Contribution Paper

Genetic diversity and structure in Arizona pronghorn following conservation efforts

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Funding information
DOI USGS, Grant/Award Number: RWO#61

Abstract
Arizona pronghorn (Antilocapra americana) population numbers have declined over the last century due to unregulated-harvest, population fragmentation, urban expansion, and habitat loss. Captive breeding, reintroductions, and translocations have helped to curb decline and boost population numbers of the endangered Sonoran subspecies (A. a. sonoriensis). To assess the effect of ongoing management actions on the Sonoran subspecies, we collected multi-locus genotype data and performed tests of genetic differentiation and population structure in comparison to the non-endangered American subspecies (A. a. americana). We provide updated estimates of genetic diversity and relatedness to serve as a benchmark for future management toward further recovery of Sonoran pronghorn. Management actions have upheld distinction between the two subspecies in Arizona and stemmed further genetic diversity loss while avoiding an increase in inbreeding within the captive-bred Sonoran population.

KEYWORDS
Antilocapra americana, Antilocapridae, captive-breeding, inbreeding, microsatellites, translocation

1 | INTRODUCTION

A major goal of species conservation programs is to maintain populations with high genetic variation while minimizing inbreeding to avoid the risk of inbreeding depression (Hedrick & Kalinowski, 2000; Witzenberger & Hochkirch, 2011). Ideally, local population extinctions would be remedied with the introduction of a large number of locally-adapted but genetically diverse individuals. Unfortunately, this is rarely possible due to limitations in sources of individuals for translocation, particularly when captive-breeding has been deemed necessary. Wildlife managers weigh the risks of creating outbred populations that may have lost local adaptations (Frankham et al., 2011) versus the benefits of genetic rescue that may come from merging populations suffering from inbreeding depression (Tallmon, Luikart, & Waples, 2004). The acts of merging and translocating populations have the effect of altering population differentiation and structure. Population history must be taken into account when interpreting genetic structure in recovering populations and repeated sampling is necessary to track the effects of management actions.

The history of pronghorn (Antilocapra americana) in North America exemplifies the need to carefully interpret genetic structure in light of long-term management actions. Four subspecies of pronghorn are currently recognized: American (A. a. americana), Mexican (A. a. mexicana), Peninsular (A. a. peninsularis), and Sonoran (A. a. sonoriensis) (Lee, Bickham, & Scott, 1994). Following the
arrival of Europeans to North America, pronghorn habitat was greatly reduced (Cancino, Ortega-Rubio, & Rodríguez-Estrella, 1998; Laliberte & Ripple, 2004) and population numbers plummeted from the tens of millions to an estimate of 30,000 in 1925 (Nelson, 1925). Over the course of the mid-20th century, re-stocking programs, predator control activities, and implementation of hunting regulations supported resurgence in pronghorn population numbers (Kitchen & O’Gara, 1982; Yoakum, 2004). In the southern terminus of the species range, however, the continued decline of pronghorn (Gedir, Cain, Harris, & Turnbull, 2015) has necessitated intervention.

The state of Arizona is on the front line of efforts to restore pronghorn to their native range. Two pronghorn subspecies, American and Sonoran, are native to Arizona (Lee et al., 1994). The nominate subspecies, American, is the most widespread of all four pronghorn subspecies, historically ranging throughout much of the western half of the continent from southern Canada to northern Arizona, northern New Mexico, and northern Texas (Lee et al., 1994; Nelson, 1925). The range of Sonoran pronghorn once encompassed southwestern Arizona, southeastern California, northeastern Baja California, and northwestern Sonora, Mexico (Arizona Game and Fish Department, 1981). The distinctiveness of the two subspecies has been contentious. The original subspecies description for Sonoran pronghorn, put forth on the basis of morphological examination of just two specimens (Goldman, 1945), noted their “smaller size; paler color; and a smaller skull that differed in detail”. Relative scarcity of Sonoran pronghorn historical specimens prohibits clarification of the Arizona subspecies on the basis of morphology.

Population numbers for Sonoran pronghorn, particularly in the United States, have been critically low for several decades, prompting the listing of the subspecies as endangered in 1967 (U.S. Fish and Wildlife Service, 1967). Following a population crash to an estimated 21 individuals in 2002, a captive breeding program was instituted at the Cabeza Prieta National Wildlife Refuge (CPNWR) in southwestern Arizona (U.S. Fish and Wildlife Service, 2010; Otte, 2006). Between 2004 and 2006, 14 wild individuals were introduced to two adjacent pens at CPNWR encompassing 260 ha (642 acres) of Sonoran Desert habitat. Six of the founding individuals were captured from Sonora, Mexico (1 male, 5 females) and 8 from southern Arizona (1 male, 7 females) in an attempt to introduce genetic diversity from multiple populations to the captive herd. In accordance with the Sonoran pronghorn recovery plan (U.S. Fish and Wildlife Service, 2016), genetic diversity has been subsequently preserved through maintenance of a large population size and gene flow between the captive pens and the released individuals. The time between 2004 and 2006 includes 2 generations for male pronghorn and 1.5 generations for female pronghorn as both sexes are capable of breeding as yearlings but young males are preventing from mating by more dominant older males. Each pen contains females and non-breeding age males. One breeding male is moved between pens to maintain gene flow. A single breeding male may breed for several seasons before being replaced and released to the wild (Jill Bright, Arizona Game and Fish Department, personal communication). Between 2010 and 2014, approximately 78 fawns were produced per 100 females (Bright & Hervert, 2005). With captive-breeding and reintroduction activity now established, the Arizona population of Sonoran pronghorn is on the rebound with an estimated 202 wild individuals as of January 2015 (U.S. Fish and Wildlife Service, 2016). Annual collection of genetic material from the captive herd now facilitates genetic monitoring to track fluctuations in genetic diversity and inbreeding. While we are not aware of any evidence to suggest that the captive herd is currently experiencing inbreeding depression, changes in estimated inbreeding over time would indicate the need for more intensive investigation into inbreeding depression risk.

The presence of the American subspecies in Arizona provides an opportunity for genetic comparison with the recovering endangered subspecies. To date, no species-wide genetic study of pronghorn has been conducted but estimations of differentiation between the subspecies have been made. A survey of mitochondrial haplotype diversity among populations of Arizona pronghorn uncovered differences between populations, correlated with reintroduction history (Rhodes Jr., Reat, Heffelfinger, & DeVos Jr., 2001). Stephen et al. (2005) conducted a direct comparison of American pronghorn to the Arizona and Sonora, Mexico populations of Sonoran pronghorn prior to the establishment of the captive herd at CPNWR. Their results supported a minor distinction of Sonoran from American pronghorn through the processes of recent isolation, repeated bottlenecks, and drift. They estimated moderate differentiation between the Sonora, Mexico, and Arizona populations of Sonoran pronghorn but little differentiation between central Arizona populations of American pronghorn. The commencement of captive breeding for Sonoran pronghorn necessitates the reassessment of essential population genetic parameters pertaining to subspecies differentiation.

Captive breeding and translocation activity have likely altered the population genetic landscape of pronghorn in Arizona (Rhodes Jr. et al., 2001). Here, we present updated estimates of genetic diversity and inbreeding within Sonoran pronghorn to benefit the continued recovery of the subspecies. With an expanded set of microsatellite loci, we tested for genetic differentiation to reevaluate the established subspecies distinction. We estimated inbreeding, effective
population size, and relatedness for Arizona pronghorn populations to provide benchmarks and guidance for future management of the species.

2 METHODS

In collaboration with the Arizona Game and Fish Department (AGFD) and the Sonoran pronghorn recovery team, we sampled captive Sonoran pronghorn within the CPNWR herd (Figure 1) in the years 2009 through 2014. To avoid repeated sampling of adult females, we included only samples from fawns each year (N = 111 individuals). Additionally, we were able to sample 6 of the 14 original founders (4 Mexico and 2 United States, all female). We received 92 American pronghorn samples from adults captured in the years 2010 through 2014 at sites across northern and central Arizona (Figure 1).

We sampled all individuals with blood, storing approximately 1 ml of whole blood in either TES buffer (100 mM Tris, 100 mM EDTA, 2% SDS; 2 parts blood to 1-part buffer) or collection tubes containing EDTA. We lysed the samples overnight at 55°C with agitation in 650 uL lysis buffer (50 mM tris pH 8.0, 50 mM EDTA, 25 mM sucrose, 100 mM NaCl, 1.0% SDS) and 25 uL 10 mg/ml proteinase K. To extract DNA from the lysate, we employed a standard phenol:chloroform:isoamylalcohol (25:24:1) protocol utilizing light and heavy Phase Lock Gel tubes (5 PRIME, Gaithersburg, MD). We precipitated DNA with 3 M NaOAc and isopropanol then performed ethanol washes to further purify the DNA before re-suspending in low TE, pH 8.

We genotyped all individuals with a panel of 16 total microsatellite loci (Data S1). We deployed two high-throughput multiplex assays as previously described (Hahn, Klimova, Munguía-Vega, Clark, & Culver, 2020) to genotype samples at 13 microsatellite loci (Carling, Passavant, & Byers, 2002; Dunn et al., 2010; Lou, 1998; Munguía-Vega, Klimova, & Culver, 2013). We ran multiplex PCRs for three more loci; Aam4, Aam6, and Aam8 (Carling et al., 2002). We conducted monoplex PCRs in 15 uL volumes containing 20 ng of DNA and a final concentration of 1 × Invitrogen Buffer, 0.25 uM each fluorescently labeled forward primer and unlabeled reverse primer, 200 uM dNTPs, either 4 mM (Aam4 and Aam8) or 2 mM (Aam6) MgCl2, and 0.5 U Invitrogen Taq polymerase. We used the following cycling protocol for PCR with these three loci: 94°C for 5 min; 30 cycles of 94°C for 30 s, 55°C/50°C/45°C (Aam4/Aam6/Aam8) for 30 s, and 72°C for 30 s; then 72°C for 5 min. We performed microsatellite fragment analysis on the ABI3730 DNA Analyzer platform and called alleles in Genemarker. We re-attempted amplification a minimum of 2 times for any samples that initially failed to amplify at a given locus. For every 96-well plate run, we included two or more replicate samples to compare the resulting genotypes with established controls. In instances where the control’s genotype differed from the previously observed genotype for that sample, we proceeded to collect triplicate runs of the entire plate from which we assigned consensus genotypes to all samples. To test for large allele drop out and the presence of null alleles, we ran Microchecker v.2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). We tested for deviation from Hardy Weinberg expectations (HWE) and calculated pairwise linkage disequilibrium (LD) in Genepop (Rousset, 2008).

We calculated summary statistics and tested for population differentiation (FST and GST) by performing permutation tests with 9,999 permutations in Genalex v6.502 (Peakall & Smouse, 2006, 2012). We calculated Nei’s distance (DA) (Nei, Tajima, & Tateno, 1983) in the R environment (R version 3.2.4, http://cran.r-project.org/, accessed March 15, 2016) using the dist.genpop function in hierfstat v0.04-22 (Goudet, 2005). We performed principal coordinate analyses (PCoA) upon genetic dissimilarity matrices calculated following Smouse and Peakall (1999) in R using the gd.smouse function in Popgenreport v2.0 (Adamack & Gruber, 2014). To estimate effective population size (Ne) for both subspecies, we ran Onesamp (Tallmon, Koyuk, Luikart, & Beaumont, 2008; Tallmon, Luikart, & Beaumont, 2004). Onesamp combines eight summary statistics in an approximate Bayesian computational framework to
produce estimates of $N_e$ for single populations. We bounded the uniform $N_e$ prior to between 2 and 5,000 for both subspecies and performed 50,000 iterations.

We investigated population structure with a Bayesian clustering algorithm in Structure v2.3.4 (Pritchard, Stephens, & Donnelly, 2000). We ran Structure on the entire dataset without sampling location priors to investigate pronghorn population structure at the subspecies level within the regions sampled in Arizona. We employed the admixture model and assumed correlated allele frequencies to test for $K = 1$–$10$ with 20 iterations of each possible $K$. After an initial 200,000 burn-in generations we ran the analysis for 800,000 generations. We visualized Structure results and plotted the best $K$ estimation (Evanno, Regnaut, & Goudet, 2005) with Clumpak (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015).

We estimated relatedness among all sampled individuals for each subspecies with the R package Related (Pew, Muir, Wang, & Frasier, 2015), which implements the code within Coancestry (Wang, 2011) and enables comparison of seven relatedness estimators. We used the compare estimators function to test relative performance of the Wang (Wang, 2002), lynchli (Li, Weeks, & Chakravarti, 1993), lynchrd (Lynch & Ritland, 1999), and quellergt (Queller & Goodnight, 1989) relatedness estimators through comparison of observed values to expected values generated from a simulated sample set of 400 individuals of known relatedness (100 each of parent-offspring, full-sib, half-sib, and unrelated pairs). To generate $p$-values for our observed relatedness estimates, we used the familysim and coancestry functions to calculate the percentage of runs within 500 iterations with the simulated data where the computed expected value was greater than or equal to our observed value.

We estimated inbreeding coefficients ($F_{IS}$) in the R package DiveRsity (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013). DiveRsity allows for the assessment of the biological significance of observed differences through the calculation of 95% confidence intervals with an integrated bootstrapping procedure. We estimated annual $F_{IS}$ within the Sonoran pronghorn captive herd to investigate differences in $F_{IS}$ over time and compare with $F_{IS}$ of the American subspecies as a whole.

3 | RESULTS

All 16 microsatellite loci were polymorphic in both subspecies. As a first round of quality control, we eliminated four Sonoran samples and eight American samples with which we had poor amplification success. All remaining samples amplified with 14 or more loci and our amplification success rate for individual loci ranged from 91.9 to 100% with 14 loci amplifying successfully in more than 97% of all samples (Supporting Information). Two loci, Anam13 and Aam17, demonstrated consistent LD ($p < .05$, Bonferroni corrected) and deviations from HWE and were subsequently removed from further analyses. Accounting for the removal of these loci and samples, our genotyping success (percentage of samples genotyped at a given locus) was greater than 95% for the remaining 14 loci. We found no evidence for null alleles or allelic drop out within our final dataset. Overall, subspecies level summary statistics indicate lower diversity in the Sonoran subspecies relative to the American subspecies (Table 1). We also calculated summary statistics for Sonoran pronghorn stratified into two populations corresponding to the north and south pens (Table 1). We estimated mean $N_e$ = 1,623.56 (95% CL = 610.56–12,541) for American pronghorn and mean $N_e$ = 49.41 (95% CL = 31.18–140.49) for Sonoran pronghorn from the posterior distribution of $N_e$ calculated by Onesamp.

We assessed significance of observed difference in $F_{IS}$ through the examination of bootstrap confidence interval size and overlap. Confidence intervals reflect the sample size as well as the standard deviation of the data and therefore encompass more information from which to draw conclusions about the biological significance of observed differences (reviewed in Ranganathan, Pramesh, & Buyse, 2015). We deemed $F_{IS}$ estimates with non-overlapping confidence intervals to be significantly different. Excluding 2009 due to insufficient sample size ($n = 6$), our annual estimates of inbreeding for Sonoran pronghorn ranged from $F_{IS} = −0.090$ in 2013 to $F_{IS} = 0.124$ in 2012 (Figure 2b). $F_{IS}$ estimates were made for the pens combined because no structure was observed between the two pens ($F_{ST} = 0.028$, $G_{ST} = 0.050$). Our estimate of inbreeding in 2012 was significantly higher than for 2013. Differences we observed in $F_{IS}$ for the remaining sampling years were not significant. We missed sampling a portion of fawns in 2012. Therefore, it is plausible that the observed increase in $F_{IS}$ in 2012 is the result of differences in the extent of sampling of the population. Our $F_{IS}$ estimates for Sonoran pronghorn in 2010, 2013, and 2014 are significantly lower than for American pronghorn ($F_{IS} = 0.128$, Figure 2a).

We observed high correlation ($R = 0.83–0.87$) between observed and expected values with each of the four estimators used to examine relatedness. We further report relatedness with the Wang estimator, which performed best with our data based on comparisons between observed and simulated values. Overall average relatedness within the captive Sonoran pronghorn fawns born between 2010 and 2014 was 0.311, which falls between that expected for half and full siblings. Relative to our estimated overall average relatedness of 0.06 within American pronghorn, relatedness in Sonoran pronghorn...
was very high. Average relatedness in Sonoran pronghorn was higher than expected \((p = 0.002)\) while in American pronghorn it was not different from expected \((p = 0.142)\).

To examine subspecies differentiation, we estimated both \(F_{ST}\) and Hedrick’s standardized \(G'_{ST}\), which was derived to overcome \(F_{ST}\)’s susceptibility to underestimation of differentiation from data containing highly variable loci (Hedrick, 2005). Our estimates of \(F_{ST}\) and \(G'_{ST}\) agreed in terms of the magnitude of differences observed between pairwise comparisons. We observed strong genetic differentiation \((F_{ST} = 0.137, G'_{ST} = 0.779, N_e = 33)\) between Sonoran and American pronghorn.

However, we caution that the assemblage of samples we obtained is not completely representative of either subspecies. We extensively sampled the captive Sonoran population, which includes representative founders from Arizona and Mexico, but we did not include samples from the Mexico populations. Likewise, our sampling of American pronghorn was limited to a portion of the wide-ranging subspecies’ range in Arizona. We observed very little differentiation between the north and south pens of the captive Sonoran population \((F_{ST} = 0.028, G'_{ST} = 0.050)\).

We performed PCoA upon distance matrices calculated from genotypes of both subspecies (Figure 3). PCoA clearly demonstrates two genotypic clusters associated with the two subspecies. In this analysis, we also...
included the genotypes of the six sampled founders but we did not detect clear differentiation between Sonora, Mexico, and Arizona, USA founders, perhaps due to our small sample size. Our Structure analysis of subspecies population structure strongly predicted two populations corresponding precisely with the two subspecies (Figure 4 and Data S2).

4 | DISCUSSION

We investigated the impact of ongoing conservation efforts upon captive-bred Sonoran pronghorn in Arizona through measurement of inbreeding, relatedness, genetic diversity and differentiation from American pronghorn in Arizona. We report no substantial differences in population genetic parameter estimations for the herds in the adjacent north and south pens, suggesting that captive management protocols have not resulted in unintentional differentiation. Inbreeding is often a significant concern for endangered species and, especially, captive bred populations. Stephen et al. (2005) previously reported higher inbreeding within Sonoran pronghorn relative to American pronghorn from samples collected in Arizona between 1996 and 2000. To measure changes in inbreeding over time, we estimated $F_{IS}$ on an annual basis from 2010 to 2014 for the endangered Sonoran population. Over this five-year period, when annual $F_{IS}$ for Sonoran pronghorn was significantly different relative to American pronghorn, it was significantly lower (Figure 2). Differences between our subspecies $F_{IS}$ estimates and those made by Stephen et al. (2005) may be a product of the difference in genetic markers used as only three loci are shared between the two studies. However, changes in relative differences in $F_{IS}$ between the two subspecies, are more likely due to admixture events occurring when the captive Sonoran pronghorn herd was founded. The 2005 study was conducted prior to formation of the captive Sonoran pronghorn herd, which involved admixture of individuals from Arizona and Sonora, Mexico. The apparent reduction in inbreeding for Sonoran pronghorn relative to American pronghorn may therefore be a direct result of the management decision to source the captive herd’s founders from multiple populations. Introduction of breeding stock from Mexico may have also contributed to an increase in genetic diversity relative to levels previously observed for Sonoran pronghorn in Arizona. Comparison of $F_{IS}$ between the two subspecies is further complicated by existing population substructure in the Arizona populations of American pronghorn. While our study design lacked sufficient sample numbers to address substructure, previous studies have observed population structure associated with habitat division by highways in Northern Arizona (Theimer, Sprague, Eddy, & Benford, 2012). To account for this substructure as well as the Sonoran pronghorn’s reintroduction history, more extensive sampling of both the United States and Mexican populations is needed to monitor inbreeding and confirm the potential effect of admixing populations within the captive herd.

Although we estimated relatively low levels of inbreeding within the captive Sonoran herd, we observed higher relatedness estimates than expected of an outbred population. Our estimated relatedness coefficient of 0.311 for all captive-born fawns born between 2010 and 2014 is consistent with breeding management practices wherein a single male sires all fawns within an individual pen each year. Thus, it is encouraging that overall average relatedness within the captive herd is only marginally greater than that expected of half-siblings. The individuals within the captive herd are also related to one another from having descended from the original 14 founders. Unfortunately, we were unable to reliably estimate relatedness, genetic diversity and $N_e$ for the founding population due to having sampled only six founding females. However, if relatedness among the founders was low to begin with and the founding population contained genetic variation from both Arizona and Mexico, then higher heterozygosity could be observed within the captive herd than may have existed in either Arizona or Mexico prior to captive breeding. Higher heterozygosity, even among related individuals, will result in lower $F_{IS}$ estimates but only if breeding of related individuals is minimized. Furthermore, $N_e$ for Sonoran

**FIGURE 4** Estimated pronghorn population structure. We carried out Bayesian Structure analysis from 14 microsatellite loci. Vertical bars represent individuals partitioned into $K$ colored segments denoting estimated membership fractions. (a) Results for $K = 2$ from analysis of Sonoran and American pronghorn.
pronghorn is substantially lower than for American pronghorn, putting the endangered population at greater risk of genetic diversity loss via drift. To preserve genetic diversity and avoid inbreeding and the effects of inbreeding depression, relatedness estimates can be taken into account when selecting individuals for the re-establishment of wild Sonoran pronghorn populations.

Genetic diversity, as measured by observed heterozygosity (\(H_0\)), average number of alleles observed/locus (\(N_a\)), Shannon’s Diversity Index (SDI) and the number of private alleles (\(N_p\)) is lower within the endangered Sonoran subspecies relative to the American subspecies (Table 1). Comparing our results to diversity estimates made prior to the formation of the captive-breeding herd at CPNWR (Stephen et al., 2005), we observed that while allelic richness (\(A_R\)) has not changed considerably, there is presently less difference in HO between the two subspecies. Several interacting factors may have contributed to our observation of reduced differences in HO. Admixture in the formation of the captive herd and subsequent direct management of breeding pairs may have caused an increase in HO relative to previous estimates for the endangered subspecies. Simultaneously, despite recent American pronghorn population growth in Arizona, prolonged reduction of \(N_e\) relative to historical levels may be suppressing \(H_0\) within the subspecies.

Although we observed less divergent \(H_0\), we observed an increase in subspecies differentiation (\(F_{ST} = 0.137\)) relative to a previous estimate of differentiation (\(F_{ST} = 0.104\)) made by Stephen et al. (2005). These seemingly contradictory results are likely due to sampling and methodological differences. Stephen et al. (2005) sampled a similar number of American pronghorn individuals in central Arizona (\(n = 83\) versus \(n = 84\)) and fewer Arizona Sonoran pronghorn individuals (\(n = 24\) vs. \(n = 107\)). Additionally, we surveyed more microsatellite loci (14 vs. 5). With our expanded survey of 14 microsatellite loci, which includes 3 of the 5 loci used by Stephen et al., we conducted additional subspecies level structure analyses including PCoA (Figure 3) and implementation of the Bayesian clustering algorithm in Structure (Figure 4 and Data S2) and both analyses indicated strong divergence between subspecies. At present, it is unknown how diversity in the rehabilitating Sonoran pronghorn population in Arizona compares with wild populations in Mexico. Exploring the significance of this observed subspecies divergence in more detail on a species-wide scale to include populations in Mexico, American pronghorn populations throughout more of the subspecies’ range, as well as Peninsular and Mexican pronghorn would be beneficial for characterizing pronghorn genetic variation.

Local extirpations and prolonged population decline have necessitated conservation intervention to support persistence of Sonoran pronghorn in Arizona. A variety of management actions have been taken to boost population numbers, including: captive-breeding, translocation, predator abatement, and habitat rehabilitation (Arizona Game and Fish Department, 2013). When population numbers of endangered species reach critically low numbers, wildlife managers often decide between two courses of action, (a) preserve population distinction that may represent local adaptation at the risk of increasing inbreeding or (b) merge populations to avoid inbreeding and risk the loss of local adaptation (Frankham et al., 2011). In the case of Sonoran pronghorn, managers weighed the options and chose to merge populations. It was determined that formation of a viable captive breeding herd required sourcing founders from Arizona as well as Sonora, Mexico. Our analyses indicate that this management decision has resulted in a captive population with stable levels of genetic diversity, relatively stable levels of inbreeding, and the retention of previously observed differentiation from American pronghorn. While the apparent reduction in \(F_{IS}\) for the Sonoran herd relative to the American subspecies compared to previous estimates may be a result of sampling differences, the stable inbreeding levels we observed within the captive Sonoran herd indicates that captive breeding practices are not contributing to an increase in inbreeding. Further study of phenotypic traits in comparison with population genetic parameters may provide additional insight into the inbreeding depression risk in pronghorn populations having undergone population bottlenecks.

**ACKNOWLEDGMENTS**

We thank USFWS and AGFD for providing sampling opportunities for *A. a. sonoriensis* and A. Justice-Allen, in particular, for providing samples for *A. a. americana*. We explicitly thank K. Vargas for assisting with laboratory work and J. Heffelfinger and T. Edwards for providing comments that greatly improved the manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Funding for this project was provided by DOI USGS RWO#61.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

Erin Hahn: Conceptualization (supporting); formal analyses; methodology (lead); visualization; writing—original draft preparation; writing—review and editing (lead). Melanie Culver: Conceptualization (lead); funding acquisition; methodology (supporting); resources; supervision; writing—review and editing (supporting).
ETHICS STATEMENT
The authors certify that the material presented here is original work which has not been previously published. All biological samples were collected during routine herd management operations as part of a study plan approved by the Arizona Game and Fish Department (AGFD).

SOCIAL MEDIA SUMMARY
Captive-breeding of Sonoran pronghorn has maintained genetic diversity and subspecies distinction whilst avoiding increased inbreeding.

DATA AVAILABILITY STATEMENT
Data generated that support the findings of this study are available from the corresponding author upon reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article**: Hahn, E. E., & Culver, M. (2021). Genetic diversity and structure in Arizona pronghorn following conservation efforts. *Conservation Science and Practice*, 3(10), e498. https://doi.org/10.1111/csp2.498