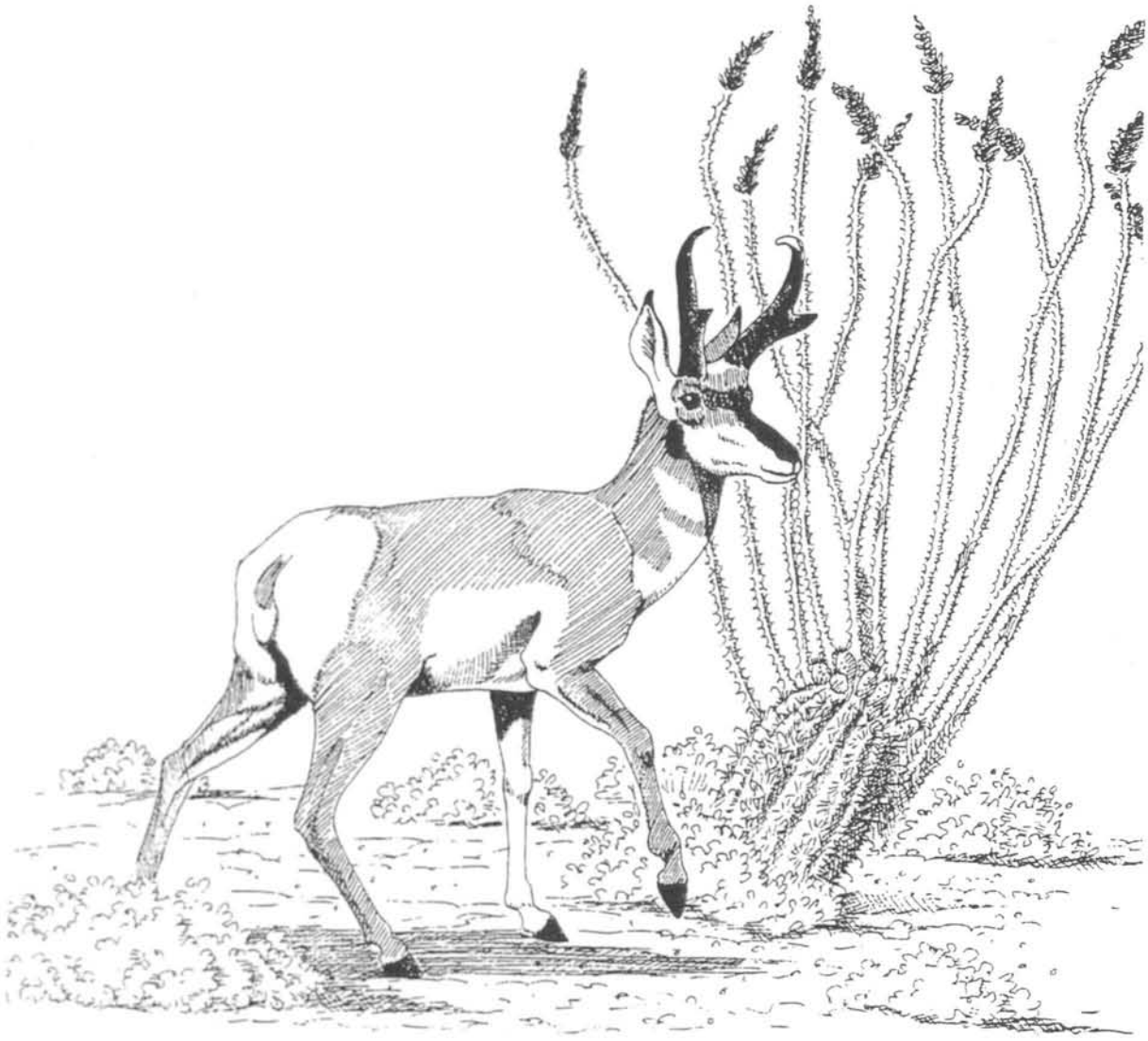
PROCEEDINGS OF THE 18th BIENNIAL PRONGHORN ANTELOPE WORKSHOP

Sanctioned by

Western Association of Fish and Wildlife Agencies

Hosted by

Arizona Game and Fish Department



Editor
Richard A. Ockenfels
December 1999

STEERING COMMITTEE

Arizona Game and Fish Department
2221 W. Greenway Road, Phoenix, AZ 85023-4312

Richard A. Ockenfels, Chair
Cindy L. Ticer, Co-Chair
James C. deVos, Jr.
Amber Alexander Munig
Raymond M. Lee

Sanctioned by

Western Association of Fish and Wildlife Agencies
c/o Game and Fish Department
5400 Bishop Boulevard
Cheyenne, WY 82006

Co-Sponsors

Arizona Antelope Foundation
P.O. Box 15501, Phoenix, AZ 85060-5501

North American Pronghorn Foundation
1905 CY Avenue, Casper, WY 82604

Contributors

Arizona Game Rangers Association
P.O. Box 41460, Phoenix, AZ 85080-1460

Arizona Bowhunters Association
P.O. Box 67084, Phoenix, AZ 85082-3894

International Association of Fish and Wildlife Agencies
444 N. Capitol NW #544, Washington, D.C. 20001

Valley of the Sun YMCA Camps
Chauncey Ranch Camp
HC 63 Box 3027, Mayer, Arizona 86333

Meeting Site

Prescott Resort Conference Center and Casino
Prescott, Arizona, March 23-27, 1998

REGIONAL GENETIC DIFFERENTIATION IN ARIZONA PRONGHORN

ERIN P. REAT, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA

OLIN E. RHODES, JR., Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA

JAMES R. HEFFELFINGER, Arizona Game and Fish Department, 555 North Greasewood Road, Tucson, AZ 85745, USA

JAMES C. deVOS, JR., Arizona Game and Fish Department, 2221 W. Greenway Road, Phoenix, AZ 85023, USA

Abstract: Mitochondrial haplotype diversity was examined in pronghorn antelope (*Antilocapra americana*) to elucidate overall levels of genetic diversity and regional differentiation of the Arizona pronghorn population. A total of 389 Arizona pronghorn were analyzed for haplotype variation using polymerase chain reaction (PCR) protocols. The resulting amplified fragments were digested using 1 of each of the following restriction enzymes: Aci-I, *Bsp*-1286, *Hha*-I, *Hinf*-I, *Rsa*-I, and *Ssp*-I. Four composite haplotypes were observed in Arizona pronghorn, including 3 composite haplotypes (**C**, **A**, and **J**) previously observed in North American pronghorn and a single haplotype (**K**) that is potentially unique to Arizona. The **A** haplotype, once proposed as a potential genetic marker associated with Mexican pronghorn (*A. a. mexicana*), was found throughout the Arizona pronghorn range in high frequency. Haplotype **K** was found in highest frequency within the central portion of the historical pronghorn range in Arizona. In addition, there was differentiation in haplotype frequencies of Arizona pronghorn among regions (Arizona strip, northwestern, central, northeastern, and southeastern) of the state.

Key Words: *Antilocapra americana*, genetic diversity, mitochondrial DNA, polymerase chain reaction, restriction enzyme.

Pronghorn Antelope Workshop Proceedings 18:25-31

Antilocapra americana currently contains 4 recognized subspecies (American pronghorn, *A. a. americana*; Mexican pronghorn; Sonoran pronghorn, *A. a. sonoriensis*; and Peninsular pronghorn, *A. a. peninsularis*). Historically, 3 of these 4 subspecies of pronghorn (American, Mexican, and Sonoran) were believed to have occupied ranges in Arizona (Hoffmeister 1986; Fig. 1). However, because of sharp declines in numbers of pronghorn around the turn of the century, resulting in both isolated and extirpated populations, the Arizona Game and Fish Department (AGFD) initiated a series of pronghorn reintroductions beginning in the 1920s and continuing through the present. These reintroductions were designed to help bolster small populations and to repopulate historic pronghorn ranges. Although successful in reestablishing pronghorn populations to respectable numbers, these reintroductions compromised the historical intraspecific patterns of genetic variation in Arizona pronghorn and may have altered the spatial distribution of subspecies within the state.

Mitochondrial haplotype analyses have been used extensively to examine issues of intraspecific differentiation of organisms (Ellsworth et al. 1994*a*,*b* ; Theimer and Keim 1994; Cronin et al. 1996; Walpole et al. 1997). Restriction Fragment Length Polymorphism analysis of mitochondrial DNA (mtDNA) is a simple procedure whereby the mitochondrial genome, or portions thereof, are digested with restriction enzymes, electrophoresed, and scored for the presence or absence of restriction sites. Mitochondrial DNA has certain properties that make it ideal for use in phylogenetic analysis at the intraspecific level. Among these are maternal inheritance, a non-recombinant method of transmission, a high rate of evolution in

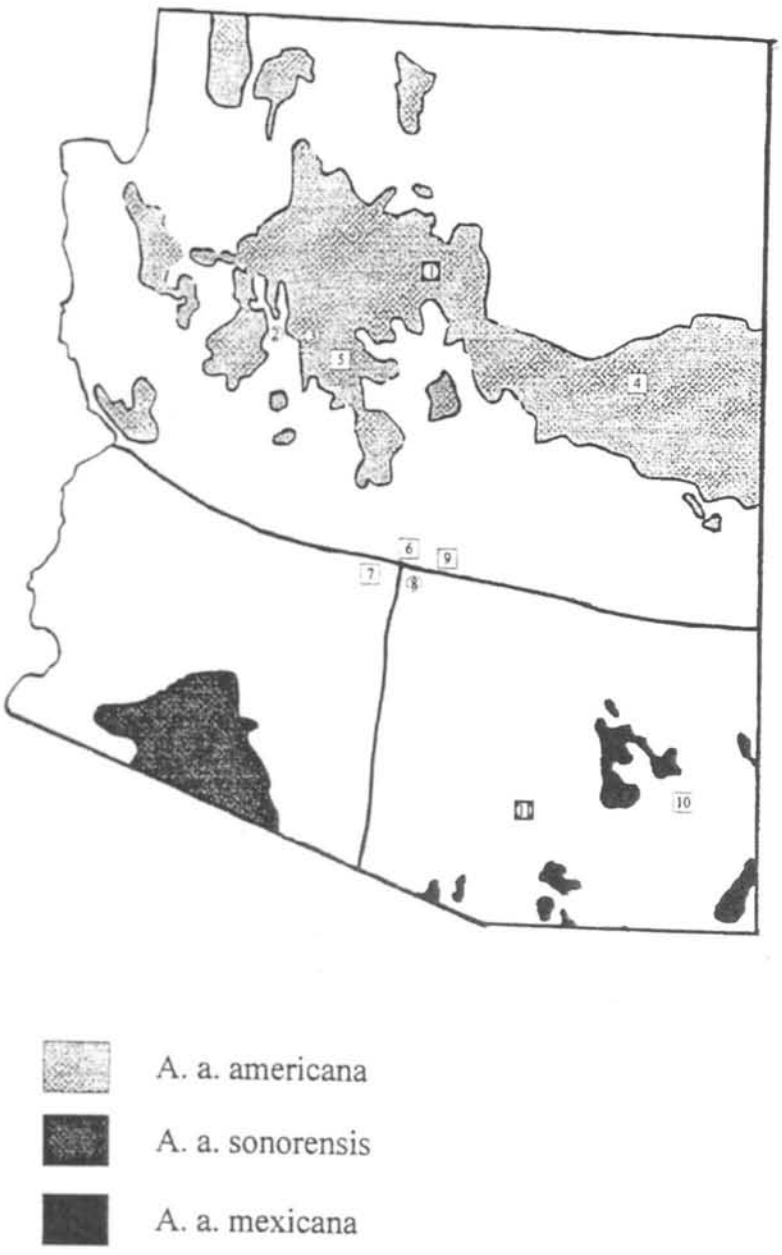


Figure 1. The distribution of pronghorn subspecies within the state of Arizona is indicated. Collection stations were run during pronghorn rifle hunts in 1996 (squares), 1997 (circles), or in both years (circle in square). Numbers within the symbols represent locations. 1) Flagstaff, 2) Wickieup, 3) Bagdad, 4) Showlow, 5) Prescott, 6) I-17 and Carefree Highway, 7) Wittmann, 8) Phoenix, 9) Beeline and Bush highways, 10) Willcox, 11) Tucson.

vertebrates (compared to the nuclear genome), and extensive intraspecific polymorphism (Avise et al. 1987).

Lee et al. (1994) reexamined subspecific differentiation of pronghorn across North America using a mitochondrial DNA haplotype analysis. Lee et al. (1994) discovered that their mitochondrial markers were useful in resolving intraspecific differentiation of pronghorn and observed unique regional haplotypes across North America, including 1, which appeared informative in resolving American pronghorn from Mexican pronghorn. Although Lee et al. (1994) were successful in surveying the majority of North American pronghorn range for haplotypic variation, Arizona, which has a mixture of recognized subspecies, was not included in their examination.

The objectives of our research were to survey pronghorn from 5 regions of Arizona using the same mitochondrial markers as Lee et al. (1994) and to describe patterns of regional haplotype variation.

METHODS

Collection

Collection kits, consisting of sample bags and instructions for tissue collection and handling, were mailed out to hunters that obtained permits to harvest pronghorn in Arizona during the 1996 and 1997 hunting seasons. In 1996, approximately 700 kits were mailed out to hunters throughout the state. In 1997, approximately 350 kits were mailed out to hunters that drew tags for areas under-represented in the 1996 sampling effort. Hunters were asked to collect liver and muscle tissue in the field and place samples on wet ice. Hunters were then asked to drop samples off at convenient collection stations located throughout Arizona at major highway intersections. In addition to roadside collection, AGFD wildlife managers in the field collected samples. 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. At Purdue University, samples were stored at -75 C until analysis.

PCR-RFLP Analysis

Following organic genomic DNA extraction, mitochondrial DNA (mtDNA) was analyzed for haplotype variation using a technique whereby a 2,290 base-pair (bp) portion of the NADH-2 gene region was amplified using standard Polymerase Chain Reaction (PCR) protocols (Lee et al. 1994). PCR is a technique where target DNA strands are copied by means of enzymatic amplification (Saiki et al. 1988). Following heat denaturation of the target DNA strands, primers anneal to their specific binding sites on the target DNA strands. Thermostable DNA polymerase (usually *Taq* DNA Polymerase) then recognizes the 5′ overhang from the primers and begins to extend the primers from the 5′ end to the 3′ end, incorporating deoxynucleotide triphosphates to their conjugate bases on the template strand. Repeated temperature fluctuations (denaturation, annealing, extension steps) produce millions of copies of target DNA sequence from only a few initial copies of template DNA.

The NADH-2 gene region was amplified using a slight variation of the protocol outlined by 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 (Reat 1998). Each fragment was amplified using the primers 5′ TAA GCT ATC GGG CCC ATA CC 3′ and 5′ ACT TCA GGG TGC CCA AAG AAT CA 3′ (Lee et al. 1994).

Resulting amplified DNA fragments were digested to completion according to manufacturer's recommendations using 1 of each of the following restriction enzymes: *Aci*-I, *Bsp*-1286, *Hha*-I, *Hinf*-I, *Rsa*-I, *Ssp*-I. Digested fragments were then electrophoresed on 1%-2% agarose gels (Sambrook et al. 1989), separating 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 record of these patterns was made using a

Stratagene Eagle-Eye II™ gel documentation system.

Composite haplotype frequencies were calculated for 5 geographic regions of Arizona (northeast, central, northwest, southeast, and the Arizona Strip; Fig. 2). Regions were selected based on their past history of reintroductions and degree of isolation. The Arizona strip and southeastern regions are isolated from the remainder of the pronghorn populations and were thought to have been entirely extirpated at some point in the past. The central region was considered the core of the pronghorn range in Arizona and had no reintroductions from other herds prior to our sampling. The northeastern region was selected based on its history of reintroductions from Wyoming, and the northwestern region was selected based on its somewhat isolated location relative to the other portions of Arizona pronghorn range.

RESULTS

During 1996 and 1997 collection efforts, 405 individual pronghorn were sampled throughout Arizona (1996, $n = 351$; 1997, $n = 54$). This represented approximate return rates of 50% in 1996 and 15% in 1997, based on number of collection kits mailed to hunters. Of 405 samples collected, 389 were analyzed for composite haplotype variation of the ND-2 gene region of the mitochondrial genome.

All restriction enzymes, except Rsa-I, proved informative. *Aci*-I, *Bsp*-1286, *Hha*-I, and *Hinf*-I each resulted in 2 distinct fragment patterns, while *Ssp*-I resulted in 3 distinct fragment patterns (Reat 1998). From these individual restriction digests, 4 composite haplotypes were observed in Arizona's pronghorn, including 1 composite haplotype, **K**, not previously seen in North America. The remaining 3 composite haplotypes were observed previously by Lee et al. (1994) in North America.

Composite haplotype **A** consists of an A digestion with *Aci*-I, C with *Bsp*-1286, C with *Hha*-I, C with *Hinf*-I, C with *Rsa*-I, and C with *Ssp*-I. Composite haplotype **K** was viewed as an A digestion with *Aci*-I, C with *Bsp*-1286, C with *Hha*-I, C with *Hinf*-I, C with *Rsa*-I, and B with *Ssp*-I. Composite haplotype **C** was observed as C digestions with all restriction enzymes, whereas composite haplotype **J** was viewed as C with *Aci*-I, A with *Bsp*-1286, A with *Hha*-I, A with *Hinf*-I, C with *Rsa*-I, and E with *Ssp*-I.

Of 389 pronghorn analyzed statewide, 267 were characterized as having the **A** haplotype. Eighty-six pronghorn were observed as having the **C** haplotype, whereas 16 and 20 pronghorn were viewed as being haplotypes **J** and **K** respectively (Table 1). In northeastern Arizona, 103 pronghorn were analyzed. Of those, 81 pronghorn were characterized by the **A** haplotype, 6 pronghorn had the **C** haplotype, whereas 15 pronghorn and 1 pronghorn were characterized with the **J** and **K** haplotypes respectively (Table 1). In central Arizona, 136 of 188 sampled pronghorn were characterized with the **A** haplotype. Thirty-seven and 15 were observed as having the **C** and **K** haplotype respectively (Table 1). Twenty-six pronghorn were analyzed in northwestern Arizona, of which 20 were observed with the **A** haplotype and 6 had the **C** haplotype. Twenty-two pronghorn were analyzed from the Arizona Strip, of which 2 were characterized with the **A** haplotype, 19 were characterized with the **C** haplotype, and 1 had the **J** haplotype (Table 1). Finally, 50 pronghorn were analyzed from southeastern Arizona, of which 28 were observed with the **A** haplotype, 18 were characterized with the **C** haplotype, and 4 had the **K** haplotype (Table 1).

DISCUSSION

Three of the 9 composite haplotypes observed over the North American range of pronghorn (Lee et al. 1994) were detected in Arizona pronghorn populations. In addition, a previously unknown composite haplotype was detected in the Arizona population. Based on surveys of pronghorn performed by Lee et al. (1994), the **C** haplotype was found to be the most common haplotype detected across their North American range. The **A** haplotype was found to be fixed in pronghorn populations in the Marathon Basin, Texas, but occurred at low frequencies in other Texas populations as well as in 1 individual from both New Mexico and Colorado. Overall, the **A** haplotype is quite rare across most of the North American pronghorn range. The third previously detected haplotype observed in Arizona, haplotype **J**, has only been

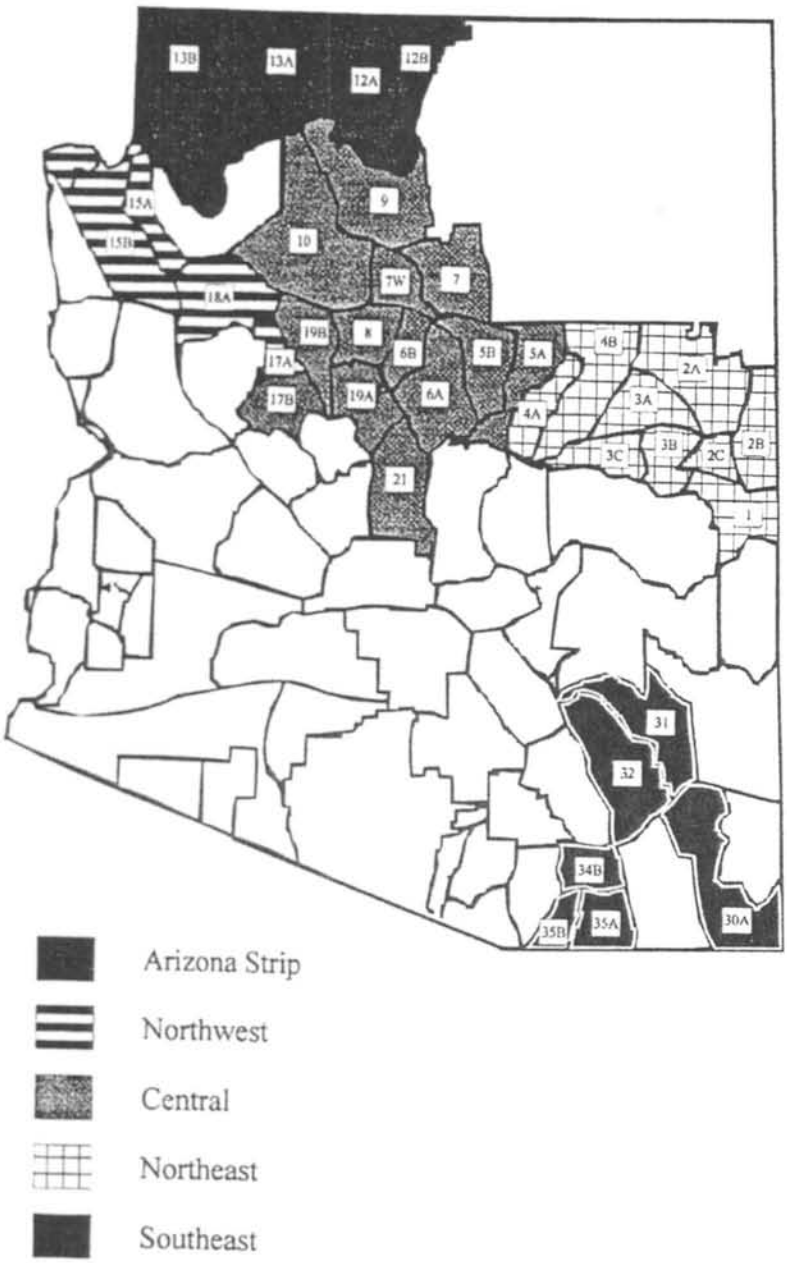


Figure 2. Samples from pronghorn were divided into 5 regional populations for analysis. Pronghorn were assigned to the Arizona strip, northwest, central, northeast, and southeast regions of Arizona. Game management unit numbers are provided within regions.

Table 1. Composite haplotype frequencies of the 2.3 kb ND-2 gene region of mtDNA are provided for pronghorn sampled in Arizona during 1996-97. Haplotype frequencies are provided for the overall herd and for 5 geographic regions of the state. Letters **A**, **C**, **J**, and **K** represent 4 composite haplotypes observed in Arizona pronghorn. Sample sizes are provided in parentheses.

	Haplotypes			
Region	A	C	J	K
Northeast	78.64 (81)	5.83 (6)	14.56(15)	0.96 (1)
Central	72.34 (136)	19.68 (37)	0.00 (0)	7.98 (15)
Northwest	76.92 (20)	23.08 (6)	0.00 (0)	0.00 (0)
Arizona Strip	9.09 (2)	86.36 (19)	4.55 (1)	0.00 (0)
Southeast	56.00 (28)	36.00 (18)	0.00 (0)	8.00 (4)
Arizona (total)	68.64 (267)	22.14 (86)	4.14 (16)	5.14 (20)

observed in 1 other population in North America, that being the Yellowstone, Wyoming, herd.

Perhaps the most striking result stemming from analysis of our data is the ubiquitous distribution of the **A** haplotype in Arizona. The **A** haplotype, proposed by Lee et al. (1994) as a potentially diagnostic marker for Mexican pronghorn, was actually the most common haplotype observed in Arizona pronghorn. This result alone does not provide the basis upon which to make a final determination on the actual utility of the **A** haplotype as a marker for Mexican pronghorn, but strongly suggests that the **A** haplotype may simply be a genetic marker that is common to pronghorn in the southern portion of this species range.

The discovery of the **K** haplotype in central and southeastern Arizona also is important. The central Arizona Game Management Units (GMU) in which the **K** haplotype was detected are generally considered to be the heart of pronghorn range in Arizona, and this region has been used extensively as a source for pronghorn transplants to the southeastern portion of the state. In addition, there have been no documented pronghorn reintroductions into the central Arizona GMUs in which the **K** haplotype was observed. Thus, it is likely that the **K** haplotype is an ancestral marker, potentially unique to Arizona, and central Arizona populations containing this haplotype should be protected from future translocations. Such protection should include limiting pronghorn reintroductions into the central region to sources within that same region. This would prevent dilution of the unique Arizona haplotype **K**. Occurrence of the **K** haplotype in southeastern Arizona, an area of the state that was considered to be entirely devoid of pronghorn at one time, is likely a consequence of extensive reintroductions to the southeast from the central portion of the state.

MANAGEMENT IMPLICATIONS

This study adds to work previously performed by Lee et al. (1994) on pronghorn of North America, by providing a clearer picture of mitochondrial DNA differentiation of the species nationwide. Our data indicate the presence of a regionally unique haplotype (**K**) within the central portion of pronghorn range in Arizona. Haplotype **A**, a marker proposed to be associated with Mexican pronghorn, was found to be distributed throughout Arizona in high frequency. In addition, there is evidence for a high degree of regional differentiation in haplotype frequencies across pronghorn range in Arizona. Using data collected in this study, Arizona biologists can make choices concerning selection of source populations for future pronghorn reintroductions based on haplotypic composition of recipient herds and can protect portions of the current Arizona pronghorn population that contain individuals with haplotypic diversity that is potentially unique in the North American pronghorn metapopulation.

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 State University, Pima Community

College, and the Arizona Antelope Foundation who helped operate sample collection stations, especially R. Barkhurst, D. N. Cagle, A. Fuller, J. Galaway, J. Goodwin, J. Hanna, B. Henry, J. Hervert, C. Jones, J. Beals, 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 R.A. Ockenfels for information on pronghorn movements and population fragmentation. Thanks also to M. Alderson for generating GIS-based maps of pronghorn distribution and GMUs in Arizona. 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 Projects W-53-M and W-78-R of the Arizona Game and Fish Department

LITERATURE CITED

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and **N. C. Saunders**. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489-522.

Cronin, M. A., J. Bodkin, B. Ballachey, J. Estes, and **J. C. Patton**. 1996. Mitochondrial-DNA variation among subspecies and populations of sea otters (*Enhydra lutris*). Journal of Mammalogy 77:546-557.

Ellsworth, D. L., R. L. Honeycutt, N. J. Silvy, K. D. Rittenhouse, and **M. H. Smith**. 1994*a*. Mitochondrial-DNA and nuclear gene differentiation in North American prairie grouse (Genus *Tympanuchus*). The Auk 111:661-671.

Ellsworth, D. L., R. L. Honeycutt, N. J. Silvy, J. W. Bickham, and **W. D. Klimstra**. 1994*b*. Historical biogeography and contemporary patterns of mitochondrial DNA variation in white-tailed deer from the southeastern United States. Evolution 48:122-136.

Hoffmeister, D. F. 1986. Mammals of Arizona. Arizona Game and Fish Department and University of Arizona Press. Tucson, Arizona, USA.

Lee, T. E., Jr., J. W. Bickham, and **M. D. Scott**. 1994. Mitochondrial DNA and allozyme analysis of North American pronghorn populations. Journal of Wildlife Management 58:307-318.

Reat, E. P. 1998. Use of genetic markers to determine the subspecific distribution of pronghorn in Arizona. M.S. Thesis, Purdue University, West Lafayette, Indiana, USA.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and **H. A. Erlich**. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491.

Sambrook, J., E. F. Fritsch, and **T. Maniatis**. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press. Plainview, New York, USA.

Theimer, T. C., and **P. Keim**. 1994. Geographic patterns of mitochondrial-DNA variation in collared peccaries. Journal of Mammalogy 75:121-128.

Walpole, D. K., S. K. Davis, and **I. F. Greenbaum**. 1997. Variation in mitochondrial DNA in populations of *Peromyscus eremicus* from the Chihuahuan and Sonoran deserts. Journal of Mammalogy 78:397-404.