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# Genetic diversity and demography of two endangered captive pronghorn subspecies from the Sonoran Desert

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Species that have experienced population reduction provide valuable case studies for understanding genetic responses to demographic change. Pronghorn (*Antilocapra americana*) were once widespread across the North American plains but were subject to drastic population reductions due to overexploitation and habitat fragmentation during the late 19th and early 20th centuries. *A. a. peninsularis* and *A. a. sonoriensis*, 2 pronghorn subspecies that inhabit the southern edge of the species' distribution, are almost extinct and now breed almost exclusively in captivity. We therefore sequenced the complete mitochondrial control region and genotyped 18 microsatellite loci in 109 individuals to evaluate the impact of population bottlenecks, captive breeding, small population sizes, and isolation on the genetic composition of captive populations of these 2 subspecies. We found extremely low levels of genetic diversity in both subspecies. The 2 subspecies showed high and significant genetic differentiation, indicating the absence of historic and recent gene flow despite their geographic proximity within the Sonoran Desert. Historical effective population size estimates for the 2 subspecies were inferred to be similar, whereas the Sonoran pronghorn has a contemporary effective size ($N_e$) more than twice as high as the Peninsular subspecies. Our findings suggest the need for careful genetic management of both subspecies in order to minimize the further loss of genetic variability.

Key words: *Antilocapra americana*, captive breeding, genetic diversity, population differentiation, Sonoran Desert


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Populations and subspecies inhabiting the edges of a species' distribution can be important because they often exhibit lower genetic diversity and greater genetic differentiation from central populations due to extended geographic isolation, small effective population sizes ($N_e$s), and the effects of population bottlenecks and drift (Johannesson and André 2006; Eckert et al. 2008). Additionally, peripheral populations are of conservation and management concern because they have a greater likelihood of suffering from a local extinction in the short term (Frankham et al. 2002). It has been widely recognized that the rate of species extinction is increasing and that many species are in imminent danger of extinction. Anthropogenic exploitation, habitat degradation and fragmentation, and the introduction of nonnative species (such as pathogens, parasites, predators, and competitors) have been identified as major factors responsible for these extinctions and declines (Wilson 2010).

The New World family Antilocapridae has only 1 extant species, the pronghorn (*Antilocapra americana*). Nevertheless,

there are fossil records of at least 18 genera dating back to around 20 million years ago (early Miocene—McKenna and Bell 1997; Heffelfinger et al. 2004). The pronghorn represents a unique evolutionary branch within the ungulate clade Pecora (Hassanin et al. 2012). It has therefore been the subject of extensive research dealing with its basic biology, ecology, taxonomy, and management, as well as with its long and intricate interactions with humans (McCabe et al. 2004; O'Gara and Yoakum 2004). Four pronghorn subspecies are currently recognized, which are distributed across the western United States, southern Canada, and northern Mexico: *A. a. americana* (American), *A. a. mexicana* (Mexican), *A. a. sonoriensis* (Sonoran), and *A. a. peninsularis* (Peninsular). Peninsular and Sonoran subspecies are listed as endangered and are legally protected in Mexico and in the United States (Endangered Species Act [United States Fish and Wildlife Service 1973; Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT) 2010]).

Like many other large North American herbivore species, pronghorn have been subjected to overexploitation and range-wide habitat destruction (Brown and Ockenfels 2007). Historically, pronghorn herds were found in the United States west of the Mississippi River and across much of northern Mexico. However, by the late 1800s, pronghorn populations were drastically reduced to small isolated groups (Yoakum 1975). Even though the historical numbers of pronghorn roaming the North American plains are still debated, they probably exceeded 40 or 60 million (Nelson 1925). It was estimated the species as a whole lost around 70% of its habitat (Laliberte and Ripple 2004), whereas some subspecies lost up to 90% (Cancino et al. 1998). As a result, fewer than 20,000 animals remained at the beginning of the 20th century (O'Gara and Yoakum 2004; SEMARNAT 2009).

The Peninsular and Sonoran subspecies inhabit separate areas of the Sonoran Desert, which represents the southern periphery of the pronghorn's main geographic distribution (Medellin et al. 2005). Historically, the distributional ranges of these subspecies are thought to have overlapped in a small area of northeastern Baja California, Mexico, near San Felipe (Fig. 1). Survey numbers for the 2 pronghorn subspecies from the Sonoran Desert have been fluctuating between 20 and 150 animals over the past few decades, which led to the creation of captive breeding herds for both subspecies (Cancino et al. 2005; Otte 2006; United States Fish and Wildlife Service 2010; Wilson et al. 2010). Table 1 summarizes some recent events affecting these pronghorn subspecies and the numbers of animals taken into captivity (Cancino et al. 1994, 1998, 2005; Otte 2006; SEMARNAT 2009; USFWS 2010; Wilson et al. 2010; V. Sánchez Sotomayor, Área de Protección de Flora y Fauna Valle de los Cirios, pers. comm. 2012).

Previous genetic analyses of pronghorn populations have focused on American, Mexican, and Sonoran pronghorn (Lee et al. 1989, 1994; Rhodes et al. 2001; Stephen et al. 2005a, 2005b). The Peninsular subspecies, however, appears to have been largely overlooked. Although Brown (2006) argues that the taxonomic status of the pronghorn subspecies is based on obsolete criteria, current subspecies definitions of pronghorn, although not clearly resolved, have had a profound effect on reintroductions, translocations, and conservation decisions in the United States and in Mexico (Stephen et al. 2005a). Lee et al. (1994), using protein and mitochondrial DNA (mtDNA) restriction fragment length polymorphism analyses, were among the first to question the subspecific status of the Oregon pronghorn (*A. a. oregona*). Another study (Lou 1998) determined that the Sonoran subspecies was not highly differentiated from American pronghorn populations in Texas or Arizona. Stephen et al. (2005a) found that the Sonoran populations in the United States and Mexico were significantly differentiated at 5 microsatellites ($F_{ST} = 0.073$), but still more related than any other pair of populations ($F_{ST}$ range = 0.104–0.205), suggesting a closer relationship between them. More recently, Theimer et al. (2012) used microsatellites to show that the genetic composition of pronghorn herds is highly influenced by highways, which can act as a barrier to contemporary gene flow.

To our knowledge, no peer-reviewed studies have been published on the population genetics of the Peninsular pronghorn. Consequently, the data needed to make important decisions regarding reintroductions, translocations, breeding, and management are lacking. We therefore sought to elucidate the status of the 2 pronghorn subspecies within captive programs in the Sonoran Desert regarding genetic diversity, inbreeding, $N_e$s, and genetic differentiation. Our goal was to obtain an improved understanding of the demographic processes responsible for the present-day genetic composition of these subspecies, as well as to provide baseline data to inform effective management and conservation.

## Materials and Methods

*Sample locations and collection.*—We collected a total of 109 pronghorn samples, 64 for *A. a. sonoriensis* and 45 for *A. a. peninsularis*. This sample size represents approximately 5–10% of each subspecies' total population size. Feces and skin tissue for the Peninsular pronghorn, and buccal swab and blood samples for the Sonoran pronghorn, were used as DNA sources. In the case of the Peninsular subspecies, all of the samples were collected during 2012 from a captive breeding herd within the Vizcaino Biosphere Reserve, Baja California Sur, Mexico, at La Choya Peninsula. We collected a minimum of 10 fecal pellets per individual. Only freshly deposited pellets were sampled when defecation was observed by 3 people to minimize the risk of inadvertently sampling multiple individuals. All samples were placed in labeled sterile paper bags, stored in a cooler, dried overnight, and transported to the laboratory, where they were kept at –20°C. Tissue samples were taken from the ears of fawns that died during the first 5 weeks after birth. Each sample was stored in 95% ethanol at room temperature. The Sonoran pronghorn samples were collected in 2010 and 2011 from captive animals at Cabeza Prieta National Wildlife Refuge, Arizona. Oral swabs and ~0.5 ml of blood were preserved in an equal

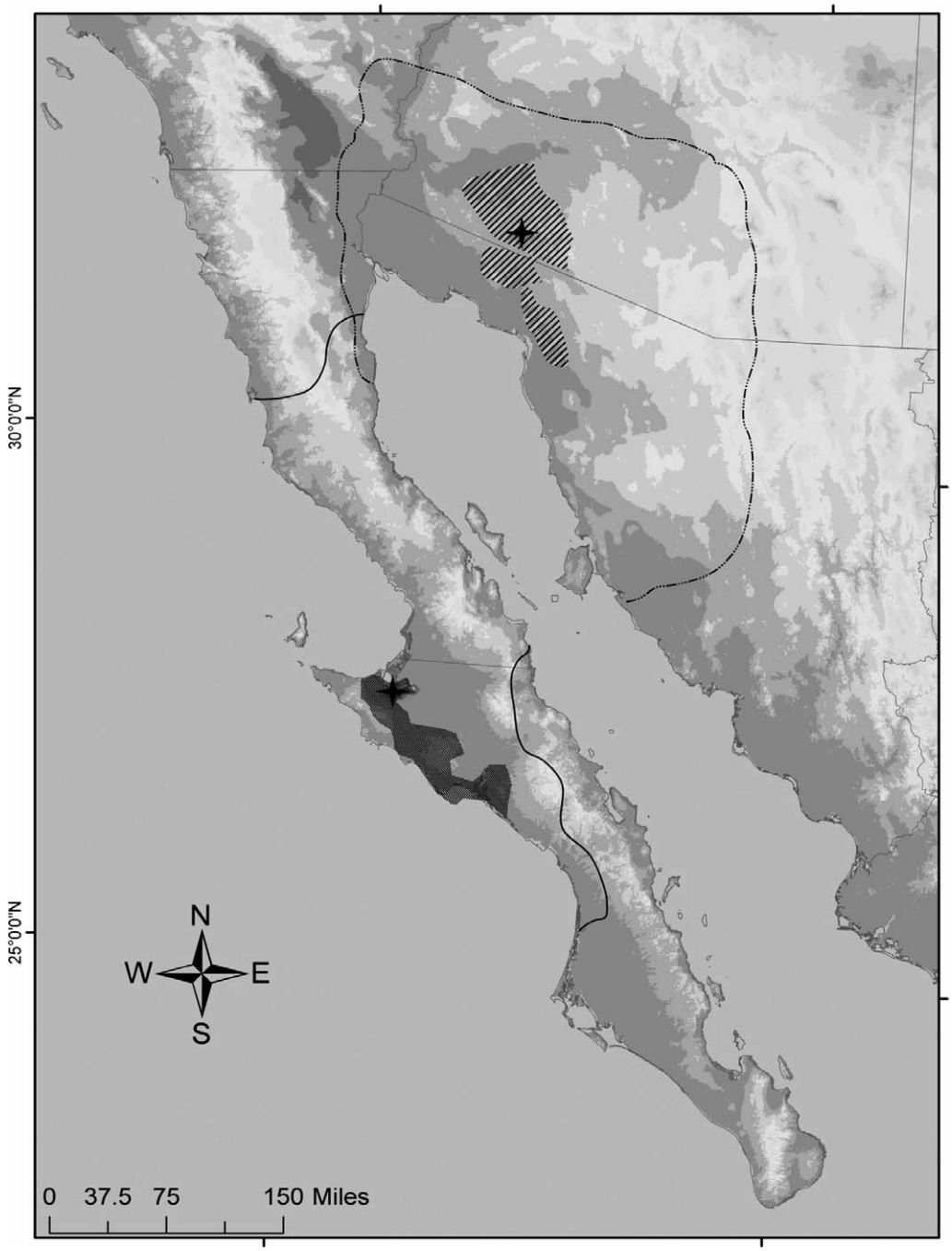


**Fig. 1.**—Historical and current distribution of the 2 pronghorn subspecies, *Antilocapra americana peninsularis* and *A. a. sonoriensis*, from the Sonoran Desert and sampling locations. The dotted line delineates the historical distribution of *A. a. sonoriensis*, and the hatched area its current range. Solid black lines mark the historical limits of *A. a. peninsularis* and the black area shows the current distribution. Modified from Cancino et al. (1998) and United States Fish and Wildlife Service (2010).

amount of STE buffer (0.1 m NaCl, 0.05 m Tris-HCl, pH 7.5, and 0.001 m ethylenediaminetetraacetic acid) at –20°C. All sampling of pronghorn individuals followed guidelines of the American Society of Mammalogists (Sikes et al. 2011) and animal protocols were approved by local authorities (V. Sánchez Sotomayor, Área de Protección de Flora y Fauna Valle de los Cirios, and J. Atkinson, United States Fish and Wildlife Service and Leader of the Sonoran pronghorn recovery team).

*Molecular techniques*.—Total DNA was extracted from 23 tissue and 22 fecal samples of the Peninsular pronghorn and 34 swab and 30 blood samples of the Sonoran pronghorn. For tissue, blood, and swab samples we used a DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, California) following the manufacturer's recommended protocols. DNA was extracted from fecal samples using a QIAamp DNA Stool Kit (Qiagen Inc.). The manufacturer's protocol was followed except for a minor modification to the wash step,

**Table 1.**—Important dates, events, and approximate numbers of Sonoran and Peninsular pronghorn (*Antilocapra americana peninsularis* and *A. a. sonoriensis*, respectively) individuals during the 20th and 21st centuries.

| Subspecies | Date | Population size/events |
|---|---|---|
| *A. a. sonoriensis* | 1920 | 100 |
| | 1994 | 282 animals, followed by steady population decline |
| | 2002 | 21 individuals, severe drought in the Sonoran Desert |
| | 2002 | Recovery actions (i.e., forage enhancements, development of water sources, and construction of a captive-breeding pen) |
| | 2006 | Captive population started from 3 adults from Sonora, Mexico (1 male) and 11 adults from Arizona (2 males) captured during 2004–2006 |
| | 2007 | Mexican population 360 individuals |
| | 2009 | United States captive and wild population of 131 animals |
| *A. a. peninsularis* | 1970s | Average 72 individuals |
| | 1980s | Average 58 individuals |
| | 1990–1996 | Average 85 individuals |
| | 1997 | 59 individuals in the wild |
| | | Start of captive breeding program in the Vizcaino Biosphere Reserve |
| | 1998 | First 5 wild animals captured |
| | 2000 | First captive births |
| | 2003 | 90 animals in captivity from 16 wild fawns and 9 wild adults captured during 1998–2003 |
| | 2012 | 400 captive population and 50 wild population |

for which we washed 5 or 6 fecal pellets for 15 min in 5 ml of the stool lysis buffer (ASL). To maximize the yield of the DNA, we performed the final step twice, using 75 μl of buffer each time.

To sequence the complete mitochondrial control region, we designed 4 sets of primers (Table 2) using CLC Genomic Workbench 5.0.1. (CLC Bio, Aarhus, Denmark). The combinations of these primers were sequenced and subsequently aligned to render the complete control region of the pronghorn (Hassanin et al. 2012). Each polymerase chain reaction was carried out in a 25-μl volume containing 1 μl of the DNA template for blood, tissue, and swab samples or 3 μl for feces. Each polymerase chain reaction contained: 1X buffer (Invitrogen, Carlsbad, California), 1 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, 0.05% bovine serum albumin, 0.5 U of *Taq* DNA Polymerase (Invitrogen), and 0.5 μM of each primer. The polymerase chain reaction profile consisted of an initial denaturalization step at 95°C for 5 min, followed by 35 cycles of 60 s each at 95°C and 58–60°C followed by 60 s at 72°C. Cycles were terminated with a final extension of 10 min at 72°C. A negative control lacking any DNA was included in each set of polymerase chain reactions to detect any potential contamination. Polymerase chain reaction products were sequenced in both directions on a 3730 Automated DNA Analyzer (Applied Biosystems, Foster City, California), using the BigDye Terminator Cycle Sequencing kit version 3.1 at the University of Arizona Genetics Core. The sequences were edited and the forward and reverse strands aligned to one another using SEQUENCHER 4.9 (Gene Codes Corporation, Ann Arbor, Michigan).

Fourteen microsatellite loci developed specifically for Sonoran and Peninsular pronghorn (Munguia-Vega et al. 2013) were amplified for 32 and 23 individuals of Sonoran and Peninsular subspecies, respectively. Four additional loci (*Aam2*, *Aam3*, *Aam7*, and *Aam8*) were chosen, based on levels of their variability and previous successful use in *A. a. americana* (Carling et al. 2003). Each polymerase chain reaction was carried out in a 15-μl volume with 20–40 ng of genomic DNA, 1X polymerase chain reaction buffer, 0.2 mM of each deoxynucleoside triphosphate, 1.5 mM of $MgCl_2$, 0.2% bovine serum albumin, 0.5 U Taq DNA polymerase (Invitrogen), 0.02 μM of the unlabeled M13-tailed forward primer, 0.2 μM of the fluorescently labeled M13 primer, and 0.2 μM of the reverse primer. The polymerase chain reaction conditions are described elsewhere (Munguia-Vega et al. 2013). The 4 additional microsatellite loci were amplified with the protocol and thermal profile of Carling et al. (2003). Polymerase chain reaction fragments were resolved on an Applied Biosystems 3730XL and alleles were scored using GENOTYPER 3.7 (Applied Biosystems).

**Table 2.**—List of newly designed primers for the amplification of the complete pronghorn (*Antilocapra americana peninsularis* and *A. a. sonoriensis*) mitochondrial control region.

| Primer name | Sequence | Primer size (base pairs) | Position on the pronghorn mitochondrial genome (Hassanin et al. 2012) |
|---|---|---|---|
| AnamF1 | 5′-ATTAATCGTGGGGGTAGC-3′ | 18 | 15826–15843 |
| AnamR1 | 5′-TCAGGTGGACTTAAAGGG-3′ | 18 | 161–144 |
| AnamF2 | 5′-AAACCAGAAAAGGAGAACGA-3′ | 20 | 15333–15352 |
| AnamR2 | 5′-AAATATCTAGGGATGAGCGT-3′ | 20 | 16267–16248 |
| AnamF3 | 5′-CAATAGCCCCACTTTCAAC-3′ | 19 | 15381–15399 |
| AnamR3 | 5′-TAAGAGGAAAGAGTGGACG-3′ | 19 | 15920–15902 |
| AnamF4 | 5′-CGAACAAACCTACCAAACT-3′ | 19 | 15491–15509 |
| AnamR4 | 5′-CGCCGTATTCCTATTAACT-3′ | 19 | 354–336 |

*Genetic diversity*.—For the control region sequences, we calculated the number of unique haplotypes, haplotype frequencies, the number of polymorphic sites, numbers of transitions and transversions, haplotype diversity ($h$), and nucleotide sequence diversity ($\pi$) using DnaSP version 5 (Librado and Rozas 2009).

Microsatellite alleles were binned and scored using FLEX-IBIN (Amos et al. 2007). Micro-checker 2.2.3 (Van Oosterhout et al. 2004) was used to test for genotyping errors and the presence of null alleles. Deviation from Hardy–Weinberg expectations and linkage equilibrium among loci were tested using Genepop 4.1.3 (Rousset 2008) and Fstat version 2.9.3 (Goudet 1992), respectively. The resulting $P$-values were corrected for multiple testing using sequential Bonferroni correction for multiple comparisons using alpha = 0.05 (Rice 1989).

Genetic variability was assessed for each population as the proportion of polymorphic loci, the mean number of alleles (allelic diversity [$A$]), the effective number of alleles ($A_e$), allelic richness ($A_r$), the presence and number of private alleles, observed heterozygosity ($H_O$), and expected heterozygosity ($H_E$). These were calculated using Fstat version 2.9.3 (Goudet 1992), GENETIX 4.05 (Belkhir et al. 1996–2004), and GENALEX 6.1 (Peakall and Smouse 2006). We also calculated Wright's inbreeding coefficient ($F_{IS}$—Weir and Cockerham 1984) using Fstat and estimates of pairwise relatedness and inbreeding as implemented in COANCESTRY version 1.0 (Wang 2011). We used different methods described by Wang (2011) in COANCESTRY to estimate pairwise relatedness between individuals and TrioML (Wang 2007) and DyadML (Milligan 2003) to estimate individual inbreeding coefficients. This strategy was used in order to evaluate the degree of discordance between different estimators, using the correlation methods implemented by COANCESTRY.

*Population structure and genetic differentiation*.—The limited number of phylogenetically informative sites in our samples precluded an unambiguous phylogenetic reconstruction. However, the relationships between haplotypes were assessed by constructing minimum spanning networks using TCS 1.21 software (Clement et al. 2000) at the default 95% connection limit. Analyses of molecular variance (AMOVAs) incorporating both $\Phi_{ST}$ and $F_{ST}$ (Weir and Cockerham 1984) were used to investigate differentiation between the subspecies (Excoffier et al. 1992). The statistical significance of these values was tested through permuting the data 10,000 times in Arlequin version 3.0 (Excoffier et al. 2005).

For the microsatellite data, we used several test statistics based on allele identity and allele size to determine genetic divergence between the subspecies: $F_{ST}$ (Weir and Cockerham 1984); Nei's unbiased genetic distance ($D_S$—Nei 1978), $G_{ST}$ (Pons and Petite 1996), and $R_{ST}$ (Goldstein et al. 1995). All of these distance measures were calculated using the software Spagedi version 1.0 (Hardy and Vekemans 2002). Significance was evaluated based on 10,000 permutations. STRUCTURE version 2.3.3 (Pritchard et al. 2000; Hubisz et al. 2009) was used to test for genetic structure among all of the pronghorn individuals without knowledge of the subspecies to which they belong. To determine the number of clusters ($K$) present within the data set, 10 independent simulations for each value of $K$ between 1 and 3 were run with 100,000 burn-in iterations followed by 500,000 iterations. We used the admixture model and allele frequencies were assumed to correlate among populations.

*Tests for reductions in population size*.—We employed 2 programs capable of detecting genetic signatures of population declines. BOTTLENECK 1.2.0.2 (Cornuet and Luikart 1996; Piry et al. 1999) tests across all the loci for a significant observed heterozygosity excess ($H_E$) caused by the quick loss of rare alleles after a population reduction relative to the heterozygosity expected in a population at equilibrium ($H_{EQ}$), a signature of a recent bottleneck (Cornuet and Luikart 1996; Piry et al. 1999). For this analysis, we choose the 2-phase model because this accounts for occasional "jump" mutations of several repeat units that can occur during microsatellite evolution (Schlotterer et al. 1998). We evaluated four 2-phase models, with 1%, 5%, 10%, and 30% multistep mutations using a default variance of 30. The number of loci for which $H_E$ was greater than $H_{EQ}$ was determined and statistical significance was tested using standardized differences and Wilcoxon signed ranks tests. To detect more ancient genetic bottlenecks, we calculated the $M$-ratio of Garza and Williamson (2001). The number of alleles declines faster than the range in allele size during a bottleneck due to chance sampling effects. Accordingly, the $M$-ratio is expected to be smaller in bottlenecked populations than in equilibrium populations. To determine significance, the mean observed $M$-ratio across loci is compared with an expected distribution generated from simulations under mutation–drift equilibrium, as implemented in CRITICAL_M (Garza and Williamson 2001). The critical $M$-ratio ($M_C$) was calculated using 10,000 simulations under 1-step mutations (ps) and the average size of multistep mutations ($\Delta g$) parameters set to 80% and 3.5, respectively, as suggested by Peery et al. (2012). The $\theta$ parameter was estimated using a mutation rate of 0.00011 (Crawford and Cuthbertson 1996) and a range of potential historical $N_e$-values (100, 200, 500, 1,000, and 2,000) expressing previous population sizes when these pronghorn subspecies were more widespread.

*Estimation of current effective population size*.—The $N_e$ can be used to predict the rate of loss of neutral genetic variation, fixation of deleterious and favorable alleles, and the rate of increase of inbreeding experienced by a population. It is therefore a central concept in evolutionary biology (Wright 1931) and conservation genetics (Mace and Lande 1991; Frankham 1996) because it can help predict the extinction risk of populations. Genetic estimates of the current $N_e$ of the 2 pronghorn subspecies were calculated using various methodological approaches to assess the robustness of the results. First a method based on linkage disequilibrium was used to estimate contemporary $N_e$ from a single temporal sample, as implemented in the software LDNe (Waples 2006; Waples and Do 2008) and Ne-estimator (Hill 1981; Ovenden et

**Table 3.**—Positions of the 7 variable sites within the 906–base pair consensus segment of the pronghorn (*Antilocapra americana peninsularis* and *A. a. sonoriensis*) mitochondrial DNA control region, defining the 6 unique haplotypes and their frequency in the 2 pronghorn subspecies. Dots (·) indicate identity to the top sequence and dashes (–) indicate insertion–deletion mutations.

| | | | Position | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Subspecies | Frequency | Haplotype | 60 | 61 | 240 | 258 | 279 | 658 | 727 |
| *A. a. sonoriensis* | 45 | Anas1 | – | – | C | T | T | T | – |
| *A. a. sonoriensis* | 5 | Anas2 | – | – | T | · | · | · | – |
| *A. a. sonoriensis* | 12 | Anas3 | – | – | T | · | · | · | T |
| *A. a. sonoriensis* | 2 | Anas6 | – | – | T | C | · | · | T |
| *A. a. peninsularis* | 33 | Anap4 | – | – | T | · | C | · | – |
| *A. a. peninsularis* | 12 | Anap5 | A | A | T | · | C | – | – |

al. 2007). The principle of the linkage disequilibrium method is that as $N_e$ decreases, genetic drift generates nonrandom associations among alleles at different loci (Hill 1981; Waples 1991a). LDNe was run excluding allele frequencies of less than 0.01 and assuming random mating (Waples 2006). Another approach widely used to estimate $N_e$ from a single temporal sample is based on an approximate Bayesian computation framework, as implemented in ONESAMP 1.1 (Tallmon et al. 2004, 2008). In contrast to linkage disequilibrium–based approaches, the ONESAMP $N_e$ estimator combines 8 summary statistics: the *M*-ratio, the difference between the natural logarithms of variance in allele length and heterozygosity, $H_E$, number of alleles per locus, Wright's $F_{IS}$, the mean and variance of multilocus homozygosity, and the square of the correlation of alleles at different loci based on linkage disequilibrium (Tallmon et al. 2008). The use of multiple summary statistics potentially improves accuracy and precision but this approach has not yet been thoroughly evaluated (Luikart et al. 2010). Bayesian estimates were produced based on uniform $N_e$ priors between 5 and 1,000. Finally, the sibship-based method infers $N_e$ from sibship frequencies using COLONY 2.0.3.3 (Wang 2009). Comparisons based on empirical data (Beebee 2009) suggest that Wang's sibship method performs better than heterozygote excess and linkage disequilibrium methods in respect to most of the criteria tested (insensitivity to locus number, correlation with other effective- and census-size estimates, and correlation with genetic diversity).

*Historical population dynamics*.—Long-term $N_e$s were estimated from microsatellite data for each subspecies using Migrate 3.3.1 (Beerli and Felsenstein 2001; Beerli 2008) based on a maximum-likelihood approach. Migrate uses coalescent theory to estimate the relative effective population size θ ($4N_e\mu$, where $N_e$ is effective population size and μ is mutation rate) and asymmetric gene flow M (m/μ) between pairs of populations (approximately $4N_e$ generations in the past—Beerli 2008) over long periods of time (approximately thousands of years). Three maximum-likelihood runs were conducted using a Brownian-motion model. The initial run used an estimate of $F_{ST}$ as a starting parameter to calculate θ and M and each subsequent run used the maximum-likelihood estimates from the previous run as new starting parameters. As suggested by authors, 4-chain heating at temperatures of 1, 1.5, 3, and 1,000,000 were implemented to increase the efficiency of the Markov chain Monte Carlo analysis (Beerli 2006, 2009). The first 2 runs were shorter (10 short chains of 50,000 sampled, 500 recorded, and 5 final long chains of 500,000 sampled, 5,000 recorded), then a final longer run was performed (10 short chains of 10,000 sampled, 500 recorded, and 5 final chains of 500,000 sampled, 25,000 recorded). Because parameter estimates from the final run were similar to the results from the shorter runs, we assumed that the final run had converged and we present results from this final run. To estimate $N_e$ from θ, we used a mutation rate of 0.00011 (Crawford and Cuthbertson 1996).

**Table 4.**—Comparative haplotype (*h*) and nucleotide diversity (π) of the Peninsular and Sonoran pronghorn subspecies (*Antilocapra americana peninsularis* and *A. a. sonoriensis*). *n* = sample size; *H* = number of haplotypes; *h* = haplotype diversity; π = nucleotide diversity.

| Subspecies | *n* | *H* | *h* | π |
|---|---|---|---|---|
| *A. a. sonoriensis* | 64 | 4 | 0.47 | 0.00054 |
| *A. a. peninsularis* | 45 | 2 | 0.40 | 0.0 |
| Total | 109 | 6 | 0.7178 | 0.00113 |

## Results

*Genetic diversity*.—A 906–base pair fragment of the mitochondrial control region was successfully amplified in 45 and 64 Peninsular and Sonoran pronghorn individuals, respectively. Seven polymorphic sites and 6 unique haplotypes were identified (Table 3). Three of the mutations identified were transition substitutions and 4 were insertion–deletions (indels). No shared haplotypes were found between the 2 subspecies. For Sonoran pronghorn, we found 4 haplotypes; the most frequent haplotype (denoted Anas1) being shared among 45 individuals (70.3%). Among 45 Peninsular pronghorn, we detected 2 haplotypes, the most common of which (Anap4) was found in 33 individuals (73.3%). The average number of nucleotide differences between the subspecies was 1.73. Because most haplotypes differ by 1 nucleotide position, the mean sequence divergence between haplotypes was low (0.1%) with a maximum of 0.2% between the Sonoran and Peninsular subspecies. Estimates of haplotype and nucleotide diversities are shown in Table 4. The 2 haplotypes found in the Peninsular pronghorn differed by only 3 indels, leading to a lack of nucleotide diversity. Sequences representing all 6 haplotypes have been deposited in GenBank (accession numbers KF806592–KF806597).

The 18 microsatellite loci carried a total of 108 alleles in 55 individuals of the 2 subspecies. We did not detect genotyping errors or the presence of null alleles in the sample. After adjustment for multiple comparisons, no evidence of linkage disequilibrium between any of the pairs of loci was found. One locus (Anam 97) deviated significantly from Hardy–Weinberg

**Table 5.**—Genetic diversity of 2 pronghorn subspecies, *Antilocapra americana peninsularis* and *A. a. sonoriensis*, measured at 18 microsatellite loci. $A$ = number of alleles, $A_r$ = allelic richness, $A_e$ = effective number of alleles, $A_p$ = number of private alleles, $H_E$ = expected heterozygosity, $H_O$= observed heterozygosity.

| | *A. a. sonoriensis* | | | | | | *A. a. peninsularis* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | $A$ | $A_r$ | $A_e$ | $A_p$ | $H_O$ | $H_E$ | $A$ | $A_r$ | $A_e$ | $A_p$ | $H_O$ | $H_E$ |
| Anam79 | 5 | 4.58 | 2.81 | 4.0 | 0.53 | 0.64 | 4 | 3.52 | 1.19 | 3.0 | 0.17 | 0.16 |
| Anam83 | 5 | 4.18 | 2.43 | 3.0 | 0.41 | 0.59 | 2 | 2 | 1.42 | 0.0 | 0.26 | 0.3 |
| Anam49 | 5 | 4.57 | 2.72 | 4.0 | 0.47 | 0.63 | 3 | 3 | 2.37 | 2.0 | 0.52 | 0.58 |
| Anam24 | 5 | 4.78 | 2.07 | 3.0 | 0.28 | 0.52 | 2 | 2 | 1.52 | 0.0 | 0.35 | 0.34 |
| Anam88 | 6 | 5.44 | 2.67 | 5.0 | 0.47 | 0.63 | 3 | 2.99 | 1.52 | 2.0 | 0.26 | 0.34 |
| Anam82 | 8 | 7.19 | 3.43 | 5.0 | 0.69 | 0.71 | 3 | 2.99 | 1.59 | 0.0 | 0.35 | 0.37 |
| Anam69 | 6 | 5.42 | 2.68 | 4.0 | 0.47 | 0.63 | 4 | 3.96 | 2.57 | 2.0 | 0.7 | 0.61 |
| Anam80 | 5 | 4.96 | 3.74 | 3.0 | 0.69 | 0.73 | 2 | 2 | 1.39 | 0.0 | 0.26 | 0.28 |
| Anam50 | 5 | 4.6 | 3.53 | 5.0 | 0.78 | 0.72 | 3 | 3 | 2.34 | 3.0 | 0.61 | 0.57 |
| Anam99 | 5 | 4.55 | 2.92 | 4.0 | 0.69 | 0.66 | 2 | 2 | 1.68 | 1.0 | 0.39 | 0.41 |
| Anam97 | 5 | 4.44 | 2.75 | 5.0 | 0.56 | 0.64 | 4 | 3.99 | 2.27 | 4.0 | 0.43 | 0.56 |
| Anam36 | 2 | 1.99 | 1.17 | 2.0 | 0.13 | 0.15 | 1 | 1 | 1.00 | 1.0 | 0 | 0 |
| Anam14 | 6 | 4.29 | 1.31 | 4.0 | 0.25 | 0.24 | 2 | 1.95 | 1.05 | 0.0 | 0.04 | 0.05 |
| Anam17 | 2 | 2 | 1.54 | 1.0 | 0.31 | 0.35 | 1 | 1 | 1.00 | 0.0 | 0 | 0 |
| Aam2 | 6 | 5.06 | 2.53 | 2.0 | 0.63 | 0.6 | 5 | 4.78 | 3.09 | 1.0 | 0.74 | 0.68 |
| Aam8 | 4 | 3.99 | 2.49 | 2.0 | 0.63 | 0.6 | 3 | 3 | 1.97 | 1.0 | 0.48 | 0.49 |
| Aam3 | 2 | 1.99 | 1.28 | 2.0 | 0.25 | 0.22 | 3 | 2.99 | 1.93 | 3.0 | 0.43 | 0.48 |
| Aam7 | 2 | 2 | 1.88 | 0.0 | 0.63 | 0.47 | 3 | 2.99 | 1.52 | 1.0 | 0.39 | 0.34 |
| $\bar{X}$ | 4.67 | 4.23 | 2.44 | 3.22 | 0.49 | 0.54 | 2.78 | 2.73 | 1.75 | 1.33 | 0.36 | 0.36 |

equilibrium after Bonferroni correction in the Peninsular but not in the Sonoran subspecies.

All 18 microsatellite loci were polymorphic in the Sonoran subspecies, whereas 2 loci (Anam 36 and Anam 17) were monomorphic in the Peninsular subspecies (Table 5). The Sonoran subspecies carried 84 alleles in total, with the mean number of alleles per locus being 4.67 and ranging from 2 to 8 alleles per locus. A total of 50 alleles were detected in the Peninsular pronghorn and the mean number of alleles per locus for this subspecies was 2.78 and ranged from 1 to 5 alleles per locus. Differences between the subspecies also were observed in allelic richness, the effective number of alleles per locus, and mean observed heterozygosity (Table 5), all of which were higher in the Sonoran subspecies. The mean number of private alleles per locus was 3.22 for Sonoran and 1.33 for the Peninsular subspecies.

The 2 methods for estimating the inbreeding coefficient implemented in COANCESTRY were highly correlated (99.1%), so we present only results based on the triadic likelihood method (TrioML—Wang 2007). The average inbreeding level was 0.061 ± 0.004 (*SE*) and 0.086 ± 0.001 for the Sonoran and Peninsular populations, respectively, with 34.8% of the Peninsular and 12.5% of the Sonoran individuals showing inbreeding levels higher than 0.125, which is equivalent to half siblings, grandparent–grandchild, uncle–niece, or double first-cousin relationships. The various relatedness estimators were also highly correlated ($r^2$ ranged from 58.5% to 98.4%) so we present results obtained with the TrioML estimator of relatedness, which takes into account genotyping errors, tends to have a reduced sampling variance, produces results that are biologically constrained, and, unlike many other maximum-likelihood methods, does not overestimate pairwise relatedness (Wang 2007). The mean relatedness coefficient was higher between individuals of the Peninsular subspecies (0.11 ± 0.03) than between animals of the Sonoran subspecies (0.079 ± 0.01).

*Population structure*.—A minimum spanning network depicting relationships among complete mitochondrial control region haplotypes is shown in Fig. 2. The haplotype Anas2, which was found in low frequency in the Sonoran subspecies, was central to the network, suggesting that it may be the most ancient haplotype. The 2 most common specific haplotypes, Anas1 and Anap4, which were found in the Sonoran and Peninsular pronghorn, respectively, appear to be derived from this ancestral haplotype. The AMOVA showed strong differentiation between the Sonoran and the Peninsular subspecies at both the haplotype and nucleotide level ($F_{ST}$ = 0.56; $\Phi_{ST} = 0.84$).

All microsatellite-based fixation indexes were large, indicating significant differences between the 2 subspecies within the nuclear genome. $F_{ST}$ and $G_{ST}$ statistics, based on the infinite allele model, were almost identical (0.402 and 0.409, respectively), whereas $R_{ST}$, which is based on the more realistic stepwise-mutation model was slightly lower at 0.346. Nei's $D$, which includes a correction for small sample sizes, gave the highest differentiation index ($D_S = 0.885$). Concordant with these values, STRUCTURE analysis found that the individuals most likely compose 2 genetically distinct populations (10 replicate simulations consistently indicate $K$ = 2), which correspond perfectly to the 2 subspecies. Cluster membership coefficients of the individuals indicate negligible admixture between the 2 subspecies.

*Tests for genetic bottlenecks*.—*M*-ratio analyses revealed support for both of the subspecies having undergone historical bottlenecks. Depending on the different assumptions of the prebottleneck $N_e$, the critical $M$ value ($M_C$) ranged from 0.80 to

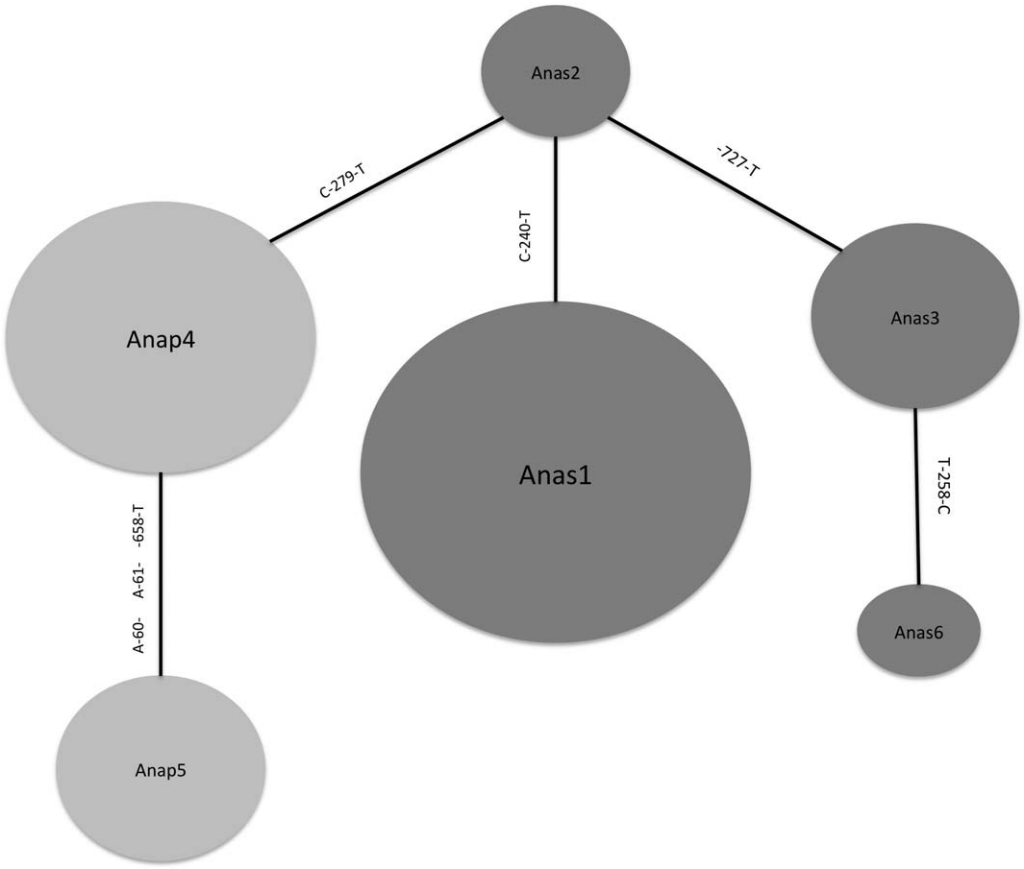


**Fig. 2.**—Minimum spanning network of the 6 pronghorn control region haplotypes. Circle sizes are proportional to the frequencies of the haplotypes in each sample. Light and dark gray circles represent Peninsular and Sonoran pronghorn, *Antilocapra americana peninsularis* and *A. a. sonoriensis*, haplotypes, respectively. The position and type of the changes are indicated for each variant site (e.g., Anas3 differs from Anas6 by a T–C change at position 258).

0.89. The far lower observed *M*-ratios for the Peninsular and Sonoran pronghorn (0.42 and 0.55, respectively) were well below the estimated $M_C$ with 95% probability (Table 6), indicating a historical population bottleneck. Independently of the proportion of the multistep mutations in 2-phase mutation models and of the probability test used, BOTTLENECK did not detect any evidence of population reduction in the Peninsular pronghorn subspecies. In the Sonoran subspecies, a bottleneck signature was only detected with the standardized differences test and only with 1%, 5%, and 10% proportions of multistep mutations (Table 6).

*Estimation of current effective population sizes*.—Point estimates of $N_e$ for the pronghorn subspecies varied depending on the choice of method, but ranged from 6 to 100 breeding individuals (Table 7). Not all of the approaches could be successfully implemented for both subspecies because of computational and data limitations. Nonetheless, ONESAMP, Colony, and the linkage disequilibrium method in Ne-estimator produced estimations of $N_e$ and confidence limits for both subspecies. For both subspecies, the highest $N_e$ was estimated using LDNe (100.4 for the Sonoran subspecies and 23.5 for the Peninsular subspecies) and the lowest values were obtained using the linkage disequilibrium method within Ne-estimator (16.6 for the Sonoran subspecies and 6.1 for the Peninsular subspecies). Overall, a trend emerges in which estimates of $N_e$ for the Sonoran subspecies are typically around 2- to 3-fold higher than the Peninsular subspecies regardless of the method used.

*Historical population dynamics*.—Estimates of historical migration rates (M) calculated using Migrate revealed little to no migration between the 2 subspecies over a long time period ($4N_e$ generations ago). Estimates of M were 0.16 from Sonoran

**Table 6.**—The results of *M*-ratio test and probabilities of heterozygote excess obtained using a range of mutational models.

| | | Standardized differences test *P*-value | | | | Wilcoxon test *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *M*-ratio | TPM70 | TPM90 | TPM95 | TPM99 | TPM70 | TPM90 | TPM95 | TPM99 |
| *Antilocapra americana sonoriensis* | 0.55 | 0.229 | 0.0167 | 0.00177 | 0.00007 | 0.533 | 0.8939 | 0.955 | 0.982 |
| *Antilocapra americana peninsularis* | 0.42 | 0.444 | 0.269 | 0.177 | 0.097 | 0.201 | 0.411 | 0.589 | 0.647 |

**Table 7.**—Pronghorn (*Antilocapra americana peninsularis* and *A. a. sonoriensis*) current effective population size ($N_e$) estimates. 95% *CI* = 95% confidence interval, LD = linkage disequilibrium.

| | $N_e$ | | | | |
|---|---|---|---|---|---|
| | | | | $N_e$-estimator | |
| | LD$N_e$ (95% *CI*s) | ONESAMP (95% *CI*s) | Colony (95% *CI*s) | LD (95% *CI*s) | Heterozygote excess |
| *A. a. sonoriensis* | 100.4 (42–infinity) | 15.6 (12.3–23.0) | 45 (27–80) | 16.6 (14.5–19.3) | 38.2 |
| *A. a. peninsularis* | 23.5 (11.4–86.9) | 9.5 (7.7–13.9) | 21 (11–47) | 6.1 (5.3–7.2) | — |

to Peninsular subspecies and 0.43 vice versa. Contrary to the current extremely low $N_e$s, historical $N_e$-values estimated using Migrate were relatively high and similar for the both subspecies. Thus, θ for the Sonoran subspecies was 1.19, equivalent to an $N_e$ of 2,704, and θ for the Peninsular subspecies was 1.12, equivalent to an $N_e$ of 2,545. These estimates were derived from formulae proposed by Beerli (2008) and assuming a microsatellite mutation rate of 0.00011, as described by Crawford and Cuthbertson (1996).

## Discussion

In this study, we used a variety of different analytical approaches to elucidate the genetic diversity, population structure, and demography of 2 threatened desert pronghorn subspecies. We found low levels of genetic diversity in both subspecies, with the Peninsular pronghorn having the lowest reported genetic diversity of any pronghorn subspecies. Both subspecies had far higher historical than contemporary $N_e$s and showed evidence of historical bottlenecks, indicating population reduction that is consistent with anthropogenic impacts over the last 2 centuries. The Sonoran and Peninsular subspecies also were found to be strongly differentiated at nuclear and mitochondrial genetic markers. We discuss the evolutionary and conservation implications of these findings below.

Our results indicate that levels of genetic diversity are low for both subspecies but the Peninsular pronghorn is particularly depauperate, both at microsatellites and mitochondrial DNA. Although the genetic diversity of the Peninsular pronghorn is the lowest of any pronghorn subspecies described to date, results for the Sonoran subspecies are similar to those reported previously (Stephen et al. 2005a).

The low levels of diversity observed may have been caused by a population bottleneck related to anthropogenic hunting, habitat destruction, and captive breeding, but also could be partly a consequence of long-term geographical isolation (Eckert et al. 2008). Studies of island and isolated ungulate populations that have undergone population collapse show similarly low levels of haplotype diversity. For example, mountain bongo antelope (*Tragelaphus eurycerus isaaci*) and Hainan Eld's deer (*Rucervus eldii siamensis*) both carry only a single control region haplotype (Pang et al. 2003; Faria et al. 2011). The same also has been found for 5 captive or semicaptive populations of Dorcas gazelle (*Gazella dorcas* spp.) in Morocco (Godinho et al. 2012).

Pairwise relatedness values and inbreeding coefficients in the Peninsular and Sonoran pronghorn suggest that mating between closely related individuals of both subspecies may be frequent and could be playing an important role in shaping the current genetic structure of these subspecies. Lower genetic diversity, higher inbreeding, and relatedness within the Peninsular subspecies does not seem to be caused by a smaller historical population size, because estimated historical effective sizes were very similar for the 2 subspecies. Small population sizes over the last 100 years also cannot explain the differences, because both subspecies have numbered fewer than 500 individuals during this period (Table 1; Cancino et al. 1994, 1998). The number of founders of the captive populations also differs little between the subspecies (~20 for the Peninsular versus 14 for the Sonoran pronghorn). One possible explanation relates to the fact that the Peninsular pronghorn captive program has been running much longer than that of the Sonoran subspecies, potentially accentuating inbreeding and the loss of diversity via genetic drift. The Sonoran pronghorn captive-breeding program also received contributions from individuals from Mexico. Additionally, it is possible that the Sonoran pronghorn may have originally been less related and inbred, if at least some level of gene exchange was present with geographically adjacent populations of *A. a. americana*, as suggested previously (Stephen et al. 2005a).

The taxonomic rank of subspecies has been the subject of a long-running controversy (Mayr 1982), primarily because of the failure of some molecular studies to identify traditional subspecies as phylogenetically distinct (Barrowclough 1980; Ball and Avise 1992; Cronin 1993; Burbrink et al. 2000). Pronghorn subspecies status also has been much debated, with several researchers questioning the validity of established subspecies designations (Lee et al. 1994; Brown 2006). For example, Brown et al. (2006) concluded that the pronghorn is remarkably monotypic on the basis of several morphological measurements, with the possible exception being *A. a. peninsularis*. Several previous studies have attempted to elucidate pronghorn subspecies designations based on different genetic markers. For example, Lee et al. (1994) found that Oregon and American pronghorn were genetically indistinguishable from one another, but clearly differentiated from the Mexican subspecies. Lou (1998) found only 2 genetically differentiated groups of pronghorn populations; a northern group (represented by samples from Colorado, Kansas, Montana, Idaho, Wyoming, and Oregon) and a southern group (comprising *A. a. sonoriensis* and *A. a. americana* from Texas and southwestern Arizona). Stephen et al. (2005a) found

evidence in support of a history of recent isolation of the Sonoran pronghorn from the American subspecies, but argued that the Sonoran pronghorn is not highly distinct from *A. a. americana*. These authors suggested that genetic differences between the Sonoran and American subspecies have likely resulted from isolation by distance in historically contiguous populations, the loss of genetic diversity due to genetic bottlenecks, and the cumulative effects of genetic drift, rather than being due to prolonged separation and the subsequent accumulation of unique genetic diversity.

Our results based on mitochondrial and nuclear markers show that the Peninsular pronghorn is highly distinct from its Sonoran conspecific. The 2 subspecies carry private alleles and the degree of genetic differentiation is very large ($F_{ST} = 0.402$, and Nei's $D = 0.885$), about twice as large as the highest values reported from any other pronghorn populations. This implies that gene flow between the 2 subspecies has been restricted, a finding consistent with the control region data showing no shared haplotypes. This pattern also is reflected in studies of other ungulates with overlapping geographic ranges such as mule deer (*Odocoileus hemionus*—Latch et al. 2009).

The Sonoran Desert formed 8–15 million years ago in the Miocene (Van Devender 2007), well before the geological separation of the Baja California Peninsula (about 5 million years ago) contributed to the genetic isolation of the terrestrial biota in the Peninsula and the evolution in situ of endemic species (Hafner and Riddle 1997; Riddle et al. 2000; Riddle and Hafner 2006). Analyses of pack rat midden fossils have shown that desert scrub communities and the geographic ranges of individual species have been dynamic in the Sonoran Desert since the end of the last glacial period around 11,000 years ago. Woodland and chaparral vegetation is restricted today to mountaintops lower than 600 m in elevation and desert scrub communities and the modern Sonoran Desert have existed only for the last 9,000 to 4,500 years (Van Devender 2007). Given that there were multiple glacial periods during the Pleistocene, there seem to have been ample opportunities for the Peninsular and Sonoran pronghorn to evolve in isolation during range shifts to southern or lower-elevation glacial refugia in the Sonoran Desert. Genetic drift in the captive breeding herds probably also has played a large role in explaining the strong differentiation of the 2 subspecies (Lacy et al. 1993; Leberg and Firmin 2008), although previous studies of other species suggest that this need not be the case. For example, Peng et al. (2008) found multiple mitochondrial haplotypes in a herd of musk deer (*Moschus berezovskii*) held in captivity for more than 50 years, which they attributed to this herd never having suffered from a severe founder effect and the founders originating from a very large wild population. In contrast, both of the pronghorn subspecies have experienced similar demographic histories and were probably never very abundant in the wild because of the harsh desert environment. It also is well known that droughts can heavily affect pronghorn survival and recruitment (Brown et al. 2002; Bright and Hervert 2005; Bender et al. 2013), suggesting that environmental factors could have played a role in shaping pronghorn genetic diversity and differentiation. Nonetheless, the effect of the strong genetic drift in extremely small and geographically isolated populations cannot be ruled out (Casas-Marce et al. 2013).

We identified strong genetic bottleneck signatures in both pronghorn subspecies using the *M*-ratio test, whereas heterozygosity-excess tests were only significant for the Sonoran subspecies. The heterozygosity-excess test is highly dependent on the mutational model assumed and is often underpowered to detect even 10- to 1,000-fold population declines (Girod et al. 2011; Peery et al. 2012). Heterozygosity excess, caused by the loss of rare alleles during a bottleneck, is a transient phenomenon, because heterozygosity quickly reaches a mutation–drift equilibrium (Luikart and Cornuet 1998). This could explain why this test detected a bottleneck in the Sonoran pronghorn, which has been subject to a more recent reduction (only ~8 years in captivity), but not in the Peninsular pronghorn subject to ~15 years in captivity. The *M*-ratio has also been shown to perform better under certain circumstances at detecting population reductions (Hundertmark and Van Daele 2010) and is particularly sensitive to older bottlenecks (Spear et al. 2006; Swatdipong et al. 2010). Results of the *M*-ratio test supported historical bottlenecks in both pronghorn subspecies. The observed ratios were smaller than obtained for several other species with known histories of population reductions, including the Mexican wolf (*Canis lupus baileyi*), Mediterranean monk seal (*Monachus monachus*), and northern elephant seal (*Mirounga angustirostris*—Garza and Williamson 2001). In our case, the bottleneck was probably caused by long-term small population sizes together with unregulated hunting in the last 100–200 years.

The effective size can be estimated with precision using pedigree information that accounts for inbreeding in the population (Falconer and Mackay 1996). However, these estimates are largely dependent on the completeness of the pedigree (Goyache et al. 2003), making confidence in the obtained values difficult in many breeding programs where the genetic relatedness of founder individuals is unknown. This means that genetic methods are often more appropriate to estimate $N_e$. Discrepancies between the different estimators may be attributed to the different assumptions upon which the various methods are based. Regardless of these methodological subtleties, however, a consistent pattern emerges in our study, in which $N_e$-values recovered for the Sonoran subspecies were 2–3 times larger relative to the Peninsular pronghorn. This may have important implications for conservation because, with smaller $N_e$-values, the Peninsular subspecies may be more prone to extinction. Our analyses also confirm the utility of comparing results from multiple estimators (Beebee 2009; Luikart et al. 2010).

Contemporary $N_e$ estimates were only about 1–5% from the historical $N_e$ estimates generated by Migrate, in line with the large and widespread population decline that pronghorn experienced over the last 2 centuries (Cancino et al. 1998, 2005; Brown and Ockenfels 2007). However, very low levels of genetic diversity preclude more-detailed analyses of our

data. Future studies may be able to overcome this limitation using next-generation sequencing-based approaches capable of screening many more loci.

Two major axes of biodiversity are generally recognized: one related to isolation and one related to adaptation (Waples 2013). Regarding isolation, an evolutionarily significant unit is a group of organisms that has been isolated from other conspecific groups for a sufficient period of time to have undergone meaningful genetic divergence from other groups (Ryder et al. 1988), and contributes substantially to the ecological or genetic diversity found within the species taxon as a whole (Waples 1991b). The genetic cutoffs for evolutionarily significant units include that they are reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci (Moritz 1994). Concerning adaptation, evolutionarily significant units have genetic attributes significant for the present and future of the species in question (Ryder et al. 1988). Although it is problematic to predict which evolutionarily significant units will be important to the future evolution of the taxon, conservation of as many evolutionarily significant units as possible should minimize anthropogenic constraints on natural evolutionary processes and maximize the probability that the taxon and some of its populations will persist into the future (Hey et al. 2003). Conservation below the species level must be guided by the general objective of preventing elements of biodiversity from becoming extinct or extirpated (Green 2005), because the loss (Moritz 2002) or merging of evolutionarily significant units are irreversible processes and are therefore not recommended (Ryder et al. 1988).

Our study showed that both pronghorn subspecies from the Sonoran Desert meet the evolutionarily significant unit criteria relating to isolation. Regarding adaptation, although we did not survey adaptive or detrimental variation directly, genetic drift in long-term small populations may have reduced nonneutral variation as well, because selection is less effective in small populations, except for genes under strong selection (Munguia-Vega et al. 2007). The Sonoran Desert is an unusual environment for pronghorn and is located at the southern edge of the species' range. This implies that the genomic diversity of pronghorn might include some adaptive genetic variants shaped during thousands of years by strong selection to drought and warmer temperatures. Considering that climate change projections suggest that both water scarcity and higher temperatures will be spreading over North America over the next decades (Prudhomme et al. 2013), adaptive variants within the Peninsular and Sonoran pronghorn could be useful in allowing the species as a whole to adapt to climate change, particularly at the edge of their distribution where extinction risk is predicted to be higher (Provan and Maggs 2012). Although this argument is highly speculative, there is some evidence to suggest that local populations of pronghorn in the Sonoran Desert may be adapted to their local environment. For instance, they are comparatively smaller and paler (Brown et al. 2006), give birth earlier, and males do not seem to form harems (Cancino et al. 2005; Wilson et al. 2008).

Because both subspecies currently possess low levels of genetic diversity, we recommend close genetic monitoring in order to minimize the effects of further inbreeding and the erosion of genetic diversity. One way to do this would be to design captive-breeding programs based on the genetic relationships between individuals. As noted previously by O'Gara and Yoakum (2004), a comprehensive study of pronghorn incorporating all of the subspecies and embracing a wider variety of genetic markers also is currently lacking. Together with information from adaptive genetic markers, this would improve our understanding of the potential for genetic rescue and the risk of outbreeding depression in pronghorn.

## Resumen

Las especies que han experimentado reducciones poblacionales son ejemplos valiosos para entender la repuesta genética al cambio demográfico. En el pasado el berrendo (*Antilocapra americana*) tenia una amplia distribución en las praderas norteamericanas, sin embargo su población sufrió una drástica reducción por caza descontrolada y fragmentación de su hábitat durante finales del siglo XIX y comienzos del siglo XX. *A. a. peninsularis* y *A. a. sonoriensis*, son 2 subespecies del berrendo que habitan el extremo sur del rango de distribución de la especie; ambas se encuentran al borde de la extinción y sobreviven casi exclusivamente en cautiverio. En este estudio, secuenciamos en su totalidad la región control del ADN mitocondrial y genotipificamos 18 loci microsatélites en 109 individuos con el propósito de evaluar el impacto de cuellos de botella poblacionales, reproducción en cautiverio, tamaños poblacionales pequeños y aislamiento sobre la composición genética de estas dos subespecies. Encontramos niveles bajos de diversidad genética en ambas subespecies, particularmente en el berrendo peninsular. Las 2 subespecies mostraron diferenciación genética alta y significativa, lo que implica ausencia de flujo genético histórico y reciente, a pesar de su cercanía geográfica dentro del Desierto Sonorense. Inferimos que el tamaño poblacional histórico efectivo para ambas subespecies fue similar, mientras que el berrendo sonorense tiene un tamaño efectivo contemporáneo 2 veces mayor que el de la subespecie peninsular. Nuestro estudio sugiere que es necesario realizar un manejo genético cuidadoso en ambas subespecies, para así minimizar la pérdida de variabilidad genética durante la reproducción en cautiverio.

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