Proceedings of the Nineteenth Biennial Pronghorn Antelope Workshop

2001

Hosted by:
Centro de Investigaciones Biológicas del Noroeste, S. C.
La Paz, Baja California Sur, México.
March 14-17, 2000

PROCEEDINGS

of the

NINETEENTH BIENNIAL

PRONGHORN ANTELOPE WORKSHOP

2001

Edited by Jorge Cancino

La Paz, Baja California Sur, Mexico

March 13 – 17, 2000

Chair
Jorge Cancino

ANALYSIS OF REINTRODUCED PRONGHORN POPULATIONS IN ARIZONA USING MITOCHONDRIAL DNA MARKERS

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Abstract: Genetic diversity was examined in pronghorn (*Antilocapra americana*) to assess relationships among Arizona populations sharing common reintroduction or translocation sources. Ninety-seven Arizona pronghorn were analyzed for mitochondrial DNA (mtDNA) haplotype variation via restriction enzyme analysis and four composite haplotypes were revealed. Comparative analyses of Arizona pronghorn populations that shared founders from Montana, Wyoming, Texas, or central Arizona were performed. In addition, analyses of differences in haplotype frequency were performed specifically for populations in the northwestern and southeastern sections of the state because these populations are thought to be composed entirely of reintroduced pronghorn. Tests for differences in haplotype frequencies among populations sharing founders were performed using Monte Carlo simulation. Populations which received translocated animals from Texas, Wyoming, or Montana showed no significant variability in haplotype frequencies. Haplotype frequencies were significantly different among populations that received reintroductions from central Arizona only when a population which also received pronghorn from Montana, was included in analyses. Overall, populations in southeastern Arizona differed significantly from each other in haplotype frequencies. However, populations within southeastern Arizona with common reintroduction sources (e.g., Texas or central Arizona only) were not different in haplotype frequencies. Populations sampled in northwestern Arizona were not different from each other in haplotype frequencies despite the wide array of sources (central Arizona, Montana, Wyoming, Colorado, and Utah) used to restock that region. Our results suggest that whenever possible, genetic data should be used to plan future reintroductions of pronghorn in Arizona.

PROCEEDINGS PRONGHORN ANTELOPE WORKSHOP 19:45-54

Key Words: *Antilocapra americana*, mitochondrial DNA, polymerase chain reaction, reintroduction.

Numerous genetic studies of reintroduced organisms document increased colonization success by individuals that most closely resemble the original genetic stocks of the region (Ellsworth et al. 1994, Leberg et al. 1994, Rhodes et al. 1995, Nedbal et al. 1997, Serfass et al. 1998). Thus, selection of source populations which closely correspond to current or historical genetic stocks of recipient populations could increase the probability of successful restocking events and may help to preserve remnants of native stocks. To manage wildlife populations at this level of resolution, baseline data on genetic diversity of potential source populations and existing remnant populations are needed (Avise 1989, Serfass et al. 1998, Williams et al. 2000).

Declines in pronghorn numbers around the turn of the century both isolated and extirpated populations and stimulated the Arizona Game and Fish Department (AGFD) to initiate a series of pronghorn reintroductions beginning in the 1920s. Continuing through the present, these reintroductions were designed to help bolster small populations and to repopulate historic pronghorn ranges. Pronghorn used in Arizona reintroductions came from central Arizona, as well as from other states such as Montana, Wyoming, Colorado, Utah, and Texas (Lee 1988).

Although successful in reestablishing pronghorn populations in Arizona, these reintroductions may have compromised the phylogeographic relationships of pronghorn throughout the state. For example, pronghorn north of the Grand Canyon (northwestern Arizona; Game Management Units [GMU] 12A, 12B, 13A & 13B; Figure 1) are believed to have been extirpated by the early twentieth century and repopulated with reintroductions from central Arizona, Colorado, Wyoming, Montana, and Utah (Alexander 2000). All present-day pronghorn within northwestern Arizona are believed to be direct descendents from these translocations. Likewise, pronghorn are believed to have been extirpated from southeastern Arizona by the early 1930s, and all pronghorn populations in this region of the state were established through reintroductions from Texas and central Arizona (Hoffmeister 1986).

Reat et al. (1999) examined mitochondrial haplotype diversity of 389 pronghorn distributed across the southeastern, central, and northern portions of their range in Arizona. Their research indicated that Arizona pronghorn exhibited 4 haplotypes, 3 observed previously in North American pronghorn and 1 unique to Arizona. In addition, their research revealed that 1 haplotype, which occurs at a relatively low frequency throughout most of the United States, was the common haplotype in Arizona. The research presented herein is an extension of the work of Reat et al. (1999) with emphasis on reintroduced pronghorn populations in Arizona. Our goal was to determine whether reintroduced Arizona pronghorn populations that shared a common source (either from a reintroduction or translocation) differed in their haplotype distributions. Additionally, we examined regional pronghorn populations in the northwestern and southeastern portions of the state to determine whether haplotype distributions in these population could be explained by their reintroduction history.

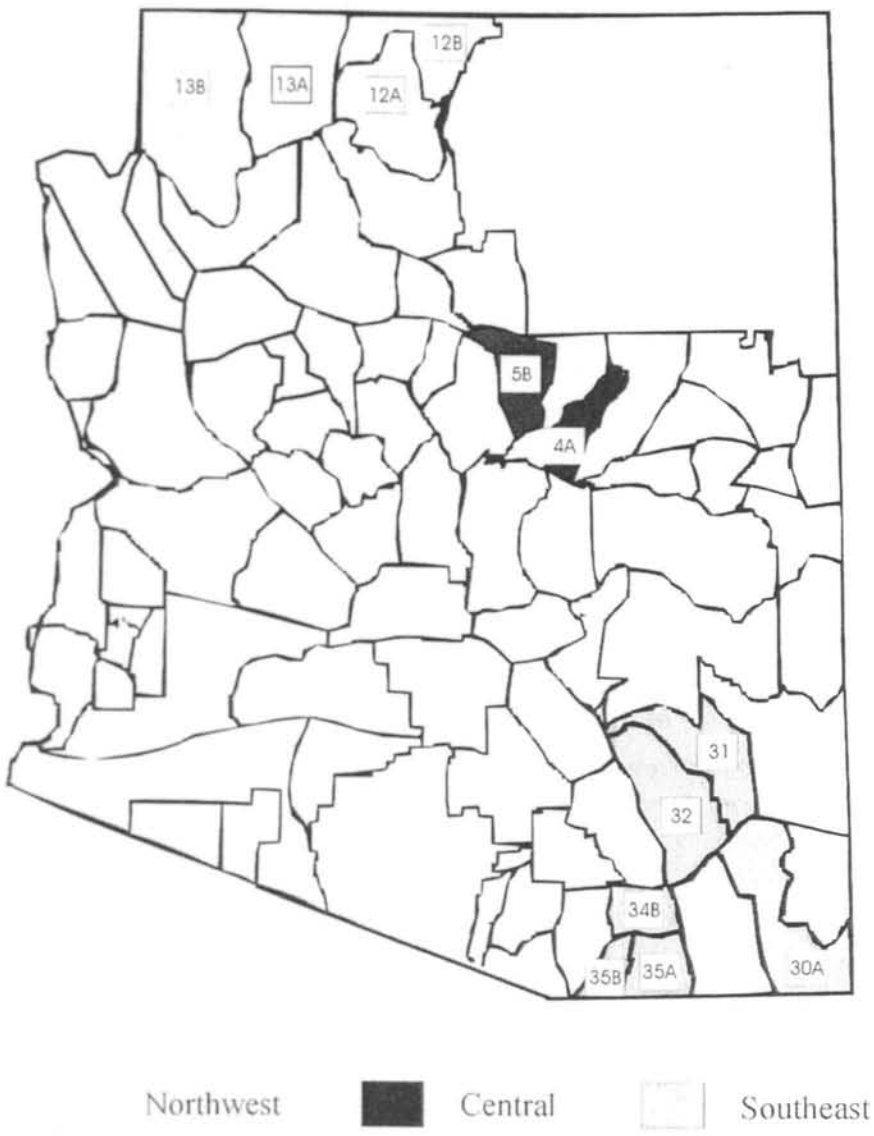

Figure. 1. Pronghorn samples were obtained from 10 Game Management Units in northwestern, central, and southeastern Arizona during the 1996 and 1997 hunting seasons. Game management unit numbers are provided within regions.

STUDY AREA AND METHODS

Collection kits, consisting of sample bags and instructions for tissue collection, were mailed to hunters that obtained permits to harvest pronghorn in Arizona during the 1996 and 1997 hunting seasons. In 1996, 755 kits were mailed to hunters throughout the state. In 1997, 334 kits were mailed to

hunters that drew tags for areas under-represented in the 1996 sampling effort. The hunters were asked to collect liver and muscle tissue in the field and place the samples on wet ice. Hunters then dropped the samples off at collection stations located throughout Arizona at major highway intersections. In addition to roadside collections, samples were collected at AGFD regional offices and by Wildlife Managers in the field. Archived samples from the AGFD were also used to bolster sample numbers from critical areas. Following collection, samples were cataloged and placed in liquid nitrogen for storage until they could be transported to Purdue University, where they were stored at -75°C until analysis.

Following genomic DNA extraction, mitochondrial DNA (mtDNA) was analyzed for haplotype variation using a technique whereby a 2290 base-pair (bp) segment of the ND-2 gene region was amplified using standard polymerase chain reaction (PCR) protocols (Saiki et al. 1988, Lee et al. 1994). Cycling times and temperatures were as follows: 1) initial denaturation for 2 min at 95°C, 2) denaturation for 1 min at 95°C, 3) annealing for 1 min at 52°C, 4) extension for 2.5 min at 72°C, 5) final extension for 7 min at 72°C, and 6) soak at 4°C. Steps 2-4 were repeated 40 times. Each fragment was amplified using the primers 562 (5′ TAA GCT ATC GGG CCC ATA CC 3′) and 452 (5′ ACT TCA GGG TGC CCA AAG AAT CA 3′; Lee et al. 1994).

The resulting amplified DNA fragments were digested to completion according to manufacturer's recommendations using 1 unit of each of the following restriction enzymes: *Aci*-I, *Bsp*-1286, *Hha*-I, *Hinf*-I, *Rsa*-I, *Ssp*-I . The digested fragments were then electrophoresed on 1%-2% agarose gels (Sambrook et al. 1989), separating the fragments according to size, and producing a scorable pattern. An *EcoR*-I, *Hind*-III-digested lambda DNA marker was used for size determination on each agarose gel. Gels were stained with ethidium bromide, and a permanent electronic record of these patterns was stored using a Stratagene Eagle-Eye II™ gel documentation system.

Data were analyzed for population haplotype frequency differentiation among sampling locations using the Monte Carlo simulation (Roff and Bentzen 1989) program found in the Restriction Enzyme Analysis Package (REAP, McElroy et al. 1992). Each Monte Carlo simulation was run with 1,000 iterations to test the hypothesis that differences in haplotype frequencies among populations were different than would be expected under random conditions. Significance values were based on an alpha of ≤ 0.05 and critical probability values were adjusted to $P \leq 0.004$ to account for multiple comparisons using the Dunn-Sidak method. To assess the impact of past relocations, populations were grouped for analysis based on known reintroduction histories. These analyses were employed for: 1) populations that had reintroductions from Wyoming, Montana, or Texas; 2) populations that received reintroductions from central Arizona [GMU 5B; Figure 1]; 3) populations in southeastern Arizona; and 4) populations in the northwestern Arizona (Figure 1; Table 1).

RESULTS

During the 1996 and 1997 collection efforts, 405 individual pronghorn were sampled throughout the state of Arizona (1996, n = 351; 1997, n = 54). This represented approximate return rates of 46.5% in 1996 and 16.2% in 1997, based on the number of collection kits mailed to hunters. Of the 405 samples collected, 97 were used in analyses of haplotype frequency differentiation among GMUs which had received pronghorn reintroductions. Samples were analyzed from: GMU 4A (n = 13), GMU 5B (n = 15), GMU 12A (n = 1), GMU 12B (n = 2), GMU 13A (n = 13), GMU 13B (n = 6), GMU 30A (n = 22), GMU 31 and 32 (n = 15), GMU 34B (n = 4), and GMU 35A/B (n = 7) (Figure 1).

All restriction enzymes proved informative, except *Rsa*-I which was monomorphic. *Aci*-I , *Bsp*-1286, *Hha*-I, and *Hinf*-I each resulted in 2 distinct fragment patterns; *Ssp*-I produced 3 distinct fragment patterns (Reat et al. 1999). From these individual fragment patterns, 4 composite haplotypes were observed in Arizona's pronghorn, including 1 haplotype (K), that has not previously been described. The remaining 3 haplotypes (A, C, J) were observed previously in North American pronghorn (Lee 1992, Lee et al. 1994).

Populations that received translocated animals from Texas, Wyoming, or Montana showed no significant variability in haplotype frequencies (Table 1). Populations which received pronghorn from Colorado (GMUs 12A & 13B) could not be tested for haplotype differentiation, as only 1 individual was collected from GMU 12A (Table 2). Haplotype frequencies were significantly different among GMUs that received reintroductions from central Arizona (GMU 5B) only when GMU 13A, which also received pronghorn from Montana, was included (Table 1). Likewise, of those populations that received pronghorn from central Arizona, only the GMU 13A population was significantly different in haplotype frequency from its source. Overall, populations in southeastern Arizona differed significantly in haplotype frequencies (Table 1). However, populations within southeastern Arizona that shared reintroduction sources (e.g., Texas or central Arizona) were not different from each other in haplotype frequencies. Populations sampled in northwestern Arizona were not different in haplotype frequencies despite the wide array of sources (central Arizona, Montana, Wyoming, Colorado, and Utah) used to restock the region (Table 1).

DISCUSSION

Our data indicated that, in general, pronghorn populations that shared a common source were similar in haplotype frequency. For example, subsets of Arizona pronghorn populations that received reintroductions from either Montana, Wyoming, or Texas were similar in haplotype frequencies. Our comparisons of haplotype frequencies among populations that shared central Arizona as a source indicated that for all comparisons, except those in which pronghorn from

Table 1. Monte Carlo simulations with 1,000 iterations were used to compare haplotype frequency distributions among pronghorn populations in Arizona. Comparisons were made between pairs of reintroduced populations (GMUs) that shared a common source from Montana, Wyoming, Texas, or Arizona. Comparisons also were made between the central Arizona population used as a source (GMU 5B) and those populations founded from that source. Additionally, comparisons of haplotype frequencies were made among the reintroduced populations that reside in the southeastern and northwestern regions of Arizona.

Comparison	*P*-value[5]
Montana reintroductions	
GMU 13A[1], 13B[2]	0.080
Wyoming reintroductions	
GMU 4A, 13B	0.135
Texas reintroductions	
GMU 34B, 30A	0.239
Arizona reintroductions	
GMU 13A, 31/32	<0.001
GMU 13A,35A/B	0.004
GMU 31/32,35A/B	0.068
Game Management Unit 5B	
GMU 13A, 5B	<0.001
GMU 31/32, 5B	0.270
GMU 35A/B, 5B	0.850
Southeast[3]	
GMU 31/32, 30A, 34B, 35A/B	0.003
Northwest[4]	
GMU 13A, 12A, 12B, 13B	0.162

[1] reintroductions also from central Arizona
[2] reintroductions also from Wyoming and Colorado
[3] reintroductions from central Arizona and Texas
[4] reintroductions from central Arizona, Montana, Wyoming, Colorado, and Utah
[5] Dunn-Sidak Corrected significance level is $P \leq 0.004$

Table 2. Composite haplotype frequencies based on PCR-RFLP analysis of the 2.3 kb ND-2 gene region of mtDNA in Arizona pronghorn populations. Sample sizes (*n*) are provided for each of 10 Game Management Units analyzed in Arizona. Data were collected during the fall hunting seasons of 1996 and 1997.

GMU	*n*	A	C	J	K
4A	13	0.615	0.308	0.000	0.077
5B	15	0.533	0.200	0.000	0.267
12A	1	1.000	0.000	0.000	0.000
12B	2	0.000	1.000	0.000	0.000
13A	13	0.000	1.000	0.000	0.000
13B	6	0.167	0.667	0.167	0.000
30A	22	0.500	0.500	0.000	0.000
31/32	14	0.852	0.000	0.000	0.143
34B	4	0.000	1.000	0.000	0.000
35A/B	7	0.430	0.285	0.000	0.285
Average	9.7	0.410	0.496	0.016	0.076

GMU 13A were included, haplotype frequencies were similar among populations. Additionally, of those populations that were established using pronghorn from central Arizona, only the pronghorn population residing in GMU 13A was significantly different from its source in haplotype frequency. The aberrant haplotype frequencies in GMU 13A are most likely due to the high frequency of the C haplotype (common in non-Arizona pronghorn) and the total absence of the K haplotype (unique to Arizona) in pronghorn from that GMU (Table 2).

The southeastern and northwestern regions of Arizona are considered to be inhabited totally by reintroduced stock. Thus, significant differences in haplotype frequencies among pronghorn populations in southeastern Arizona are likely a consequence of differences between the Texas (K haplotype absent) and central Arizona (K haplotype present) source populations used to restock the region. Pairwise comparisons of populations sharing the same sources in the southeastern region of the state (i.e., central Arizona stock versus Texas stock) were non-significant. Differences in haplotype frequencies within the southeastern region populations are probably a consequence of historic differences between reintroduction sources (i.e., Texas versus Arizona), despite the evidence that the (A) haplotype is the common haplotype for both sources. Alternatively, the wide variety of sources and ubiquitous placement of pronghorn used to restock the Arizona Strip, as well as low samples sizes in GMUs 12A and 12B, probably contributed to the lack of observable differentiation among

pronghorn populations sampled in that region.

Several researchers have used genetic tools to assess the colonization success of reintroduced populations relative to the success of remnant populations residing in their vicinity (Ellsworth et al. 1994, Leberg et al. 1994, Nedbal et al. 1997). In some instances, remnant genetic material has clearly been maintained even in the presence of repeated translocations of individuals with different genetic characteristics (Ellsworth et al. 1994). In other cases, there is evidence that reintroduced populations have maintained the genetic characteristics of their source populations, even many generations after the reintroduction event (Leberg et al. 1994). Our analysis of reintroduced pronghorn populations indicate that Arizona populations sharing a common source do retain the genetic characteristics of those sources, despite the effects of sampling error (founder effect) during the translocation events and random changes in gene frequencies over time (genetic drift) after establishment.

Our results may not seem surprising given that many of the focal study populations were established in areas where pronghorn were assumed to have been extirpated. However, our data do serve to support the premise that pronghorn were extirpated in northwestern and southeastern Arizona. In particular, the absence of the K haplotype in northwestern Arizona (which received no intraArizona translocations) and, the presence of the K haplotype in all GMU's in southeastern Arizona except GMUs 30A and 34B (which received pronghorn from Texas only) suggest that the current genetic distributions of these regional populations were primarily influenced by their reintroduction sources.

MANAGEMENT IMPLICATIONS

Data on regional gene frequency distributions offer wildlife managers the opportunity to select source populations for reintroduction programs that are appropriate to their management goals. For instance, our data suggest that a genetic legacy of past reintroductions is maintained in Arizona's current pronghorn populations. Thus, it is clear that decisions pertaining to pronghorn sources have long lasting genetic impacts and that pronghorn reintroductions have probably changed the distribution of genetic diversity in the Arizona population. Alternatively, Arizona has a unique opportunity to preserve pronghorn stocks native to the state (e.g., K haplotype) as they make decisions regarding source populations for future translocations. In addition, using the haplotype frequency data generated in this research, Arizona biologists can make informed decisions regarding future translocations of pronghorn in the state.

ACKNOWLEDGEMENTS

Thanks to all the volunteers from Arizona Game and Fish Department, Arizona State University, University of Arizona, Northern Arizona University,

Yavapai College, Grand Canyon University, Pima Community College, and the Arizona Antelope Foundation who helped operate the sample collection stations, especially R. Barkhurst, J. Beals , D. N. Cagle, A. Fuller, J. Galaway, J. Goodwin, J. Hanna, B. Henry, J. Hervert, C. A. Jones, M. A. McKenzie, R. M. Lee, A. Munig, J. B. Pickrell, P. Shaw, J. Simmons, J. Vassel, B. F. Wakeling, and many others. The Southern Arizona Bowhunting Chapter of Safari Club International provided funding for necessary field supplies. We thank Purdue University for providing funding in support of this research and we express our appreciation to B. Shen for his assistance with laboratory analyses. Our appreciation goes to all the Arizona Game and Fish Department Employees who went out of their way to collect samples from far reaching areas. We also thank Richard Ockenfels for information on pronghorn movements and population fragmentation. Arizona pronghorn hunters played an integral role in sample collection, sometimes going far out of their way to return samples in good condition. This is a contribution of the Federal Aid in Wildlife Restoration Act Project W-53-M and W-78-R of the Arizona Game and Fish Department.

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