of these are included in the 100 genetically sampled individuals examined in this study) that we used to estimate abundance of adult Gila monsters inhabiting the transect area. Between July and September (the summer monsoon season when Gila monsters are most likely encountered after dark) 2011 we surveyed the road 108 times to generate encounter histories for each individual. Using a closed capture-recapture approach, we fit models in Program MARK based on the Huggins likelihood and parameterization (White and Burnham, 1999) to estimate abundance. Because Gila monsters are site faithful (Beck, 2005 and references therein), we thought it reasonable to assume that the area was closed to changes in abundance during the survey period.

Molecular techniques

We isolated total DNA from buccal swabs (Goldberg et al., 2003), whole blood, or tissue by overnight lysis with proteinase K at 55°C, followed by robotic extraction using a QIAGEN BioSprint 96 robotic magnetic-particle purification system (Qiagen, Valencia, California, USA) and Invitrogen Dynal bead extraction chemistry (Life Technologies, Carlsbad, California, USA). We quantified recovered DNA using a BioTEK Synergy HT (BioTEK, Vermont, USA) and diluted working stocks to 5 ng/μl for polymerase chain reaction (PCR).

PCR conditions and multiplexing. For each individual, we amplified sixteen microsatellite loci (STRs) developed for *H. suspectum* as previously described by Hess et al. (2013; HESU001-HESU016), as well as two microsatellite loci (HELO 1, HELO 4) identified in *Heloderma horridum* but found to be variable in *H. suspectum* (Feltoun et al., 2007; M. Weiss, unpublished data). We performed PCR for STRs following Hess et al. (2013). We also sequenced the 12S region of the mitochondrial genome using primers designed by M. Weiss (unpublished data) using available GenBank reference sequence (accession no. AB167711.1): HESU12S-f, CTAAAGCACGGCACTGAAA; HESU12S-r, GGCAGGCTAAAGTAACGCT. For the mtDNA locus, we performed PCR in 30 μl volumes with 10 mM Tris-HCl pH 8.3, 3.0 mM MgCl₂, 50 mM KCl, 1.2 units of Platinum[®] Taq DNA Polymerase (Life Technologies, Grand Island, NY, USA), 0.25 mM of each dNTP, and 0.4 μM of each primer and 10 ng of DNA. We cycled the PCR reactions for 3 min at 94°C initial denaturation, followed by 35 cycles of 1 min at 94°C, 2 min at 59°C, 3 min at 72°C, and a final 5 min extension at 72°C. Fragment analysis and DNA sequencing were performed by the University of Arizona Genetics Core. We analyzed STR fragment analysis data using Genemarker 1.85 (SoftGenetics, State College, Pennsylvania, USA). We aligned a 1195 bp mtDNA 12S sequence using CLC DNA Workbench ver. 5.7.1 (CLC Bio, Denmark).

Analyses

STR analysis. We assessed the dataset for evidence of null alleles, large allele dropout, and scoring error due to stuttering using MICRO-CHECKER v.2.2.3 (van Oosterhout et al., 2004). We also ran a probability of identity P(ID) analysis using GIMLET v.1.3.3 (Válie, 2002). P(ID) quantifies the power of molecular markers to choose between two individuals and represents the probability that two individuals drawn at random from a population would have the same genotype at multiple loci (Waits et al., 2001). We used an unbiased P(ID) estimator for a population where individuals randomly mate (Paetkau and Strobeck, 1994) and calculated the matching probability (the probability of drawing a given genotype from the population) following Woods et al. (1999).

We used ARLEQUIN v.3.11 (Excoffier et al., 2007) to detect significant departures from Hardy-Weinberg expectations and to test for linkage disequilibrium (nonrandom association between loci) among all pairs of loci (Slatkin and Excoffier, 1996). We employed a Bonferroni adjustment for multiple tests to account for false positives (Rice, 1989). We generated estimates of gene diversity per locus and allelic richness per locus using FSTAT v.2.9.3.2 (Goudet, 1995). We used default parameters in FSTAT and ARLEQUIN for all Markov-chain tests and permutations. We estimated individual inbreeding coefficient (F_i ; Ley and Hardy, 2013) using SPAGeDi v.1.4 (Hardy and Vekemans, 2002) and assessed significance after 10 000 randomizations of gene copies among individuals.

We used STRUCTURE v.2.3.4 (Pritchard et al., 2000) to define populations in our STR dataset. We ran a multi-locus STR analysis for all 100 samples. We tested for $K = 1-10$ with 10 trials per K , each run for 500 000 iterations following a burn-in period of 100 000 MCMC under the admixture model, assuming allele frequencies were correlated between populations. We used STRUCTURE HARVESTER Online (Earl and vonHoldt, 2012) to evaluate STRUCTURE results. We compiled independent STRUCTURE runs using the Greedy K algorithm in CLUMPP 1.1.1 (Jakobsson and Rosenberg, 2007) and we visualized these combined outputs using DISTRUCT 1.1 (Rosenberg, 2004). In addition, we also used the Bayesian model-based clustering method in GENELAND (Guillot et al., 2005) which also incorporates geographic information in determining population structure. We ran GENELAND for the STR dataset and performed 10 independent runs testing for $K = 1-10$ populations for 500 000 iterations each, thinning every 100. We took into account the putative presence of the null allele in our model choice and used a 100 m coordinate uncertainty.

We used BOTTLENECK (Piry et al., 1999) to test for evidence of historical changes in effective population sizes and deviations from equilibrium conditions. This test assumes that a population with recent reductions in effective population size will show an excess of heterozygosity over that expected under mutation-drift equilibrium (Cornuet and Luikart, 1996). We ran 100 000 replications for both the Sign Test and Wilcoxon Test, under IAM, TPM, and SMM models.

We used SPAGeDi v.1.4 to generate pairwise Rousset's distance ($\hat{a}$; Rousset, 2000) among all individuals. Rousset's

distance measure is analogous to the ratio $F_{ST}/(1 - F_{ST})$ using pairs of individuals instead of populations (Rousset, 2000). This distance measure does not require a reference population, unlike other methods for generating kinship estimators. We used NTSYSpC (v.2.02h, Applied Biostatistics Inc.) to perform Mantel tests to assess correlation between genetic distances among individuals calculated from Rousset's distance ($\hat{a}$) and geographic distances (Slatkin and Maddison, 1990). We performed tests using both true distances and natural logarithm-transformed spatial distances as well as for gender-specific subsets of the data.

We used NEESTIMATOR 2.01 with LDNe (Do et al., 2014) to estimate effective population size (N_e) in our population. NEESTIMATOR with LDNe was found to be one the most robust estimators of N_e in a study comparing the performance of various programs on simulated data (Gilbert and Whitlock, 2016). Genetically, N_e is defined as the equivalent census size of a population, in ideal Hardy-Weinberg conditions, that would exhibit the same signatures of genetic drift as seen in the current population (Frankham et al., 2010). To calculate this, we used the linkage disequilibrium method, and also the heterozygote excess method, to estimate N_e . For each of these approaches we omitted allele frequencies less than 0.01 and calculated 95% confidence intervals for the effective population size with the jack-knifing method.

MtDNA analysis. We used DNASP v.5.10.01 (Librado and Rozas, 2009) to estimate nucleotide diversity, polymorphism, haplotype diversity, and other descriptive statistics among maternal lineages. We then used NETWORK 4.6.1.2 (fluxus-engineering.com; Fluxus Technology Ltd., Suffolk, England) to produce an unrooted, median-joining haplotype network for mitochondrial haplotypes seen within our population (Bandelt et al., 1999).

Results

Samples

In total, we obtained 100 geo-referenced Gila monster DNA samples that we used for genetic analyses. Nine samples were collected outside the boundaries of the National Park, with most found on roads (five of which were found dead as road kill) on the east side of the Tucson Metropolitan area near the park entrance. Of these samples, 36 were female, 11 were male, 19 were considered juveniles, and for 53 the gender could not be determined.

Abundance

From the 108 evening surveys conducted in late-summer 2011, we encountered 17 unique adult

Gila monsters, three of which we encountered more than once. Because the recapture rate was low, we kept models for detection probability simple. Ultimately, the most parsimonious model was one where detection probability was constant ($p[.]$), which is the model we used for inference. We estimated 80 adult Gila monsters (95% confidence interval: 37-225) inhabited the 14 km transect (approx. 6/km); probability of detecting resident individuals was very low (0.0027; 95% CI = 0.00088-0.0081).

STR data

We generated STR data for all 100 individuals; 7 individuals were missing data for 3 of the 18 loci and only 4 individuals in the dataset were missing data for ≥ 4 loci. We did not find evidence of either scoring errors or large allele drop-out in our data using MICRO-CHECKER. Two loci (HESU006 and HESU010) exhibited homozygote excess, suggesting possible null alleles, and were excluded in analyses where only loci that met Hardy-Weinberg equilibrium expectations were employed. P(ID), the probability of drawing a given genotype from the population, was calculated for each individual and ranged from 3.27×10^{-12} to 7.34×10^{-26} ; this is exceptionally low for wildlife studies (Waits et al., 2001) (table 1).

Diversity indicators were generally high (allelic richness, 4.0-18.3; mean $H = 0.75 \pm 0.10$) and most loci meet HW expectations (table 1), though six were found to be outside of Hardy-Weinberg equilibrium. We detected significant linkage disequilibrium in only 4 of the 153 pairwise comparisons after applying the Bonferroni correction, each of which involved a combination of HESU005 and HESU006. These two loci also did not meet Hardy-Weinberg expectations. There was no evidence of increased levels of inbreeding in this population (table 1). We detected significant indication of recent reductions in effective population size under some of the evaluated models; Sign test (IAM model) and the Wilcoxon Test (IAM and TPM models). These bottleneck results were the same

Table 1. Population descriptive statistics for 18 STR loci. *n*: number of individuals genotyped, A: number of observed alleles, Range: allelic range, Theta(H): population parameter where $\theta = 4N_e\mu$ for diploids (Ohta and Kimura, 1973), Hobs: observed heterozygosity, Hexp: expected heterozygosity, SD: standard deviation of randomization tests for Hardy-Weinberg equilibrium, Richness: allelic richness, Diversity: Gene Diversity, P(ID): unbiased Probability of Identity (where lower values suggest more informative loci), F_i : individual inbreeding coefficient (Ley and Hardy, 2013; *p* generated from 10 000 gene copy randomizations). Six loci indicated in bold exhibited heterozygosity that was significantly different than that expected by Hardy-Weinberg equilibrium.

Locus	<i>n</i>	A	Range	Theta(H)*	Hobs*	Hexp*	<i>p-value</i>	SD	Richness ⁺	Diversity ⁺	P(ID) [^]	$F_i^{\pm}$	<i>p-value</i>
HELO1	96	7	8	4.08	0.729	0.670	0.011	0	6.91	0.67	2.47E-15	-0.089	0.168
HELO4	98	4	5	2.38	0.551	0.583	0.469	0	4.00	0.58	1.88E-18	0.055	0.440
HESU001	99	6	5	12.30	0.879	0.802	0.384	0	6.00	0.80	6.62E-05	-0.096	0.042
HESU002	99	8	7	4.06	0.707	0.669	0.372	0	7.27	0.67	1.78E-09	-0.057	0.373
HESU003	98	8	7	8.03	0.796	0.758	0.293	0	7.91	0.76	4.86E-03	-0.050	0.341
HESU004	98	7	6	18.03	0.857	0.836	0.811	0	7.00	0.84	1.60E-07	-0.026	0.572
HESU005	98	19	19	79.37	0.888	0.921	0.036	0	18.25	0.92	2.06E-12	0.036	0.229
HESU006	92	6	6	8.15	0.663	0.760	0.012	0	5.74	0.76	1.96E-13	0.128	0.031
HESU007	100	9	8	24.56	0.850	0.859	0.258	0	8.97	0.86	6.41E-20	0.010	0.781
HESU008	98	10	9	20.43	0.908	0.845	0.546	0	9.57	0.85	1.01E-16	-0.075	0.069
HESU009	97	10	12	8.23	0.742	0.761	0.003	0	9.61	0.76	1.52E-14	0.024	0.612
HESU010	91	9	8	9.47	0.670	0.776	0.018	0	8.93	0.78	1.15E-08	0.137	0.006
HESU011	86	6	5	7.72	0.756	0.753	0.861	0	5.99	0.75	6.46E-21	-0.003	0.953
HESU012	97	9	8	15.48	0.753	0.823	0.000	0	8.68	0.82	3.47E-06	0.086	0.071
HESU013	96	7	6	15.23	0.781	0.822	0.180	0	7.00	0.82	5.39E-02	0.049	0.299
HESU014	99	6	5	2.74	0.566	0.607	0.417	0.001	5.59	0.61	9.92E-04	0.068	0.325
HESU015	68	6	5	6.64	0.662	0.735	0.136	0	6.00	0.74	1.98E-10	0.101	0.141
HESU016	98	8	7	9.51	0.724	0.777	0.954	0	7.98	0.78	8.16E-18	0.067	0.204
Mean	94.9	8.06	7.56	14.24	0.749	0.764						0.020	0.102
SD	7.56	3.17	3.38	17.93	0.104	0.088							

* Calculated with ARLEQUIN.

⁺ Calculated with FSTAT.

[^] Calculated with GIMLET.

[±] Calculated with SPAGeDi.

when we excluded from analyses the six loci found to be out of Hardy-Weinberg equilibrium.

The STRUCTURE analysis performed on the complete STR dataset obtained a best fit model of $K = 1$ when evaluating the mean of the log-likelihood of the number of populations [$L(K)$; fig. 2]. STRUCTURE HARVESTER also reports DeltaK following Evanno et al. (2005) and using this method, the best fit was $K = 6$. In evaluating the STRUCTURE plots for $K = 3-6$, only 11 individuals were assigned to clusters with a Q value > 0.85 and consisted of individuals clustered at a single location Northeast of the loop road ($n = 3$) and others from near the park entrance and outside the park boundary. Of these 11 individuals with $Q > 0.85$, 7 had missing data for at least one locus. When

we ran the STRUCTURE analysis excluding the six loci found to deviate from Hardy-Weinberg equilibrium and to exhibit linkage disequilibrium (table 1; HELO01, HESU005, HESU006, HESU009, HESU010, HESU012), we again obtained a best fit model of $K = 1$ evaluating $L(K)$. The best fit was $K = 2$ following the DeltaK method (fig. 2).

In the spatial analysis using GENELAND the optimal number of clusters was $K = 2$ based on likelihood estimates (fig. 3). However, the vast majority of samples fell into a single cluster with two sample locations placed within a second cluster. We did not detect a significant correlation between genetic distance and geographic distance for analyses performed among all individuals or for gender-specific analyses. When GENELAND was run using only the

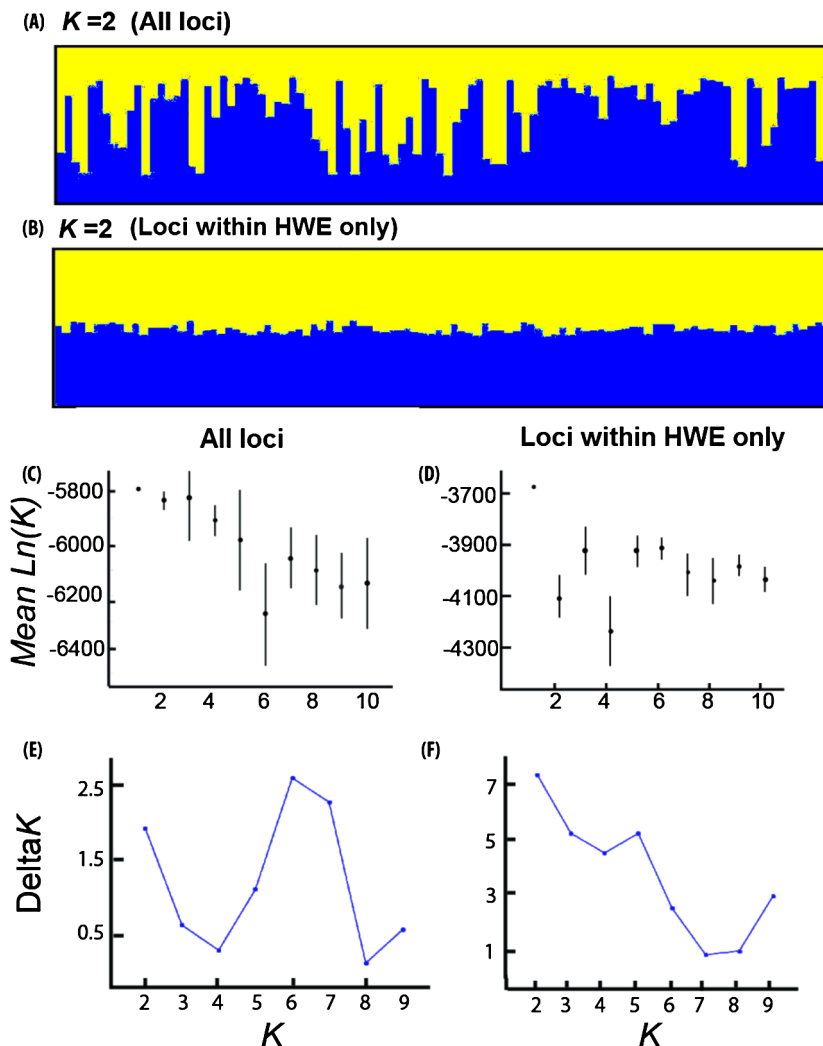


Figure 2. STRUCTURE analyses were run using both all 18 loci and 12 loci, excluding six loci that deviated from Hardy-Weinberg equilibrium and which exhibited linkage disequilibrium (table 1). Histograms show assignment to genetic clusters when $K = 2$ for runs informed by all 18 loci (A) and only the 12 loci in Hardy-Weinberg equilibrium (B). The mean log-likelihood of the number of populations (ranging from 1-10) are shown for each K , with error bars representing the standard deviation around the mean for 10 iterations using (C) all 18 loci and (D) only the 12 loci in HWE. DeltaK, derived through the Evanno et al., 2005 method, is shown for each $K = 1-10$ for analyses using all 18 loci (E) and only those 12 loci in HWE (F). This figure is published in colour in the online version.

12 loci that were unlinked and within Hardy-Weinberg equilibrium, results were the same.

Using NEESTIMATOR, we estimated the effective population size (N_e), using the linkage disequilibrium method, to be 94 individuals within the $\sim 80 \text{ km}^2$ sample area (95% CI = 80.7-111.2). When the six loci out of Hardy-Weinberg equilibrium and which exhibited linkage disequilibrium were excluded from

this analysis, we estimated N_e to be 101 individuals (95% CI = 78.0-137.0). When using the heterozygote excess method, N_e was reported as infinite, which was uninformative to our analyses. Seventy-nine of our 100 genetic samples were collected along the loop road which was the focal area for the detectability and abundance estimates using captures and recaptures in summer 2011.

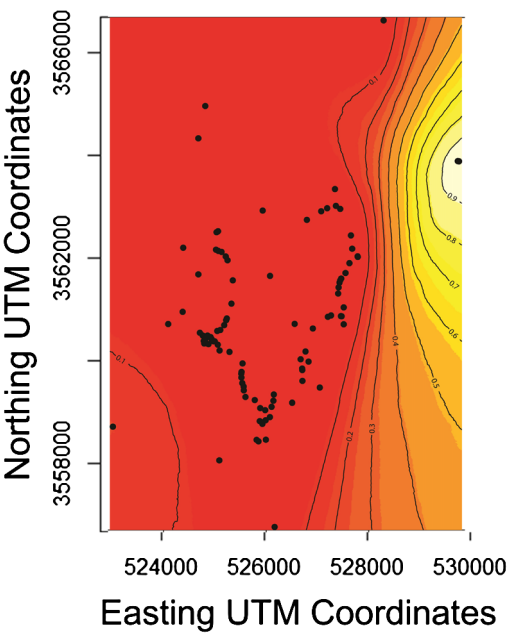


Figure 3. Spatially inferred genetic discontinuities between populations visualized using GENELAND (Guillot et al., 2005) for 100 samples in our dataset. Units on the *x* and *y* axes are UTM coordinates; datum GS84, zone 12S. Labeled contour lines represent probabilities of population membership. This figure is published in colour in the online version.

MtDNA

We recovered viable sequence data for 82 individuals and detected three unique mitochondrial haplotypes with four different variable nucleotide sites for the 12S mitochondrial region (GenBank accession numbers: KP972455-KP972457). One haplotype, HESU_12S_Hap01, comprised the majority ($n = 69$) of the individuals sampled, with the other two haplotypes (HESU_12S_Hap02 and HESU_12S_Hap03) observed in lower frequency ($n = 12$ and $n = 1$, respectively) (fig. 4). When mapped across the sampling area, these haplotypes did not cluster geographically, nor did they correspond with the clustering found in the STRUCTURE or GENELAND analyses. We found θ to be equal to π in our calculations, indicating a Tajima’s *D* of 0.0, meeting expectations of neutrality (table 2; Tajima, 1989).

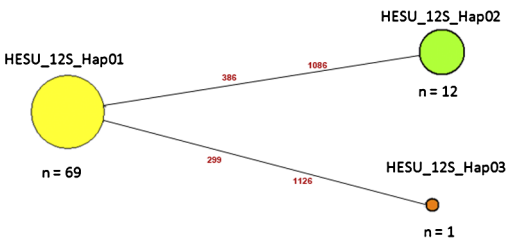


Figure 4. An unrooted, median-joining haplotype network for the three 12S haplotypes (1195 bp) identified in our population, created with NETWORK 4.6.1.2. Numbers below the nodes indicate the number of individuals with that haplotype, and numbers noted on the connections represent the nucleotide positions of mutational differences between the haplotypes. This figure is published in colour in the online version.

Table 2. Population haplotype data for the 12S mitochondrial DNA region. The population parameter $\Theta = 2N_e\mu$ for a haploid marker inherited maternally (Watterson, 1975). Calculations were completed in the program DNASP. Standard deviations are reported after values.

Number of Haplotypes	3
Proportion of haplotype of total	
HESU_12S_Hap01	0.841
HESU_12S_Hap02	0.146
HESU_12S_Hap03	0.012
Haplotype diversity (<i>h</i>)	1.0000 ± 0.272
Haplotype richness (Haps/ <i>N</i>)	0.037
Nucleotide diversity (per site) (π)	0.0022 ± 0.0007
Average number of nucleotide differences (<i>k</i>)	2.6667
Theta (per site) from <i>S</i>	2.6667 ± 0.0016
Theta (per sequence) from <i>S</i>	0.0223

Discussion

The results of these genetic analyses provide evidence for one relatively ubiquitous population of Gila monsters in our sample area in Saguaro National Park. We observed high genetic diversity without significant evidence for current inbreeding or population genetic structure within the park. Despite being considered an elusive and thus potentially rare species, these genetic data reveal that, at present, this Gila monster population appears to be healthy and robust within this protected environment and does not exhibit evidence of small population size or limits to gene flow from habitat fragmentation.

Our population structure analyses in STRUCTURE and GENELAND suggest one unified population with the majority of individuals assigned to one genetic grouping. The GENELAND analysis (and those few individuals that obtained Q values > 0.85 in the STRUCTURE results for $K = 3-6$) suggest another genetic cluster on the northeastern side of our sampling area. This site consisted of just a few samples ($n = 6$) from a higher-elevation drainage, geographically separate from the low-elevation areas around the loop road. This cluster was informed by very few individuals, so is not likely to represent true structure within our overall population. The individuals sampled here may be related, biasing genetic structure estimates, or more likely, may represent genetic differences along a geographic gradient, where we failed to sample the full extent of variation across the gradient. Our survey sampled a relatively limited area within the much larger contiguous population in Saguaro National Park. Additional sampling beyond our study area is necessary to understand the extent (or inhibition) of gene flow throughout the larger geographic area.

Interpretation of STRUCTURE results can be very subjective and Pritchard et al. (2000) recommend that the biology and history of the study organism be taken into consideration. The discrepancy we observed between using $L(K)$ and DeltaK to determine the best fit K we attribute to the sampled population having very limited genetic differentiation. This method has limited ability to evaluate the true number of subpopulations when population differentiation is low (Latch et al., 2006). In addition, the DeltaK method is a second-order derivative of the likelihood function of K which cannot evaluate $K = 1$ (Evanno et al., 2005), which we suggest is the best description for this nearly panmictic population. It is also possible that the signature of a population undergoing balancing selection could be indistinguishable from panmixia if the neutral markers being evaluated are linked to the genomic regions under selection

(Roy et al., 2014). This, however, is unlikely in this case since our STRUCTURE analysis excluding six loci found to deviate from Hardy-Weinberg equilibrium and which exhibited linkage disequilibrium obtained an even better fit of $K = 1$ (fig. 2). The subpopulation structure observed in the GENELAND analysis (and those few individuals that obtained Q values > 0.85 in the STRUCTURE results for $K = 3-6$) may be related to the non-random way we obtained samples. The small cluster of samples we obtained northeast of the loop road (figs 1 and 3) relative to the high density of samples obtained near the park entrance is consistent with neighbor mating forming local areas of relatedness (Schwartz and McKelvey, 2009). We favor a biologically realistic interpretation of these data: that Gila monsters in Saguaro National Park-Rincon Mountain District consist of a single, large population with relatively uninhibited gene flow (Meirns, 2015). If the 80 km² area sampled was laid out 8 km $\times$ 10 km then annual movements of >1 km by individual animals (Sullivan et al., 2004) could readily facilitate the gene flow suggested by our data for this species.

Because we only have data from one population, we cannot easily determine the root of the deviations from Hardy-Weinberg proportions we detected in six loci. However, there are multiple demographic explanations for this observation. First, Gila monster mating behavior is non-random, due to high site-fidelity of individuals. Gila monsters have been observed to return to familiar shelters and areas, especially those used by conspecifics, for mating (Beck, 2005), increasing the chances of recurring matings between individuals. Second, differences between effective population size (N_e) and purported census population size (N) suggest that not all individuals contribute equally to the next generation. Using a) the 2011 capture-recapture abundance estimate of 80 individuals along our primary 14-km sampling transect, b) a previously reported mean home range (48.1 ha;

Beck, 2005) in southern Arizona, and c) measured movements that readily include 1 km distances (Sullivan et al., 2004), we can roughly estimate (assuming the 14-km transect bisects each home range and animals occupy 0.5 km on either side) 5.7 Gila monsters (approx. 95% CI = 2.7-16.1) per km² (consistent with previously reported density estimates of 3.8-7.5 individuals/km²; see Beck, 2005) yielding an estimate of $N = 456$ (approx. 95% CI = 216-1288) for our 80 km² study area. Although sampling was somewhat different in time and space, and confidence intervals are large, if we utilize our linkage-disequilibrium estimate of effective population size ($N_e = 94$), the N_e/N ratio would be 0.21 (approx. 95% CI = 0.07-0.43), similar to the median N_e/N ratio reported across other studies (0.14; Palstra and Ruzzante, 2008). Third, Gila monsters have long life-spans (up to 17 years in the wild; Beck, 2005) throughout which they are reproductively active. This high survivorship leads to overlapping reproductive generations within a population, yet another violation of Hardy-Weinberg equilibrium assumptions. Fourth, apparent skewed sex ratios in our dataset may have led to the observed deviations, though this sex bias towards females may not represent actual sex ratios of Gila monsters in the park (and sex determination in the field is difficult in this species). Lastly, our sampling methods were targeted and opportunistic instead of random, as we searched for new specimens while tracking radio-tagged individuals or surveying in prime habitat areas.

Our non-random sample-collection strategy was necessary for an elusive species; probability of detection during 3 months of monsoon-season, summer-evening surveys was <0.01 . The majority of our sampling occurred along the Cactus loop road, a scenic 14-km loop road highly trafficked by park visitors and staff, that traverses only lowland desert habitat, but allowed us to focus our sampling efforts to areas where Gila monsters were more likely to be detected. Within our study, search effort ranged from <5 hours per Gila monster sighted along

the loop road in peak season of activity, to >30 hours per Gila monster in higher elevation, lower quality habitat, or off-season surveying.

Importantly, sampling along the loop road allowed us to examine the effects of within-park vehicle traffic on population structure. Previous studies of wildlife road mortality within Saguaro National Park report high mortality for herpetofauna, affecting wildlife conservation goals of the park (Gerow et al., 2010). Within the Gila monster population, we did not detect an association of the loop road and population structure or habitat fragmentation. The management characteristics of this road (one-way, closed at night to visitor traffic) may allow for a compromise between the park goals of conservation and accessibility, while mitigating risks to wildlife. In the adjacent suburban areas outside of National Park protection, risks are higher, as exemplified by the fact that most of our samples collected from outside of the park boundaries were roadkill.

Our findings of uninhibited gene flow within continuous habitat corroborate those from studies of comparable desert animals native to the Sonoran Desert. The Gila monster shares habitat and water sources in drainages, arroyos, and crevices with many desert fauna (Goode, 2002; Bradford et al., 2003; Edwards et al., 2004), and uses these other species as food sources (Barrett and Humphrey, 1986). Analyses of desert tortoises (*Gopherus morafkai*) in the same National Park also found evidence of high gene flow within the park (Edwards et al., 2004). Desert tortoises and Gila monsters experience similar physiological and ecological constraints in this environment and both have similar generation times, home range sizes, and shared habitat use (Barrett, 1990; Bailey, 1992; Beck, 2005). The tiger rattlesnake (*Crotalus tigris*) also shares similar life history traits and geographical distribution with the Gila monster. Goode (2002) observed high dispersal rates in tiger rattlesnakes through the rocky drainages at Saguaro National Park, which may help maintain high diversity and gene flow.

Our assessment of a stable population of Gila monsters with high diversity and uninhibited within-population gene flow has important conservation applications for this species. Our N/N_e ratio range overlaps the range recommended for conservation by Frankham et al. (2014; 0.1-0.2), and barring future population size declines, our estimated N_e is consistent with that recommended to avoid short-term inbreeding depression (Frankham et al., 2014). In addition, our estimates of N_e (and N) are from a relatively limited sampling area within a much larger contiguous population. Because the sampled population is not 'closed', pending a reduction in population size or a restriction to gene flow caused by habitat fragmentation, the greater population of Gila Monsters in Saguaro National Park and the surrounding Rincon Mountains appears to be robust. Our population, buffered from habitat loss by its inclusion within National Park boundaries and protected under Arizona law (AGFD, 2012) can act as a reference point for assessing the genetic status of other Gila monster populations under different habitat conditions. This includes populations at the edge of the species' range in Utah, USA where numbers have dropped significantly due to rapid development, habitat loss, and commercial trade of Gila monster specimens (Beck, 2005). Other populations, such as those in Maricopa County, Arizona, USA, may be undergoing significant habitat fragmentation and reduced gene flow due to increased traffic and urban sprawl from the Phoenix metropolitan area (Beck, 2005; Kwiatkowski et al., 2008). That the specimens included in this study from outside of the National Park were predominately roadkill suggests that the park may serve as a source population, now somewhat isolated from adjacent areas along its western boundary. In addition, the highly informative P(ID) of the STR markers could also be used to assess populations of origin for Gila monsters recovered from poaching and illegal trade activities.

It is important for resource managers to make the distinction between rare and elusive and this

study provides a model for working with species whose populations are challenging to estimate. Overall, the findings for this protected population of Gila monsters define a population genetic baseline for the notoriously elusive species useful for future monitoring of this protected population as well as for comparative genetic assessments of other at-risk populations. The positive implications of the protected status of this National Park population reinforce the benefits the National Park system has for preserving biodiversity (Franklin, 1993). The Gila monster stands as a charismatic, although elusive, representative for southwestern biodiversity and this new understanding of its population genetic signature provides a comparison point for future management decisions.

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References

- Arizona Game and Fish Department [AGFD] (2012): Arizona's State Wildlife Action Plan: 2012-2022. Arizona Game and Fish Department, Phoenix, Arizona.
- Bailey, S.J. (1992): *Hibemacula* use and home range of the desert tortoise (*Gopherus agassizii*) in the San Pedro Valley, Arizona. M.Sc. Thesis, University of Arizona, Tucson.
- Bandelt, H.J., Forster, P., Röhl, A. (1999): Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**: 37-48.

- Barrett, S.L. (1990): Home range and habitat of the desert tortoise (*Xerobates agassizii*) in the Picacho Mountains of Arizona. *Herpetologica* **46**: 202-206.
- Barrett, S.L., Humphrey, J.H. (1986): Agonistic interactions between *Gopherus agassizii* (Testudinidae) and *Heloderma suspectum* (Helodermatidae). *Southeast. Nat.* **31**: 261-263.
- Beck, D.D. (2005): *Biology of Gila Monsters and Beaded Lizards*. University of California Press, Berkeley.
- Bonnet, X., Guy, N., Shine, R. (1999): The dangers of leaving home: dispersal and mortality in snakes. *Biol. Conserv.* **89**: 39-50.
- Bradford, D.F., Neale, A.C., Nash, M.S., Sada, D.W., Jaeger, J.R. (2003): Habitat patch occupancy by toads (*Bufo punctatus*) in a naturally fragmented desert landscape. *Ecology* **84**: 1012-1023.
- Carson, E.W., De la Maza-Benignos, M.D., de Lourdes Lozano-Vilano, M., Vela-Valladares, L., Banda-Villanueva, I., Turner, T.F. (2014): Conservation genetic assessment of the critically endangered Julimes pupfish, *Cyprinodon julimes*. *Conserv. Genet.* **15**: 483-488.
- Cornuet, J.M., Luikart, G. (1996): Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001-2014.
- Culver, M., Johnson, W.E., Pecon-Slattery, J., O'Brien, S.J. (2000): Genomic ancestry of the American puma (*Puma concolor*). *J. Hered.* **91**: 186-197.
- Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., Ovenden, J.R. (2014): NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour.* **14**: 209-214.
- Earl, D.A., vonHoldt, B.M. (2012): STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**: 359-361.
- Edwards, T., Schwalbe, C.R., Swann, D.E., Goldberg, C.S. (2004): Implications of anthropogenic landscape change on inter-population movements of the desert tortoise (*Gopherus agassizii*). *Conserv. Genet.* **5**: 485-499.
- 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., Laval, G., Schneider, S. (2007): Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**: 47-50.
- Eyre, T.J., Wang, J., Venz, M.F., Chilcott, C., Whish, G. (2009): Buffel grass in Queensland's semi-arid woodlands: response to local and landscape scale variables, and relationship with grass, forb and reptile species. *The Rangeland Journal* **31**: 293-305.
- Feltoon, A.R., Guerra, T., Lehn, C., Forstner, M.R.J. (2007): Isolation and characterization of six microsatellites in the Mexican beaded lizard *Heloderma horridum*. *Mol. Ecol. Notes* **7**: 433-435.
- Flesch, A.D., Swann, D.E., Turner, D.S., Powell, B.F. (2010): Herpetofauna of the Rincon Mountains, Arizona. *The Southwestern Naturalist* **55**: 240-253.
- Frankham, R., Ballou, J.D., Briscoe, D.A. (2010): *Introduction to Conservation Genetics*. Cambridge University Press, New York.
- Frankham, R., Bradshaw, C.J.A., Brook, B.W. (2014): Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria, and population viability analyses. *Biol. Conserv.* **170**: 56-63.
- Franklin, J.F. (1993): Preserving biodiversity: species, ecosystems, or landscapes? *Ecol. Appl.* **3**: 202-205.
- Friar, E.A., Ladoux, T., Roalson, E.H., Robichaux, R.H. (2000): Microsatellite analysis of a population crash and bottleneck in the Mauna Kea silversword, *Argyroxiphium sandwicense* ssp. *Sandwicense* (Asteraceae), and its implications for reintroduction. *Mol. Ecol.* **9**: 2027-2034.
- Garcia-Moreno, J., Matocq, M.D., Roy, M.S., Geffen, E., Wayne, R.K. (1996): Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. *Conserv. Biol.* **10**: 376-389.
- Gerow, K., Kline, N.C., Swann, D.E., Pokorny, M. (2010): Estimating annual vertebrate mortality on roads at Saguaro National Park, Arizona. *Human-Wildlife Interactions* **4**: 283-292.
- Gilbert, K.J., Whitlock, M.C. (2016): Evaluating methods for estimating local effective population size with and without migration. *Evolution* **69**: 2154-2166.
- Goldberg, C.S., Kaplan, M.E., Schwalbe, C.R. (2003): From the frog's mouth: buccal swabs for collection of DNA from amphibians. *Herp. Rev.* **34**: 220-221.
- Goode, M. (2002): Ecology and management of tiger rattlesnakes at Rocking K Development site near Saguaro National Park. Western National Parks Association, Oro Valley, Arizona, USA. Unpublished report. <http://cals.arizona.edu/research/goode/Papers/Final%20Report-WPNA%201998.pdf>.
- Goudet, J. (1995): FSTAT (version 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**: 485-486.
- Grativol, A.D., Ballou, J.D., Fleischer, R.C. (2001): Microsatellite variation within and among recently fragmented populations of the golden lion tamarin (*Leontopithecus rosalia*). *Conserv. Genet.* **2**: 1-9.
- Guillot, G., Mortier, F., Estoup, A. (2005): GENELAND: a computer package for landscape genetics. *Mol. Ecol. Notes* **5**: 712-715.
- Gutierrez-Espeleta, G.A., Kalinowski, S.T., Boyce, W.M., Hedrick, P.W. (2000): Genetic variation and population structure in desert bighorn sheep: implications for conservation. *Conserv. Genet.* **1**: 3-15.
- Haig, S.M., Wagner, R.S., Forsman, E.D., Mullins, T.D. (2001): Geographic variation and genetic structure in spotted owls. *Conserv. Genet.* **2**: 25-40.
- Hammerson, G.A., Frost, D.R., Gadsden, H. (2007): *Heloderma suspectum*. The IUCN Red List of Threatened Species. Version 2014.3. <http://www.iucnredlist.org/details/9865/0>. Accessed 22 December 2014.
- Hardy, O.J., Vekemans, X. (2002): SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Notes* **2**: 618-620.

- Helgen, K.M., Pinto, C.M., Kays, R., Helgen, L.E., Tsuchiya, M.T.N., Quinn, A., Wilson, D.E., Maldonado, J.E. (2013): Taxonomic revision of the olingos (*Bassaricyon*), with description of a new species, the olinguito. *Zookeys* **324**: 1-83.
- Hess, M.R., Edwards, T., Edmunds, D.A., Bonine, K.E. (2013): Characterization of STR/microsatellite primers for the Gila monster, *Heloderma suspectum* screened from paired-end Illumina shotgun sequencing. *Conserv. Genet. Resour.* **5**: 1121-1123.
- Jakobsson, M., Rosenberg, N.A. (2007): CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801-1806.
- Kwiatkowski, M.A., Schuett, G.W., Repp, R.A., Nowak, E.M., Sullivan, B.K. (2008): Does urbanization influence the spatial ecology of Gila monsters in the Sonoran Desert? *J. Zool.* **276**: 350-357.
- Latch, E.K., Dharmarajan, G., Glaubitz, J.C., Rhodes, O.E. (2006): Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv. Genet.* **7**: 295-302.
- Ley, A., Hardy, O.J. (2013): Improving AFLP analysis of large-scale patterns of genetic variation – a case study with the Central African lianas *Haumania* spp (Marantaceae) showing interspecific gene flow. *Mol. Ecol.* **22**: 1984-1997.
- Librado, P., Rozas, J. (2009): DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451-1452.
- Lowe, C.H., Schwalbe, C.R., Johnson, T.B. (1986): The Venomous Reptiles of Arizona. Arizona Game and Fish Department, Phoenix, AZ, USA.
- Marshall, T.C., Spalton, J.A. (2000): Simultaneous inbreeding and outbreeding depression in reintroduced Arabian oryx. *Animal. Conserv.* **3**: 241-248.
- Martínez-Cruz, B., Godoy, J.A., Negro, J.J. (2007): Population fragmentation leads to spatial and temporal genetic structure in the endangered Spanish imperial eagle. *Mol. Ecol.* **16**: 477-486.
- Miermans, P.G. (2015): Seven common mistakes in population genetics and how to avoid them. *Mol. Ecol.* **24**: 3223-3231.
- Ohta, T., Kimura, M. (1973): Model of mutation appropriate to estimate number of electrophoretically detectable alleles in a finite population. *Genet. Res.* **22**: 201-204.
- O'Ryan, C., Flamand, J.R.B., Harley, E.H. (1994): Mitochondrial DNA variation in black rhinoceros (*Diceros bicornis*): conservation management implications. *Conserv. Biol.* **8**: 495-500.
- Paetkau, D., Strobeck, C. (1994): Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.* **3**: 489-495.
- Palstra, F.P., Ruzzante, D.E. (2008): Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol. Ecol.* **17**: 3428-3447.
- Piry, S., Luikart, G., Cornuet, J.M. (1999): BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered.* **90**: 502-503.
- Pritchard, J.K., Stephens, M., Donnelly, P. (2000): Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959.
- Rasoloarison, R.M., Weisrock, D.W., Yoder, A.D., Rakoton-dravony, D., Kappeler, P.M. (2013): Two new species of mouse lemurs (Cheirogaleidae: *Microcebus*) from eastern Madagascar. *Int. J. Primatol.* **34**: 455-469.
- Rhodes, O.E., Reat, E.P., Heffelfinger, J.R., deVos, J.C. (2001): Analysis of reintroduced pronghorn populations in Arizona using mitochondrial DNA markers. In: Proceedings of the 19th Biennial Pronghorn Antelope Workshop, vol. 19, p. 45-54. Cancino, J., Ed., Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico.
- Rice, W.R. (1989): Analyzing tables of statistical tests. *Evolution* **43**: 223-225.
- Robichaux, R.H., Friar, E.A., Mount, D.W. (1997): Molecular genetic consequences of a population bottleneck associated with reintroduction of the Mauna Kea silversword (*Argyroxiphium sandwicense* ssp. *sandwicense* [Asteraceae]). *Conserv. Biol.* **11**: 1140-1146.
- Rosenberg, N.A. (2004): DISTRUCT: a program for the graphical display of population structure. *Mol. Ecol. Notes* **4**: 137-138.
- Rousset, F. (2000): Genetic differentiation between individuals. *J. Evol. Biol.* **13**: 58-62.
- Roy, D., Hardie, D.C., Treble, M.A., Reist, J.D., Ruzzante, D.E. (2014): Evidence supporting panmixia in Greenland halibut (*Reinhardtius hippoglossoides*) in the north-west Atlantic. *Can. J. Fish. Aquat. Sci.* **71**: 763-774.
- Schwartz, M., McKelvey, K. (2009): Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. *Conserv. Genet.* **10**: 441-452.
- Sirios, A.M., Gibbs, J.P., Whitlock, A.L., Erb, L.A. (2014): Effects of habitat alterations on bog turtles (*Glyptemys mühlenbergii*): a comparison of two populations. *J. Herpetol.* **48**: 455-460.
- Slatkin, M., Maddison, W.P. (1990): Detecting isolation by distance using phylogenies of genes. *Genetics* **126**: 249-260.
- Slatkin, M., Excoffier, L. (1996): Testing for linkage disequilibrium in genotypic data using the expectation-maximization algorithm. *Heredity* **76**: 377-383.
- Sullivan, B.K., Kwiatkowski, M.A., Schuett, G.W. (2004): Translocation of urban Gila monsters: a problematic conservation tool. *Biol. Conserv.* **117**: 235-242.
- Tajima, F. (1989): Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.
- Valière, N. (2002): GIMLET: a computer program for analysing genetic individual identification data. *Mol. Ecol. Notes* **2**: 377-379.

- van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P. (2004): MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**: 535-538.
- Waits, L.P., Luikart, G., Taberlet, P. (2001): Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol. Ecol.* **10**: 249-256.
- Watterson, G.A. (1975): On the number of segregating sites in genetical models without recombination. *Theor. Popul. Biol.* **7**: 256-276.
- White, G.C., Burnham, K.P. (1999): Program MARK: survival estimation from populations of marked animals. *Bird Study* **46** (Supplement): 120-138.
- Woods, J., Paetkau, D., Lewis, D., McLellan, B., Proctor, M., Strobeck, C. (1999): Genetic tagging of free-ranging black and brown bears. *Wildl. Soc. Bull.* **27**: 616-627.
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