

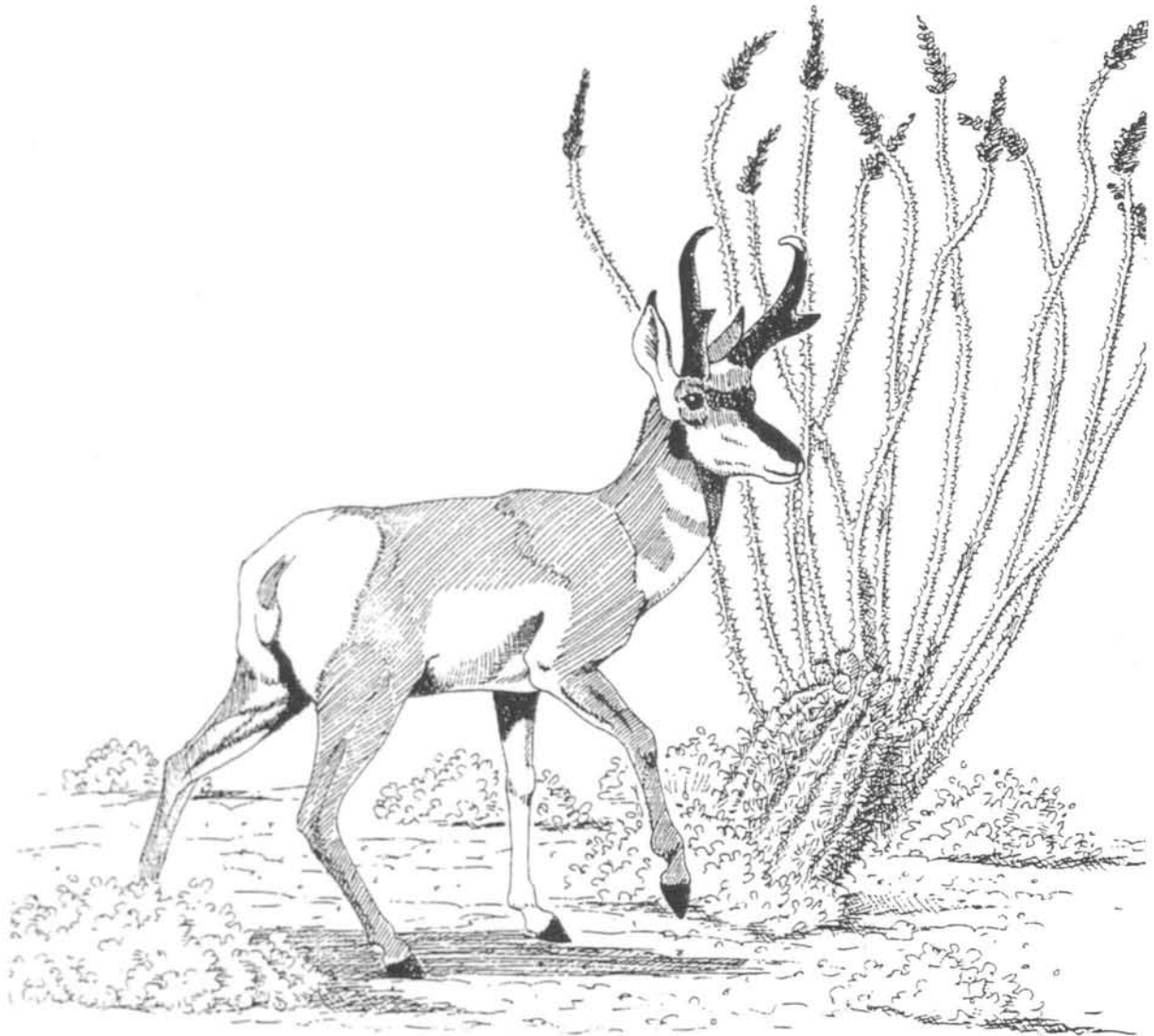
# PROCEEDINGS OF THE 18<sup>th</sup> BIENNIAL PRONGHORN ANTELOPE WORKSHOP

Sanctioned by

*Western Association of Fish and Wildlife Agencies*

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## GENETIC VARIATION IN PRONGHORN FROM ARIZONA

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**Abstract:** Three hundred twenty-two pronghorn antelope (*Antilocapra americana*) were surveyed for genetic variation at 22 allozyme loci by means of starch gel electrophoresis of allozymes. Genetic diversity measures, including heterozygosity, number of alleles per locus, and percent loci polymorphic, were estimated for the overall Arizona pronghorn sample and for selected regional populations. Pronghorn from Arizona exhibited levels of genetic diversity that were greater than average values for other North American pronghorn populations. Analyses of pronghorn populations in the Arizona Strip, northwestern, central, northeastern, and southeastern portions of Arizona indicated that there was significant differentiation in allele frequencies among herds inhabiting these regions. The significant genetic structuring observed among pronghorn populations in Arizona should be taken into account when sources are selected for future translocations of pronghorn into the state.

**Key words:** allozyme, *Antilocapra americana*, heterozygosity, nuclear DNA.

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Both mitochondrial and nuclear genetic markers have been surveyed in pronghorn antelope throughout much of the central and northern portions of this species range in North America (Lee et al. 1989, Lee et al. 1994). However, few genetic data exist for pronghorn populations inhabiting the southern portion of the North American pronghorn range, including areas in Arizona and Mexico. Arizona in particular is an area of considerable interest to pronghorn biologists, given that 3 of the 4 currently recognized subspecies of pronghorn in North America historically occurred in this state (Hall 1981, O'Gara 1978). Despite the unique opportunity for analysis of genetic structure and taxonomy represented by study of pronghorn in Arizona, only recently have genetic investigations been initiated in this area (Reat 1998).

The first substantial population level analysis of genetic structure in pronghorn was performed by Lee et al. (1989). Lee et al. (1989) used allozyme markers at 24 presumptive gene loci to assess genetic relationships and patterns of genetic variation in western Texas pronghorn populations ( $n = 65$ ). This study indicated that western Texas pronghorn populations were characterized by relatively low levels of genetic diversity (mean multilocus heterozygosity of 0.027) and moderately high levels of genetic differentiation among populations ( $F_{ST} = 0.103$ ).

Lee et al. (1994) followed up this initial work in Texas with a more comprehensive examination of genetic diversity and structure in pronghorn from 29 locations across northern and central portions of the North American pronghorn range. In this second study of pronghorn genetics, Lee et al. (1994) used both

mitochondrial DNA and allozyme markers to examine effects of past population declines, isolation, and translocation on current genetic structure of North American pronghorn populations. Analysis of allozyme data generated from their research indicated low levels of genetic diversity (mean multilocus heterozygosity of 0.024), Roger's distance values ranging from 0.01 to 0.07, and significant differentiation of allele frequencies among North American pronghorn populations at only 2 loci (Lee et al. 1994).

Arizona pronghorn, because of the historical presence of 3 subspecies in the state, are an excellent choice for initial analysis of the southern portion of the North American pronghorn population. Although extensive pronghorn reintroductions have occurred in portions of Arizona (Lee 1988), segments of the statewide population have remained undisturbed by translocation events (Hoffmeister 1986). A preliminary examination of genetic diversity in Arizona pronghorn is needed to assess genetic parameters of these animals within the context of the larger North American perspective. Furthermore, elucidation of regional variation in genetic diversity of Arizona pronghorn should provide insights into consequences of pronghorn reintroductions to allelic differentiation of pronghorn across the state. Thus, objectives of this research were to assess basic genetic parameters of pronghorn populations in Arizona relative to values reported by Lee et al. (1994) for other North American pronghorn herds and, to evaluate levels of genetic differentiation among regional pronghorn populations within the state.

## METHODS

### Sample Collection and Location

Collection kits, consisting of sample bags and instructions for tissue collection and handling, were mailed to hunters that obtained permits to harvest pronghorn in Arizona during the 1996 hunting season. Seven hundred kits were mailed out to hunters throughout the state. We asked hunters to collect liver and muscle tissue in the field and place the samples on wet ice. Hunters were then asked to drop samples off at collection stations located throughout Arizona at major highway intersections (Reat 1998). In addition to roadside collection,

samples were collected by Arizona Game and Fish Department wildlife managers in the field. On day of collection, samples were cataloged and placed in liquid nitrogen for storage until they could be transported to Purdue University on dry ice. At Purdue University, samples were stored at -75°C until analysis.

We classified pronghorn samples as to 5 geographic regions of Arizona (northeast, central, northwest, southeast, and the Arizona Strip) for analysis (Fig. 1). Regions were selected based on their past history of reintroductions and degree of isolation. The Arizona Strip and southeastern regions were isolated from the remainder of the pronghorn populations and were thought have been entirely extirpated at some point in the past. The central region was considered the core of 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.

### Starch Gel Electrophoresis

We used starch gel electrophoresis of allozymes as described in Lee et al. (1989), Rhodes et al. (1991), and Lee et al. (1994). Thirty-seven biochemical loci were surveyed using liver and/or muscle tissue. Twenty-two loci were selected for further analysis, based on reliability of scoring, and surveyed for genetic variation in pronghorn (Table 1). Less than 2 percent of individuals scored over all loci surveyed could not be assigned genotypes. We calculated allele frequencies, percent polymorphic loci, and single locus heterozygosities. The overall measure of multilocus heterozygosity ( $H$ ) was calculated from direct counts of observed heterozygotes at all loci divided by the product of the number of pronghorn analyzed and the total number of loci analyzed.

Contingency table chi-square statistics (Workman and Niswander 1970) were calculated for each variable locus (and for all loci combined) and used to analyze differences in genetic characteristics among pronghorn sampled from different regions within the state.

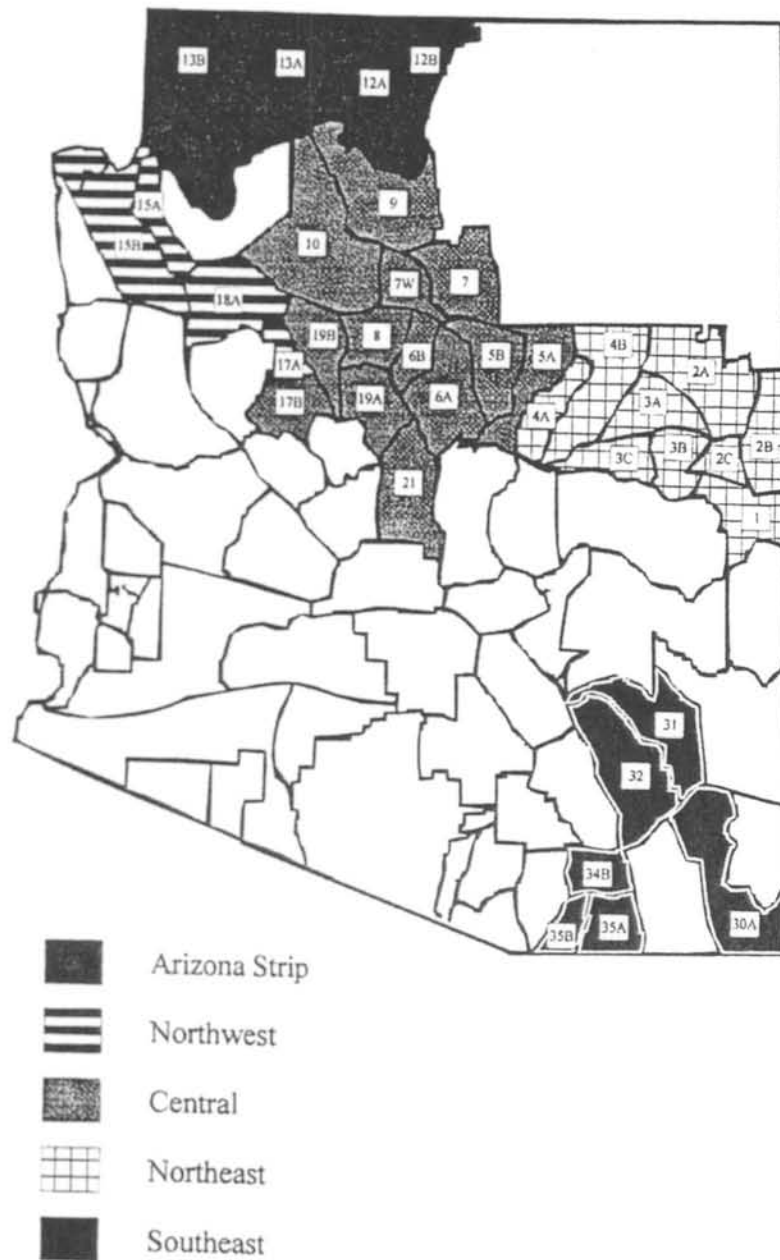


Figure 1. Samples from pronghorn were divided into 5 regional populations for analysis. Pronghorn were assigned to the Arizona Strip, northwest, central, northeast, and southeast regions of Arizona. Game Management Unit numbers are provided within regions.

Statistical genetic analyses were performed using BIOSYS (Swofford and Selander 1981).

In order to compare levels of genetic variation between Arizona pronghorn and data published for other North American populations, we reanalyzed allele frequency data reported in Lee (1992) using only those loci common to his study and our own. Sixteen loci were common to both studies (Table 1). Based on allele

frequencies reported in Lee (1992), we calculated average numbers of alleles per locus, percent polymorphic loci, and expected single locus heterozygosities for 29 North American pronghorn populations. Overall average values for numbers of alleles per locus, percent polymorphic loci and expected heterozygosities across the 29 North American pronghorn populations were calculated as weighted (by sample size) means.

Table 1. A list of electrophoretic conditions used for the 22 loci studied in pronghorn antelope. Locus acronyms, enzyme commission numbers (E.C.), tissue types, and gel buffers with respective pH's are presented.

Loci		E. C.	Tissue <sup>a</sup>	Buffer <sup>b</sup>
AAT-1 <sup>c</sup>	Aspartate aminotransferase-1	2.6.1.1	L	TC 8.0
AAT-2 <sup>c</sup>	Aspartate aminotransferase-2	2.6.1.1	L	TC 8.0
ACP <sup>c</sup>	Acid phosphatase	3.1.3.2	L	AC 6.1
ACON <sup>c</sup>	Aconitase	4.2.1.3	L	AC 6.1
EST-2	Esterase-2	3.1.1.1	L	RW 8.5
IDDH <sup>c</sup>	L-idoitol dehydrogenase	1.1.1.14	L	AC 6.1
IDHP-1 <sup>c</sup>	Isocitrate dehydrogenase-1	1.1.1.41	M	TM 7.4
IDHP-2	Isocitrate dehydrogenase-2	1.1.1.41	M	TM 7.4
LDH-1 <sup>c</sup>	Lactate dehydrogenase-1	1.1.1.27	M	TC 8.0
LDH-2	Lactate dehydrogenase-2	1.1.1.27	M	TC 8.0
MDH-1 <sup>c</sup>	Malate dehydrogenase-1	1.1.1.37	L	TC 8.0
MDH-2 <sup>c</sup>	Malate dehydrogenase-2	1.1.1.37	L	TC 8.0
ME-1 <sup>c</sup>	Malic enzyme-1	1.1.1.38	M	TM 7.4
ME-2	Malic enzyme-2	1.1.1.38	M	TM 7.4
MPI <sup>c</sup>	Mannose-6-phosphate isomerase	5.3.1.8	M	TC 8.0
NP <sup>c</sup>	Nucleoside phosphorylase	2.4.2.1	M	TM 7.4
PEP-1 <sup>c</sup>	Peptidase-1	3.4.11.-	M	TM 7.4
PEP-2 <sup>c</sup>	Peptidase-2	3.4.11.-	M	TM 7.4
PGDH	6-Phosphogluconate dehydrogenase	1.1.1.44	M	TM 7.4
PGM-1 <sup>c</sup>	Phosphoglucomutase-1	5.4.2.2	L	PK 8.2
PGM-2	Phosphoglucomutase-2	5.4.2.2	L	PK 8.2
PGI <sup>c</sup>	Phosphoglucose isomerase	5.3.1.9	L	PK 8.2

<sup>a</sup>L = liver, M = muscle

<sup>b</sup>AC 6.1 = Amine-Citrate 6.1, TC 8.0 = Tris-Citrate 8.0, TM 7.4 = Tris- Maleate 7.4, PK 8.2 = Poulik discontinuous Tris-Citrate 8.2, RW 8.5 = Ridgeway 8.5

<sup>c</sup>Loci common to both Lee (1992) and our study.

## RESULTS

During the 1996 collection efforts, 351 individual pronghorn were sampled throughout Arizona. This represented approximate return rates of 50%, based on the number of collection kits mailed to hunters. Of 351 samples collected, 322 were analyzed for genetic variation using starch gel electrophoresis of allozymes. Sample sizes from the 5 regions of Arizona were as follows: Arizona Strip ( $n = 19$ ), northwest ( $n = 19$ ), central ( $n = 166$ ), northeast ( $n = 97$ ), and southeast ( $n = 19$ ).

Mean multilocus heterozygosity, average number of alleles per locus, and proportion of polymorphic loci (0.95% criterion) were 0.064, 2.14, and 0.23, respectively, in the pooled Arizona pronghorn population. Single locus heterozygosities in the pooled population ranged from 0.006 to 0.36 over the polymorphic loci surveyed. Of 22 loci surveyed, only 6 were monomorphic (malate dehydrogenase-2, isocitric dehydrogenase-1&-2, peptidase-1&-2, and lactate dehydrogenase-1).

Allele frequencies and mean single locus heterozygosities for 5 regional pronghorn populations sampled are provided for polymorphic loci (Appendix 1). Among 5 regional pronghorn populations surveyed, mean multilocus heterozygosities ranged from 0.05 to 0.09, mean number of alleles per locus ranged from 1.5 to 1.9, and proportion of polymorphic

loci (0.95 criterion) ranged from 0.18 to 0.36 (Table 2). Regional populations containing unique alleles (i.e., alleles not observed in other populations) ranged from zero unique alleles observed in the Arizona Strip and southeastern regions to 4 unique alleles in the northeastern region (Table 2).

Over all loci, differences in allele frequencies were detected between the 5 regions of Arizona surveyed ( $\chi^2 = 360.97$ ,  $df = 100$ ,  $P < 0.00001$ ). Differences in specific allele frequencies among the 5 regions were detected at the acid phosphatase, aconitase, glucose phosphate isomerase, phosphoglucumutase-1&-2, aspartate aminotransferase-2, esterase-3, malic enzyme-2, nucleoside phosphorylase, and mannose phosphate isomerase loci ( $P < 0.05$  for all significant loci).

When only the 16 loci common to both Lee (1992) and our study were analyzed, Arizona pronghorn population had an average of 2.2 alleles per locus, 25.0 percent polymorphic loci (0.95 criterion), and an overall expected heterozygosity of 0.101. Data from these same 16 loci in the 29 North American herds surveyed by Lee (1992) indicated an average of 1.38 alleles per locus, 13.7 percent polymorphic loci (0.95 criterion), and an overall expected heterozygosity of 0.043.

Table 2. Genetic variability measures for the 5 pronghorn populations surveyed in Arizona based on analysis of 22 loci. Standard errors are given in parentheses.

Population	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic (0.95 criterion)	Mean multilocus heterozygosity (direct count)	Number of unique alleles
Northeast	94.7 (0.4)	1.9 (0.2)	18.2	0.063 (0.026)	4.0
Central	163.9 (0.6)	1.8 (0.1)	27.3	0.064 (0.020)	1.0
Northwest	18.6 (0.2)	1.6 (0.1)	22.7	0.050 (0.016)	1.0
Arizona Strip	18.0 (0.2)	1.6 (0.2)	22.7	0.062 (0.021)	0.0
Southeast	17.9 (0.3)	1.5 (0.2)	36.4	0.090 (0.033)	0.0

## DISCUSSION

The suite of loci examined in this research differs from those used by Lee et al. (1994) in only a few ways. Both studies encompass the majority of the most variable loci observed in pronghorn populations across North America. However, our analysis involved several loci that were unresolved by Lee and his colleagues (i.e., isocitric dehydrogenase-2, lactate dehydrogenase-2, malic enzyme-2, 6-phosphogluconate dehydrogenase, and phosphoglucomutase-2). In addition, our analyses revealed extensive polymorphism at the mannose phosphate dehydrogenase locus in pronghorn from Arizona. This was not the case for North American pronghorn populations surveyed by Lee et al. (1994). Alternatively, we did not survey Arizona pronghorn populations at several loci that exhibited low levels of variation in North American pronghorn examined by Lee et al. (1994; i.e., peptidase-3, esterase-1, esterase-4, superoxide dismutase, hemoglobin, and glucose phosphate isomerase).

Using data from loci common to both studies, the statewide Arizona pronghorn herd exhibited relatively high levels of genetic variation relative to estimates for other North American herds (Lee et al. 1992, Lee 1994). For instance, the expected multilocus heterozygosity value of 0.10 is substantially higher than average for other North American herds (0.04), and falls outside the range of values observed (0.00 to 0.094; based on our reanalysis of the data from Lee 1992). Values for average number of alleles per locus (2.2) and percentage of loci polymorphic (25.0; 0.95 criterion) for pronghorn in Arizona were high relative to mean values for these parameters in other North American populations (1.38, range 1.00-1.80; and 13.7 range 0.00-31.3, respectively).

Analysis of genetic diversity measures from the 5 regional pronghorn populations indicated that levels of heterozygosity and gene polymorphism vary across the state. Pronghorn from the southeastern habitat exhibited the highest level of heterozygosity and polymorphic loci observed in any of the regional populations, but had the lowest number of alleles per locus of any region. These pronghorn are believed to be entirely composed of reintroduced pronghorn from multiple sources (Lee 1988).

A potential explanation for the pattern of high heterozygosity, high percentage of polymorphic loci, and low number of alleles per locus in the southeastern portion of the state stems from its reintroduction history. Several sources of pronghorn were used to repopulate the southeastern pronghorn range in Arizona, including animals from various locations in central Arizona and 2 locations in Texas. Pronghorn introduced from these sources probably contributed to the high percentage of polymorphic loci, through their individual contributions of new polymorphic loci into the reintroduced population. Thus, the higher level of heterozygosity stems from the inflated proportion of polymorphic loci in the southeastern population.

A second noteworthy result is the high number of unique alleles observed in the northeastern population. The northeastern population had 4 low-frequency, unique alleles (i.e., undetected in any of the other regional populations) distributed over 3 loci (AAT-1, AAT-2, and PGI). Although this region received reintroductions from Wyoming (A. Munig and R. M. Lee, Arizona Game and Fish Department, Unpub. data), data from Lee et al. (1994) did not indicate presence of these rare alleles in the Wyoming populations sampled ( $n = 12$  individuals). While it is possible that these rare alleles may have been introduced into the northeastern herd from Wyoming, it is also possible that they may actually be representative of the historical genetic stock of this region. Further analysis of the actual gene frequency data indicated that 1 individual, sampled from Arizona Game Management Unit (GMU) 4A, which received pronghorn from Wyoming, was responsible for the occurrence of rare alleles in 2 of the 4 cases. However, the remaining 2 rare alleles were found in northeastern GMUs with no recorded pronghorn transplants. Presence of these rare alleles is probably indicative of the reintroduction history of this region, as well as the historical genetic structure of pronghorn in this population.

Our analysis of allele frequency differences among the 5 regions sampled in Arizona revealed highly significant differences in allele frequencies across all loci, with  $\geq 10$  different loci contributing to this differentiation. This is not surprising considering the large geographic

area over which samples were collected and the wide variety of interstate reintroduction sources that have been used to supplement Arizona's pronghorn population (i.e., Colorado, Montana, Texas, Utah, and Wyoming). The high degree of differentiation observed in allele frequencies indicates that there are allele frequency shifts over the broad geographic scale at which we sampled. Potential impacts to the genetic structure revealed by our analyses should be considered prior to further pronghorn reintroductions in Arizona. Further analyses at a finer scale of geographic resolution (e.g., Arizona GMUs) would provide an even clearer picture of gene dynamics in Arizona pronghorn.

#### MANAGEMENT IMPLICATIONS

Genetic diversity data collected in this study indicate that there are higher than average levels of genetic heterozygosity, numbers of alleles per locus, and percentages of polymorphic loci in Arizona than would be expected for pronghorn in North America. There are significant shifts in allele frequencies among regional pronghorn populations in Arizona, and within regional populations, local gene dynamics may partially reflect recent reintroduction events. Significant genetic variation exists in the Arizona pronghorn population distributed in a nonrandom fashion throughout the state. Future transplant efforts into existing Arizona pronghorn populations should take into consideration potential genetic impacts. Further genetic analysis of the Arizona pronghorn population that specifically takes into account reintroduction history and that focuses on a finer scale of geographic resolution (e.g., GMUs or local herds) is needed to elucidate current patterns of genetic diversity in Arizona pronghorn.

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Appendix 1. Allele frequencies and single locus heterozygosities (direct count) for the variable loci examined in pronghorn antelope sampled in Arizona during 1996.

Locus	Northeast	Central	Northwest	Arizona Strip	Southeast
AAT-1					
(n)	97	164	19	19	19
A	0.990	0.951	0.974	0.974	0.895
B	0.005	0.000	0.000	0.000	0.000
C	0.005	0.049	0.026	0.026	0.105
(h)	0.021	0.085	0.053	0.053	0.105
AAT-2					
(n)	97	164	19	19	19
A	0.979	0.936	0.868	0.974	1.000
B	0.005	0.000	0.000	0.000	0.000
C	0.015	0.064	0.132	0.026	0.000
(h)	0.041	0.055	0.158	0.053	0.000
ACP					
(n)	97	166	19	19	19
A	0.974	0.970	0.974	0.974	0.947
B	0.000	0.000	0.026	0.000	0.000
C	0.026	0.030	0.000	0.026	0.053
(h)	0.052	0.060	0.053	0.053	0.105
ACON					
(n)	95	165	19	19	19
A	1.000	0.994	0.947	1.000	1.000
B	0.000	0.006	0.000	0.000	0.000
C	0.000	0.000	0.053	0.000	0.000
(h)	0.000	0.000	0.053	0.000	0.000
EST-2					
(n)	92	158	19	19	19
A	0.717	0.915	0.868	0.789	0.816
B	0.283	0.085	0.132	0.211	0.184
(h)	0.152	0.120	0.158	0.316	0.053
IDDH					
(n)	96	164	19	19	19
A	0.995	1.000	0.974	1.000	1.000
B	0.005	0.000	0.026	0.000	0.000
(h)	0.010	0.000	0.053	0.000	0.000

## Appendix 1. Continued.

Locus	Northeast	Central	Northwest	Arizona Strip	Southeast
LDH-2					
(n)	92	161	17	17	15
A	0.995	0.991	1.000	1.000	1.000
B	0.005	0.009	0.000	0.000	0.000
(h)	0.011	0.019	0.000	0.000	0.000
MDH-1					
(n)	97	165	19	19	19
A	0.995	0.988	1.000	1.000	1.000
B	0.005	0.012	0.000	0.000	0.000
(h)	0.010	0.024	0.000	0.000	0.000
ME-1					
(n)	94	166	19	17	17
A	1.000	0.982	1.000	0.971	1.000
B	0.000	0.018	0.000	0.029	0.000
(h)	0.000	0.036	0.000	0.059	0.000
ME-2					
(n)	93	160	18	17	17
A	0.984	0.984	1.000	0.912	0.765
B	0.016	0.016	0.000	0.059	0.206
C	0.000	0.000	0.000	0.029	0.029
(h)	0.032	0.031	0.000	0.059	0.353
MPI					
(n)	94	159	16	17	16
A	0.447	0.651	0.625	0.265	0.719
B	0.005	0.013	0.031	0.029	0.031
C	0.548	0.336	0.344	0.706	0.250
(h)	0.468	0.321	0.250	0.294	0.313
NP					
(n)	94	166	19	17	17
A	0.830	0.843	0.974	0.971	0.706
C	0.170	0.157	0.026	0.029	0.294
(h)	0.255	0.253	0.053	0.059	0.471
PGDH					
(n)	94	166	19	17	17
A	1.000	0.994	1.000	1.000	1.000
B	0.000	0.006	0.000	0.000	0.000
(h)	0.000	0.012	0.000	0.000	0.000

## Appendix 1. Continued.

Locus	Northeast	Central	Northwest	Arizona Strip	Southeast
PGM-1					
(n)	96	165	19	19	19
A	0.615	0.779	0.789	0.579	0.500
B	0.245	0.124	0.053	0.053	0.316
C	0.141	0.097	0.158	0.368	0.184
(h)	0.281	0.206	0.211	0.211	0.421
PGM-2					
(n)	96	165	19	19	19
A	0.953	0.997	0.974	0.842	1.000
B	0.047	0.003	0.026	0.158	0.000
(h)	0.031	0.006	0.053	0.211	0.000
PGI					
(n)	97	166	18	19	19
A	0.985	0.916	0.972	1.000	0.921
B	0.005	0.000	0.000	0.000	0.000
C	0.005	0.084	0.028	0.000	0.079
D	0.005	0.000	0.000	0.000	0.000
(h)	0.031	0.169	0.056	0.000	0.158