Genetic Variation of Pronghorn across US Route 89 and State Route 64

Final Report 659
March 2012

Arizona Department of Transportation
Research Center

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16. Abstract

This study investigated whether highways acted as barriers to gene flow for pronghorn in northern Arizona. DNA samples from 132 pronghorn were analyzed using eight polymorphic microsatellite loci. Samples represented animals living on opposite sides of US Route 89 (US 89) and State Route 64 (SR 64). Two different modeling approaches indicated that both US 89 and SR 64, and to a lesser extent US Route 180 (US 180), acted as barriers to gene flow. The genetic structuring caused by highways, especially across US 89, is consistent with behavioral data that demonstrated pronghorn rarely cross this highway. This study found no evidence of inbreeding or reduced genetic variation in any of the populations examined, but those effects may take longer to appear. Based on these results, the researchers recommend future genetic monitoring of these populations or assessment of genetic variation across highways with larger traffic volumes or longer histories to determine whether the barrier effects documented here lead to loss of genetic diversity.

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SI* (MODERN METRIC) CONVERSION FACTORS

APPROXIMATE CONVERSIONS TO SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
in	inches	25.4	millimeters	mm
ft	feet	0.305	meters	m
yd	yards	0.914	meters	m
mi	miles	1.61	kilometers	km
		AREA		
in^2	square inches	645.2	square millimeters	mm^2
ft^2	square feet	0.093	square meters	m^2
yd^2	square yard	0.836	square meters	m^2
ac	acres	0.405	hectares	ha
mi^2	square miles	2.59	square kilometers	km^2
		VOLUME		
fl oz	fluid ounces	29.57	milliliters	mL
gal	gallons	3.785	liters	L
ft^3	cubic feet	0.028	cubic meters	m^3
yd^3	cubic yards	0.765	cubic meters	m^3
		NOTE: volumes greater than 1000 L shall be shown in m^3		
		MASS		
oz	ounces	28.35	grams	g
lb	pounds	0.454	kilograms	kg
T	short tons (2000 lb)	0.907	megagrams (or "metric ton")	Mg (or "t")
		TEMPERATURE (exact degrees)		
^oF	Fahrenheit	5 (F-32)/9 or (F-32)/1.8	Celsius	^oC
		ILLUMINATION		
fc	foot-candles	10.76	lux	lx
fl	foot-Lamberts	3.426	$candela/m^2$	cd/m^2
		FORCE and PRESSURE or STRESS		
lbf	poundforce	4.45	newtons	N
lbf/in^2	poundforce per square inch	6.89	kilopascals	kPa

APPROXIMATE CONVERSIONS FROM SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
mm	millimeters	0.039	inches	in
m	meters	3.28	feet	ft
m	meters	1.09	yards	yd
km	kilometers	0.621	miles	mi
		AREA		
mm^2	square millimeters	0.0016	square inches	in^2
m^2	square meters	10.764	square feet	ft^2
m^2	square meters	1.195	square yards	yd^2
ha	hectares	2.47	acres	ac
km^2	square kilometers	0.386	square miles	mi^2
		VOLUME		
mL	milliliters	0.034	fluid ounces	fl oz
L	liters	0.264	gallons	gal
m^3	cubic meters	35.314	cubic feet	ft^3
m^3	cubic meters	1.307	cubic yards	yd^3
		MASS		
g	grams	0.035	ounces	oz
kg	kilograms	2.202	pounds	lb
Mg (or "t")	megagrams (or "metric ton")	1.103	short tons (2000 lb)	T
		TEMPERATURE (exact degrees)		
^oC	Celsius	1.8C+32	Fahrenheit	^oF
		ILLUMINATION		
lx	lux	0.0929	foot-candles	fc
cd/m^2	$candela/m^2$	0.2919	foot-Lamberts	fl
		FORCE and PRESSURE or STRESS		
N	newtons	0.225	poundforce	lbf
kPa	kilopascals	0.145	poundforce per square inch	lbf/in^2

*SI is the symbol for the International System of Units. Appropriate rounding should be made to comply with Section 4 of ASTM E380.
(Revised March 2003)

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I. EXECUTIVE SUMMARY

This study investigated whether highways acted as barriers to gene flow for pronghorn in northern Arizona. DNA samples from 132 pronghorn were analyzed using eight polymorphic microsatellite loci. Samples represented animals living on opposite sides of US Route 89 (US 89) and State Route 64 (SR 64). Two different modeling approaches indicated that both US 89 and SR 64, and to a much lesser extent US Route 180 (US 180), acted as barriers to gene flow. The genetic structuring caused by highways, especially across US 89, is consistent with behavioral data collected by Arizona Game and Fish Department (AZGFD) that demonstrated pronghorn rarely cross this highway.

This study found no evidence of inbreeding or reduced genetic variation in any of the populations examined, but those effects may take longer to appear. The data reported here provide a solid baseline of current genetic diversity and population structure that can be used in future comparisons. Future evaluation of genetic variation in these populations or in those separated by highways with greater traffic volumes or longer histories could clarify whether isolation by highways eventually leads to loss of genetic diversity. Future studies may suggest that certain types of habitat mitigation would alleviate the barrier effects documented here, but this study alone does not support that recommendation.

II. INTRODUCTION

BACKGROUND

Highways can block animal movements between seasonal ranges or other vital habitats and limit the movement of individuals between subpopulations (Forman and Alexander 1998). Population isolation and fragmentation caused by roads could result in reduced genetic variation that could lead to both short-term genetic effects, such as lower fertility and higher juvenile mortality, and long-term inability to adapt to stochastic environmental challenges (Lacy 1997). Isolating effects of roads have been documented in amphibians (Reh and Seiz 1990), terrestrial insects (Keller and Largiadér 2003; Vandergast et al. 2007), rodents (Gerlach and Musolf 2000; Metcalfe et al. 2001), bobcats and coyotes (Riley et al. 2006), grizzly bears (Proctor et al. 2005), and bighorn sheep (Epps et al. 2005).

One caveat of studies demonstrating the impact of highways on the genetic structure of animal populations is that other factors may conceal the effect of highways. Highways often follow physiographic features such as rivers, valleys, and escarpments that could have historically acted as barriers to animal movement or are concordant with geologic discontinuities that could have historically shaped genetic structure (e.g. fault lines, ancient shorelines) (Vandergast et al. 2007). Thus, distinguishing the relative importance to evolutionary history on population structure from recent anthropogenic effects like highways can be difficult (Vandergast et al. 2007).

American pronghorn (*Antilocapra americana*) (Fig. 1) avoid crossing highways, particularly those with fenced right-of-ways (Ockenfels et al. 1994; Van Riper and Ockenfells 1998; Ockenfels et al. 2006). They live on extensive grassland habitats many of which are crossed by highways. Thus, they represent a species in which the effects of highways on genetic structure are less likely to be concealed by other factors.

FIGURE 1. A Female (Left) and Male (Right) American Pronghorn (*Antilocapra americana*). Photographed in Northern Arizona.

Although pronghorn are sometimes referred to as "antelope," they are a uniquely American species in the family Antilocapridae, evolutionarily distinct from African and Asian antelope in the family Bovidae (Baccus et al. 1983; Kraus and Miyamoto 1991; Matthee et al. 2001). With an appearance somewhere between a white-tailed deer (*Odocoileus virginianus*) and a bighorn sheep (*Ovis canadensis*), pronghorn are typically smaller than either. Pronghorn primarily feed on forbs and supplement their diet with browse and grass species. Keen eyesight and the capacity to reach and sustain speeds unequalled by other North American land mammals help these grassland-dwelling ruminants avoid predation (O'Gara and Yoakum 2004*a*).

Pronghorn band composition in uninterrupted habitat has a fluidity that is marked by general seasonal trends. Leading up to a late summer breeding period, mature males establish territories and attempt to hold together groups of up to a dozen or so mature females. Bachelor bands are comprised of younger males unable to defend a territory. Over the fall and winter, pronghorn tend to form larger herds, often moving to wintering grounds distinct from summer home ranges. As the end of winter and gestation approach, winter herds break up into smaller bands as they move to summer grounds. Pregnant females ("does") disperse to bear their young ("fawns"). After a few weeks, does and their fawns come together to form nursery bands. Despite these general trends, a healthy pronghorn population has significant variability in size and individual make-up of groups on a day-to-day basis (O'Gara and Yoakum 2004*b*); therefore, designating specific herds or herd locations requires long-term monitoring of many individuals across large landscapes.

Behavioral observations suggest that pronghorn are more sensitive to highways' barrier effects than most other large mammal species. Extensive VHF-telemetry studies in northern Arizona over several years never documented pronghorn crossing a paved and fenced highway (Ockenfels et al. 1994; Van Riper and Ockenfels 1998; Ockenfels et al. 2006). More recently, ADOT-funded telemetry studies of pronghorn along US 89 have demonstrated extremely low rates of highway crossing compared to elk and white-tailed deer (Dodd and Gagnon pers. comm.).

Two aspects of pronghorn behavior likely contribute to the low passage rates across highways compared to other native ungulates. First is a reluctance to jump over intact barbed-wire fencing. Pronghorn cross under fences and are known to run along fence lines for long distances seeking areas where the lower fence wire is high enough to crawl under. Fence structure can make suitable crossings very rare to absent. Thus highways bordered by fencing can act as a double barrier to pronghorn movement (Ockenfels et al. 2006). Second, unlike deer and elk, which are often active at night and can therefore cross highways when traffic volumes are typically at their lowest, pronghorn are active during the day (O'Gara and Yoakum 2004*c*) and therefore must attempt highway crossings when traffic volumes are often high.

Recent declines in pronghorn populations have been attributed, in part, to fragmentation and isolation of pronghorn herds by highways, railways, and canals

(O'Gara and Yoakum 1992; Sawyer and Rudd 2005). Several studies, within and outside of Arizona, have examined pronghorn genetics (Rhodes et al. 1999, 2001; Carling et al. 2003b; Stephen et al. 2005), but none have investigated the impacts of roadways on genetic structure.

RESEARCH JUSTIFICATION

The final *US 89 Antelope Hills – Junction US 160 Environmental Assessment* (ADOT 2005) notes that the primary environmental effect of a proposed reconstruction of US 89 on pronghorn populations would be an increase in the barrier effect of the widened highway and increased traffic, which could contribute to a higher degree of population fragmentation and potential impacts on genetic structure. To date, no studies have assessed whether highways in Arizona may be retarding gene flow among populations. This project represents the first attempt to do so.

RESEARCH OBJECTIVES

The objectives of this project were to determine the following:

1) Whether northern Arizona pronghorn populations exhibited evidence of reduced genetic diversity or increased inbreeding caused by the isolating effects of highways.

2) Whether pronghorn populations in northern Arizona exhibited patterns of genetic structuring consistent with reduced gene flow across highways.

III. METHODS

STUDY AREA

This study focused on US 89 north of Flagstaff and SR 64 north of Williams in northern Arizona. US 89 is the primary highway connecting Flagstaff and Interstate 40 (I-40) with Utah, while also serving the Navajo Nation and popular recreation areas north of Flagstaff (e.g., Sunset Crater Volcano and Wupatki national monuments, Grand Canyon National Park, Page, and Lake Powell). US 89 was built in 1932 and is primarily two lanes, with traffic volume currently averaging 7500 vehicles per day with a modal speed limit of 65 mph along the areas where samples were collected. SR 64 is the entrance road to the South Rim of Grand Canyon National Park, connecting Williams to Grand Canyon Village. Two lanes wide, it also was built in 1932 and averages 4700 vehicles per day with a modal speed limit of 65 mph in the areas sampled. Additional samples were collected in the area between I-40 and US 180 northwest of Flagstaff. US 180 is the major connection between Flagstaff and SR 64, linking Flagstaff with Grand Canyon Village and the South Rim of the Grand Canyon. Built in 1960, US 180 is two lanes and averages 1900 vehicles per day with a modal speed of 65 mph. In each case, these highways pass through continuous pronghorn habitat, dividing that habitat independent of other physiographic features that likely influence pronghorn movements (Fig. 2).

FIGURE 2. US 89 North of Flagstaff, Arizona, Bisects Continuous Grassland Habitat Appropriate for Pronghorn. (Route of Highway Is Shown by the Dark Line).

SAMPLE COLLECTION

Muscle tissue from hunter-killed pronghorn and ear-punches from animals captured as part of radio-telemetry studies carried out by the Arizona Game and Fish Department (AZGFD) were the sources of tissue samples. Sample collection was concentrated east and west of US 89 north of Flagstaff and east and west of SR 64 (Fig. 3). Thirteen samples were from animals in the area bounded by I-40 and US 180 and SR 64. Samples were assigned to one of eight arbitrarily designated *a priori* "populations" (A-W on Fig. 3).

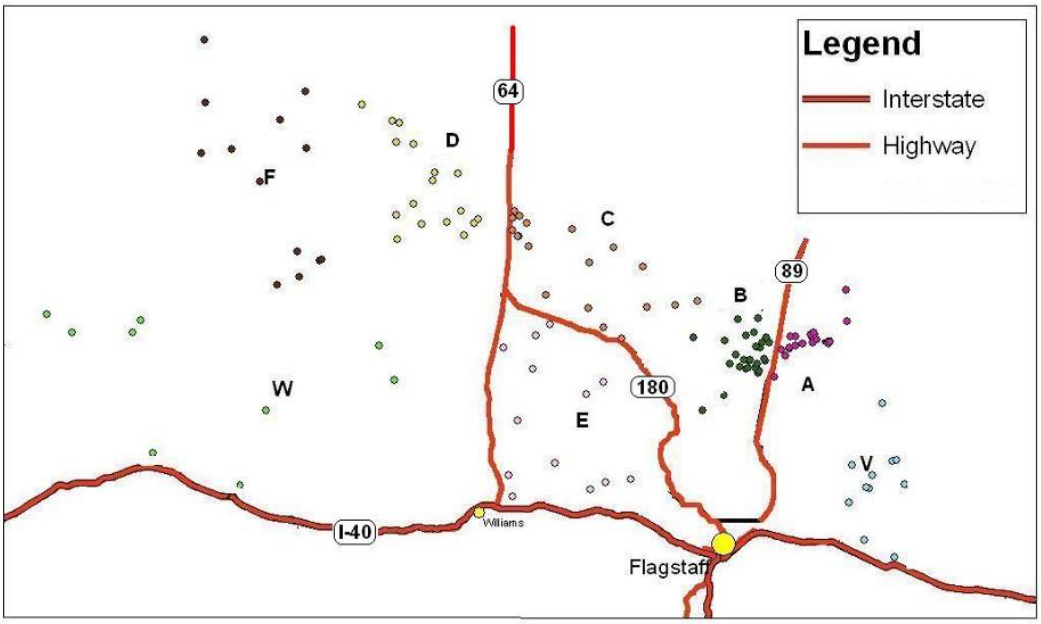

FIGURE 3. Capture/Kill Locations for the 132 Pronghorn from Northern Arizona Used in This Study. Letters Indicate the Eight "Populations" to Which Samples Were Assigned. Each Circle Represents a Pronghorn and Circles of the Same Color Were Assigned to the Same Population.

GENETIC ANALYSES

DNA was extracted from ear and muscle samples using standard techniques (Appendix A). Eight microsatellite DNA markers previously developed for pronghorn (Carling et al. 2003a, Stephen et al. 2005) were used to type all pronghorn samples (listed in Table 1 in Appendix B).

If highways reduced gene flow among populations, the geographic pattern of genetic variation would reflect this. The samples were tested two ways to see if highways affected the population's genetic structure. First, the Bayesian clustering algorithm in program STRUCTURE (Pritchard et al. 2000) was used to assign individuals to gene-based populations. STRUCTURE calculates the probability of each individual's

population membership based on any number (K) of hypothesized populations. The study team tested values from K = 1 (all samples were part of a single population) to K = 8 (samples represented eight different populations). Results for each K value can be compared by computing a ΔK statistic and the K giving the highest ΔK value is considered the most likely number of distinct genetic populations (Evanno et al. 2005). The ΔK for this test showed the highest probability that the pronghorn came from three distinct gene pools. Outputs of these analyses are in the form of bar graphs, in which each vertical bar represents an individual pronghorn and different shades in each panel represent the K different populations. The relative proportion of each individual's bar that is a given shade represents the probability of that individual being a member of that population, as depicted in Results - Figure 4.

Second, the program GENELAND was used to produce maps of genetically distinct populations across geographic space (Francois et al. 2006; Guillot et al. 2008). This program uses a different modeling approach (colored tessellation) to determine the geographic distribution of genetic population clusters. This method differs from that used in STRUCTURE because it uses the spatially explicit Universal Transverse Mercator (UTM) coordinates of each sample rather than any *a priori* population assignments. As in the program STRUCTURE, GENELAND uses repeated iterations to generate probabilities for each hypothesized number of populations (K). The output of this program is a map that shows the number of genetically distinct populations and their geographic extent. Most relevant to this study was whether boundaries between the estimated populations coincided with highways, as would be expected if highways were barriers to gene flow.

Reduced gene flow among populations can result in loss of genetic variation, both within populations (genetic or allelic diversity) and within individuals (heterozygosity). Evidence of reduced genetic diversity caused by isolation was examined by comparing genetic (allelic) diversity across the eight sample populations. If genetic diversity had been reduced in one or more of these populations, then this would be reflected in lower allelic diversity across all eight microsatellites in that population.

When populations are small and isolated, inbreeding will lead to reduced within-individual genetic variation (increased homozygosity). Evidence of this was tested by comparing levels of within-individual genetic variation (heterozygosity) to that expected if all individuals were mating randomly, without barriers to gene flow. Levels of within-individual genetic variation (heterozygosity) were compared to those expected if all individuals were randomly mating without barriers to gene flow to see if there were evidence of high homozygosity.

IV. RESULTS

Program STRUCTURE found greatest support for the hypothesis that the pronghorn samples in this study were drawn from three genetically distinct clusters (Fig.3; Table 2, Appendix B). The two populations on the eastern side of US 89 (A and V) were consistently and strongly grouped in the same cluster (Fig. 4). Though individuals from other populations were sometimes also assigned to this cluster, it was never with the consistently high probability that these two populations showed. Individuals from population B were consistently assigned to a cluster distinct from those east of US 89 or west of SR 64. Other individuals from populations between US 89 and SR 64 (C and E) were not strongly associated with any one of the three clusters, while populations west of SR 64 were associated with the same cluster roughly 60-70% of the time (Fig. 5). These results were consistent with the hypothesis that both US 89 and SR 64 acted as barriers to gene flow but that this effect was greater for US 89.

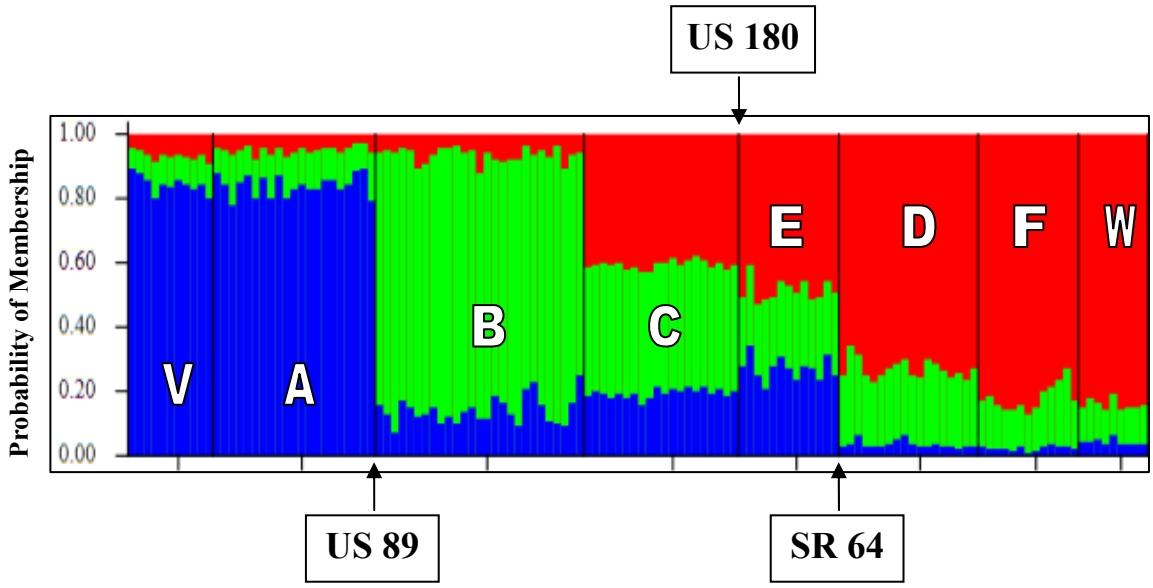

FIGURE 4. Probability of Population Membership of 132 Pronghorn Based on the Modeling Program STRUCTURE

Note: The blue, green, and red columns represent the likelihood of membership to each of the three clusters, represented by a probability value that is greater than 0 and less than 1 for each animal.

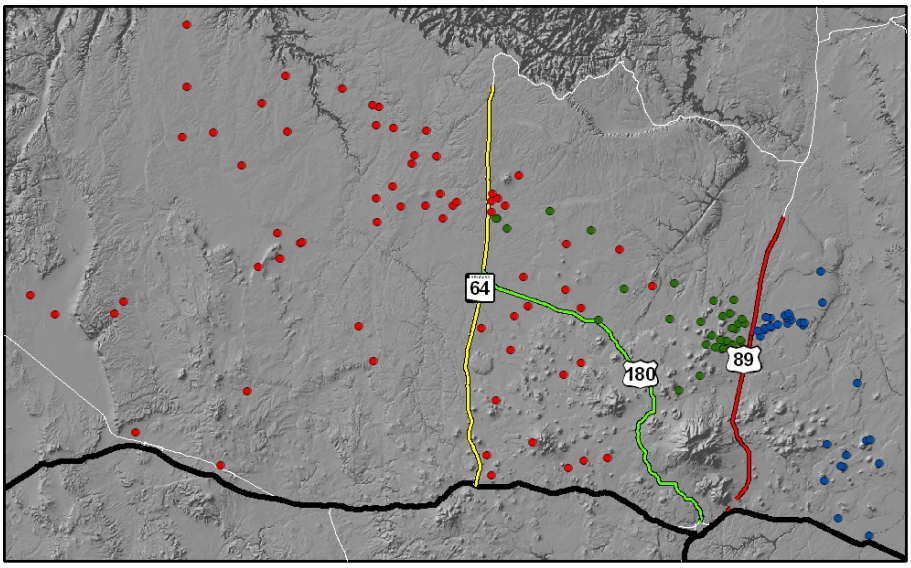

FIGURE 5. Population Assignment of Individual Pronghorn Based on Results Using the Program STRUCTURE.

Notes: Circles with the same colors indicate that those animals were consistently grouped together based on genetic similarity. Note that one genetically distinct group (blue) lies on the eastern side of US 89 while another lies on the western side (green).

When geographic patterns of genetic variation were estimated based on individual UTM locations for each sample using the program GENELAND, iterations yielded most support for either three or four populations (Appendix C). All iterations had a consistent population boundary between individuals occupying opposite sides of US 89 (Fig. 6 B and C). A population boundary was also roughly concordant with SR 64, though the placement of this boundary varied more than that of US 89. The pattern of genetic structuring in some iterations was strikingly concordant with all three highways (Fig 6 B).

The analyses found no evidence of reduced genetic variation or inbreeding in any of the eight populations examined**.** Allelic richness averaged across the eight microsatellite markers in each population ranged from 3.2 to 3.9, reflecting similar genetic diversity across populations (Table 3, Appendix B). Likewise, no significant reduction in within-individual variation (heterozygosity) was detected in any of the eight populations (Table 4, Appendix B).

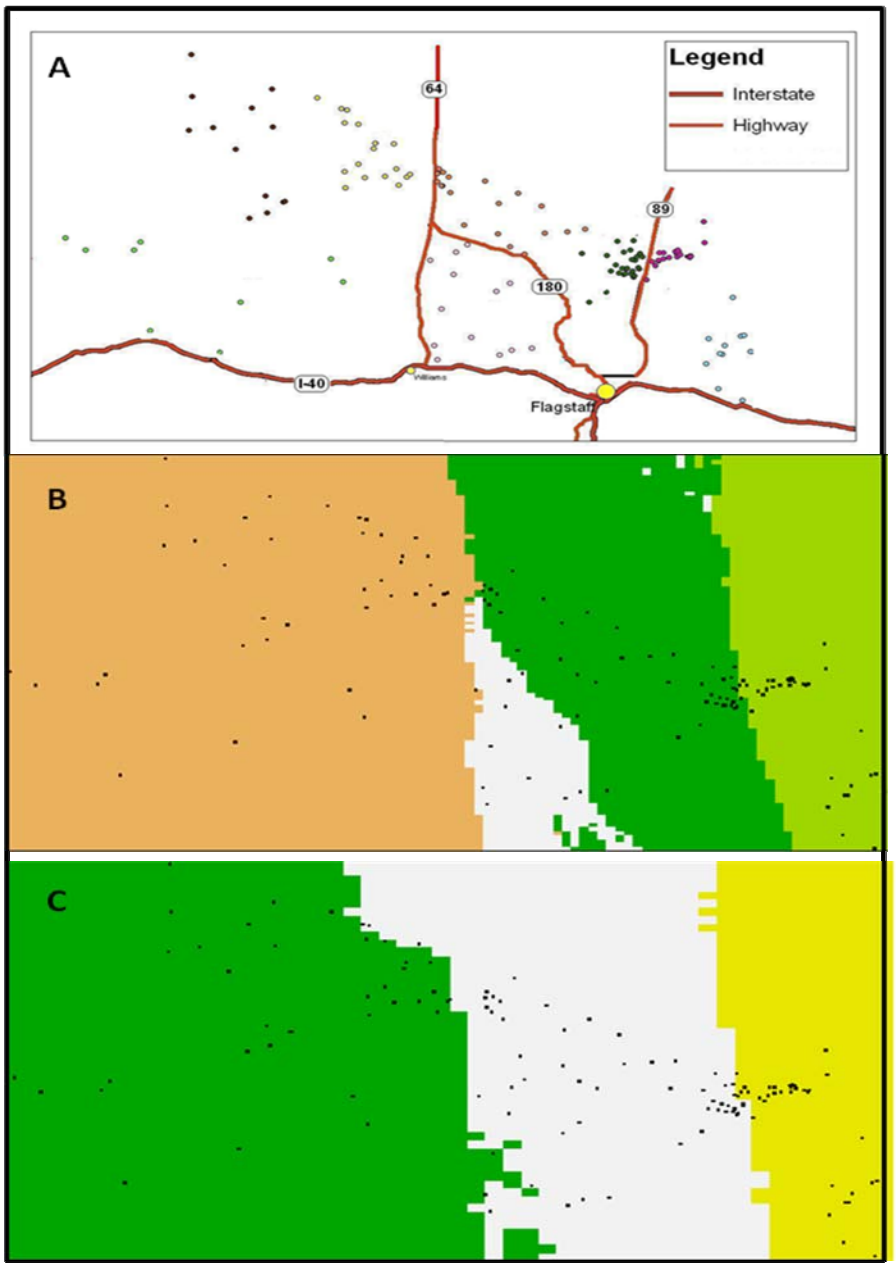

FIGURE 6. A. Collection Locations of 132 Pronghorn.

B. Pronghorn Populations for an Iteration When Modeling Program GENELAND Yielded Four Populations as Most Likely.

C. Pronghorn Populations for an Iteration that Yielded Three Populations as the Most Likely.

V. DISCUSSION

Two of the major concerns about genetically isolated populations is that they will lose genetic variation from the population overall (reduced genetic diversity) and that they will lose genetic diversity within individuals (reduced heterozygosity). Reduced genetic variation leaves populations less able to respond evolutionarily to changes in their environment, thereby increasing the chance of population extinction. Reduced heterozygosity (or its reciprocal, increased homozygosity) due to inbreeding allows expression of deleterious recessive forms of genes, leading to reduced fertility and other genetic anomalies. None of the eight populations examined exhibited evidence of reduced genetic variation or loss of heterozygosity. Therefore, pronghorn in the study area do not appear to be threatened by these genetic consequences of reduced gene flow at this time. However, reduced genetic variation and deleterious effects of inbreeding may take considerable time to be expressed, for which no timeline is available. The relatively young age of highways may mean that isolation effects simply have not yet developed.

The geographic pattern of genetic structuring concordant with highways documented here, especially along US 89, indicates that highways are acting as barriers to gene flow. The alternative hypothesis, that location of highways is correlated with some other physiographic feature that limits movement, seems highly unlikely given the uniformity of the habitat between sampling locations. The genetic structuring of populations on either side of US 89 is consistent with recent behavioral data indicating that pronghorn rarely cross this highway.

The patterns of population structuring concordant with highways were strongest across US 89, were weaker for SR 64 and weakest for US 180. The ages of US 89 and SR 64 are similar (roughly 75 years) but traffic volume on US 89 is roughly 1.5 times higher. Recent improvements to US 89 have also widened the roadway along many stretches. US 180 is younger and has lower traffic volume than either US 89 or SR 64. Collaborative efforts between ADOT and AZGFD in the 1990s also shifted highway fencing farther from US 180 to improve the ability of pronghorn to cross the highway. Both roads and fences can act as barriers to pronghorn movement, but close proximity of the two can make crossing even less likely. Taken together, these patterns suggest an increasing barrier effect of highways with increasing age and traffic volume. If true, the effects of highways on pronghorn population subdivision would increase as traffic volumes increase and highways are upgraded.

It is difficult to predict the genetic consequences of the genetic structuring detected in this study. Gene flow, though reduced by highways, may still be high enough to prevent further population differentiation and offset deleterious effects of reduced genetic variation and inbreeding. Populations on the east side of US 89 may exchange genes with populations farther east while populations on the west side of SR 64 may do the same with populations farther west. The populations of greatest concern would therefore be those between the two highways. Allelic richness in these populations was as high as that in any of the other populations, as were levels of heterozygosity. Thus, the

data do not show loss of genetic diversity in these populations due to reduced gene flow. Whether this will remain the case over longer periods, especially in the face of increasing traffic volumes and highway modifications, is unknown.

Deleterious effects of reduced gene flow and increased isolation are only a few of many biologically important impacts that highway-caused reduced movement of pronghorn could have. Most wild populations are not continuous across the landscape, but rather are comprised of a set of smaller subpopulations connected by animal movement, often termed "metapopulations" (Hanski 1998). Maintaining connectivity among these subunits is important not only for maintaining genetic diversity and avoiding deleterious effects of inbreeding, but also for maintaining subpopulations through time and "rescuing" those that undergo local extinction due to catastrophic events such as drought or heavy snowfall. Minimizing the barrier effect of highways through construction of effective wildlife crossing structures would be prudent to guard against potential effect on pronghorn genetics and also shorter-term demographic challenges (Jackson and Griffen 2000).

VI. CONCLUSIONS

This project was initiated to investigate effects of SR 64 and US 89 on gene flow among pronghorn populations on either side of these highways. The key conclusions from this research project are:

- Two independent modeling approaches revealed geographic patterns of genetic variation consistent with US 89, SR 64, and US 180 acting as barriers to gene flow. The barrier effect was strongest for US 89, weaker for SR 64, and weakest for US 180. This pattern could suggest that barrier effects of roadways increase mostly with traffic volume but also to a lesser extent with highway age and width. This would be consistent with behavioral data on highways and pronghorn movement.

- Populations examined did not differ in genetic diversity nor show excess homozygosity that would indicate inbreeding caused by population isolation. However, these effects may take longer to manifest than the length of time highways have been present.

- Consequences of highway barrier effects are difficult to predict. Over time, reduced gene flow could lead to deleterious genetic effects, especially if increased traffic or highway upgrades increases the barrier effect. Alternatively, the reduction in gene flow caused by highways may not be great enough to cause significant losses of genetic diversity. The data reported here provide a baseline of current genetic diversity and population structure that can be used in future comparisons to determine which of these outcomes occurs.

VII. RECOMMENDATIONS

This project investigated effects of SR 64 and US 89 on gene flow among pronghorn populations on either side of these highways. The key recommendation from this research project is to undertake future genetic analyses of pronghorn populations, either in this study area or across highways with a longer history or higher traffic volumes, for an assessment of whether the barrier effects documented here lead to reduced genetic diversity.

APPENDIX A

DETAILED GENETIC METHODS

Genomic DNA was extracted from approximately 5 mg of ear or muscle tissue using DNEasy Tissue Kits™ (Qiagen). Eight microsatellite loci developed or modified for pronghorn (Carling et al. 2003a, Stephen et al. 2005) were used to type all pronghorn samples (loci are listed in Appendix B). Microsatellites were amplified using polymerase chain reactions (PCR) with samples heated to 94°C for 5 minutes followed by 35 cycles of 94°C for 20 seconds, 60°C for 30 seconds, and 72°C for 5 minutes. The process utilized a fluorescent dye-labeled forward primer and unlabeled reverse primers. The resulting PCR products were sized on Applied Biosystems ABI 3100 genetic sequencer. Electropherograms were analyzed and manually scored using Genescan® (version 3.7, Applied Biosystems 2001) and Genotyper® (version 3.7, Applied Biosystems 2000).

STRUCTURE analyses were run using 30,000 iterations for burn-in followed by 100,000 repetitions (Pritchard et al 2000). The "Model with prior population information" was used with individual samples grouped by population name, as recommended when inferring weak population structure (Hubisz et al. 2009). K values ranging from 1-8 were tested with four iterations at each value of K to confirm that log-likelihood values had converged. The most likely number of population clusters (K) was assumed to be that which resulted in the highest mean log-likelihood value across the four iterations (Pritchard et al. 2000).

GENELAND estimates of population structure were based on 100,000 Markov Chain Monte Carlo iterations, with thinning set to 100, and K ranging from 1-8 (Francois et al. 2006; Guillot et al. 2008). Allele frequencies were assumed to be correlated, as this is a more likely scenario for populations arising from the same ancestral panmictic population (Balding 2003).

Allelic diversity, observed and expected heterozygosity, deviation from Hardy–Weinberg expectations, and fixation indices were calculated for each sample population using the program GenAlEx (Peakall and Smouse 2006). Allelic richness with correction for variable sample size was calculated for each locus in each population using the program FSTAT (Goudet 2001). Tests for heterozygote deficiency in each population were carried out using the program GENEPOP (Raymond and Rousset 1995).

APPENDIX B
DETAILED GENETIC RESULTS

TABLE 1. Summary of Genetic Results.

Pop	Locus	N	Na	Ho	He	HW	F
A	Aam1	21	2.000	0.571	0.499	ns	-0.145
	Aam2	21	5.000	0.857	0.719	ns	-0.192
	Aam3	21	6.000	0.524	0.529	ns	0.011
	PrM65	21	3.000	0.429	0.398	ns	-0.077
	Aam5	21	4.000	0.524	0.502	***	-0.043
	Aam6	21	2.000	0.571	0.499	ns	-0.145
	Aam7	21	5.000	0.524	0.593	ns	0.117
	Aam8	21	4.000	0.619	0.700	ns	0.115
B	Aam1	27	3.000	0.667	0.504	ns	-0.322
	Aam2	27	7.000	0.815	0.757	ns	-0.077
	Aam3	27	10.000	0.667	0.750	**	0.111
	PrM65	27	4.000	0.593	0.571	*	-0.037
	Aam5	27	2.000	0.370	0.417	ns	0.112
	Aam6	27	2.000	0.407	0.475	ns	0.143
	Aam7	27	6.000	0.778	0.706	ns	-0.102
	Aam8	27	5.000	0.741	0.666	ns	-0.112
C	Aam1	20	4.000	0.700	0.578	ns	-0.212
	Aam2	20	7.000	0.800	0.789	ns	-0.014
	Aam3	20	7.000	0.600	0.656	ns	0.086
	PrM65	20	4.000	0.300	0.303	ns	0.008
	Aam5	20	4.000	0.350	0.336	***	-0.041
	Aam6	20	2.000	0.600	0.495	ns	-0.212
	Aam7	20	6.000	0.300	0.458	***	0.344
	Aam8	20	4.000	0.500	0.636	ns	0.214
D	Aam1	18	4.000	0.611	0.611	**	0.000
	Aam2	18	7.000	0.833	0.792	ns	-0.053
	Aam3	18	7.000	0.667	0.694	***	0.040
	PrM65	18	5.000	0.500	0.481	ns	-0.038
	Aam5	18	3.000	0.333	0.356	ns	0.065
	Aam6	18	2.000	0.444	0.500	ns	0.111
	Aam7	18	5.000	0.500	0.634	ns	0.212
	Aam8	18	3.000	0.611	0.508	ns	-0.204
E	Aam1	13	2.000	0.308	0.260	ns	-0.182
	Aam2	13	8.000	0.846	0.754	ns	-0.122
	Aam3	13	5.000	0.538	0.491	ns	-0.096
	PrM65	13	4.000	0.692	0.530	ns	-0.307
	Aam5	13	2.000	0.462	0.355	ns	-0.300
	Aam6	13	2.000	0.462	0.497	ns	0.071
	Aam7	13	4.000	0.308	0.435	ns	0.293
	Aam8	13	4.000	0.846	0.663	ns	-0.277

Table I
(cont)

Pop	Locus	N	Na	Ho	He	HW	F
F	Aam1	13	4.000	0.462	0.530	ns	0.128
	Aam2	13	6.000	0.692	0.796	ns	0.130
	Aam3	13	5.000	0.385	0.444	ns	0.133
	PrM65	13	4.000	0.231	0.388	ns	0.405
	Aam5	13	2.000	0.385	0.311	ns	-0.238
	Aam6	13	2.000	0.538	0.500	ns	-0.077
	Aam7	13	5.000	0.538	0.494	ns	-0.090
	Aam8	13	4.000	0.231	0.275	***	0.161
V	Aam1	11	2.000	0.545	0.496	ns	-0.100
	Aam2	11	5.000	0.545	0.504	ns	-0.082
	Aam3	11	4.000	0.455	0.442	ns	-0.028
	PrM65	11	3.000	0.636	0.525	ns	-0.213
	Aam5	11	2.000	0.273	0.236	ns	-0.158
	Aam6	11	2.000	0.364	0.496	ns	0.267
	Aam7	11	5.000	0.636	0.616	ns	-0.034
	Aam8	11	4.000	0.636	0.678	ns	0.061
W	Aam1	9	3.000	0.222	0.204	ns	-0.091
	Aam2	9	5.000	0.667	0.765	ns	0.129
	Aam3	9	4.000	0.667	0.512	ns	-0.301
	PrM65	9	5.000	0.667	0.580	*	-0.149
	Aam5	9	2.000	0.333	0.278	ns	-0.200
	Aam6	9	2.000	0.333	0.278	ns	-0.200
	Aam7	9	5.000	0.778	0.679	ns	-0.145
	Aam8	9	4.000	0.556	0.562	ns	0.011

Pop = population designation as in Fig. 1, Locus = each of the eight microsatellite loci, N = sample size, Na = number of alleles, Ho = observed heterozygosity, He = expected heterozygosity, HW = probability of G-test for deviation from Hardy-Weinberg expectations, F = fixation index. Ns = not significantly different from expectations, * different at $p<0.05$, ** $p<0.01$, ***$p<0.001$.

TABLE 2. ΔK Values Produced by Program Structure for K Values from 1-8.

K	Run1 L(K)	L'(K)	L"(K)	Run2 L(K)	Run3 L(K)	Run4 L(K)	Run5 L(K)	ΔK
1	-2406.4	-2406.4	2445.3	-2406.6	-2406.5	-2406.6	-2406.4	24389
2	-2367.5	38.9	31.9	-2378.5	-2368.4	-2377.4	-2378.7	3.596
3	-2360.5	7	24.1	-2360.1	-2363.1	-2366.1	-2360.2	**12.881**
4	-2377.6	-17.1	29.4	-2385.4	-2375.6	-2369.6	-2409	1.220
5	-2424.1	-46.5	71.5	-2419.8	-2388	-2387.9	-2417.5	1.580
6	-2399.1	25	51.1	-2428	-2415.2	-2415.1	-2405.5	3.060
7	-2425.2	-26.1	2.6	-2378	-2420.1	-2428.3	-2416	1.377
8	-2448.7	-23.5	23.5	-2387.6	-2411.6	-2406.5	-2396.6	0.707

Results from 20 iterations of STRUCTURE (5 each at $K = 1 – 8$) and calculation of ΔK for estimation of the true number of population clusters. The modal ΔK is in bold, indicating the true value of K is 3. K = the number of inferred population clusters, L(K) = Ln P(D)' = the maximum posterior probability of the data returned for each run in STRUCTURE, L'(K) and L"(K) = intermediate stages in the calculation of ΔK as described by Evanno et al. (2005)

TABLE 3. Allelic Richness Across All Loci for Each Population Based on Minimum Sample Size of Nine Individuals.

	POPULATION							
LOCUS	A	B	C	D	E	F	V	W
AAM1	2.000	3.000	2.333	3.294	3.746	1.995	3.888	2.000
AAM2	4.862	5.000	5.599	5.828	5.902	6.882	5.866	4.608
AAM3	4.742	4.000	6.722	5.112	4.990	4.342	4.277	3.790
PRM650	2.427	5.000	3.767	2.860	3.876	3.888	3.585	2.997
AAM5	3.108	2.000	2.000	2.881	2.496	2.000	1.999	1.997
AAM6	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
AAM7	4.012	5.000	4.993	4.546	4.611	3.601	4.498	4.610
AAM8	3.946	4.000	4.225	3.764	2.886	3.691	3.298	3.818

TABLE 4. Results of Tests of Heterozygote Deficiency.

POPULATION	P-VALUE
A	0.8223
B	0.3648
C	0.9954
D	0.8963
E	0.1388
F	0.9619
V	0.4529
W	0.3648

Markov chain parameters for all tests: Dememorization:10000; Batches:20; Iterations per batch:5000

REFERENCES

Arizona Department of Transportation. 2005. *US 89 Antelope Hills – Junction US 160 draft environmental assessment*. Phoenix, AZ: Environmental and Enhancement Group.

Baccus, R., N. Ryman, M. H. Smith, C. Reuterwall, and D. Cameron. 1983. Genetic variability and differentiation of large grazing mammals. *Journal of Mammalogy* 64:109-120.

Balding, D. J. 2003. Likelihood-based inference for genetic correlation coefficients. *Theoretical Population Biology* 63:221-230.

Carling, M. D., C. Passavant, and J. A. Byers. 2003a. DNA microsatellites of pronghorn (*Antilocapra americana*). *Molecular Ecology Notes* 3:10-11.

Carling, M. D., P. A. Wiseman, and J. A. Byers. 2003b. Microsatellite analysis reveals multiple paternity in a population of wild pronghorn antelopes (*Antilocapra americana*). *Journal of Mammalogy* 84:1237-1243.

Epps, C. W., P. J. Palsboll, J. D. Wehausen, G. K. Roderick, R. R. Ramey II, and D. R. McCullough. 2005. Highways block gene flow and cause rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters* 8: 1029-1038.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611-2620.

Forman R. T. T. and L. E. Alexander. 1998. Roads and their major ecological effects. *Annual Review of Ecology and Systematics* 29:207-231.

Francois O., S. Ancelet, and G. Guillot. 2006. Bayesian clustering using hidden Markov random fields in spatial population genetics. *Genetics* 174: 805-816.

Gerlach, G. and K. Musolf. 2000. Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology* 14: 1066-1074.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (v. 2.9.3). Lausanne, Switzerland. Information accessed Oct. 2011 at http://www2.unil.ch/popgen/softwares/fstat.htm

Guillot G., F. Santos, and A. Estoup. 2008. Analysing georeferenced population genetics data with GENELAND: a new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics* 24:1406-1407.

Hanski, I. 1998. Metapopulation dynamics. *Nature* 396: 41-49.

Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9:1322-1332

Jackson, S.D. and C.R. Griffin. 2000. A Strategy for Mitigating Highway Impacts on Wildlife. In *Wildlife and Highways: Seeking Solutions to an Ecological and Socio-economic Dilemma*, ed. T. A. Messmer and B. West, 143-159. Amherst, MA: The Wildlife Society, University of Massachusetts. Accessed Oct. 2011 at http://www.umass.edu/nrec/pdf_files/tws_strategy_ms.pdf

Keller, I. and C.R. Largiadér. 2003. Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 270:417-423.

Kraus, F. and M. M. Miyamoto. 1991. Rapid cladogenesis among the pecoran ruminants: evidence from mitochondrial DNA sequences. *Systematic Zoology* 40:117-130.

Lacy, R. C. 1997. Importance of genetic variation to the viability of mammalian populations. *Journal of Mammalogy* 78:320-335.

Matthee, C. A., J. D. Burzlaff, J. F. Taylor, and S. K. Davis. 2001. Mining the mammalian genome for artiodactyl systematics. *Systematic Biology* 50:367-390.

Metcalf A. E., L. Nunney, and B. C. Hyman. 2001. Geographic patterns of genetic differentiation within the restricted range of the endangered Stephens' kangaroo rat *Dipodomys stephensi*. *Evolution* 55:1233-1244.

Ockenfels, R. A., A. Alexander, C. L. Dorothy-Ticer, and W. K. Carrel. 1994. *Home Ranges, Movement Patterns, and Habitat Selection of Pronghorn in Central Arizona.* Technical Report 13. Phoenix, AZ: Arizona Game and Fish Department.

Ockenfels, R. A., J. C. deVos Jr., and J. J. Hervert. 2006. Transportation corridors and pronghorn: what do we know and what can we do about it. In *Pronghorn symposium*, ed. K. A. Cearley and S. Nelle, 85. College Station, TX: Texas Cooperative Extension.

O'Gara, B. W. and J. D. Yoakum. 1992. Pronghorn management guides; a compendium of biological and management principles and practices to sustain pronghorn populations and habitat from Canada to Mexico. *Proceedings of the Biennial Pronghorn Antelope Workshop 15*: pp 101.

O'Gara, B. W. and J. D. Yoakum. 2004*a*. Physical characteristics. In *Pronghorn: Ecology and Management*, 109-143. Boulder, CO: University Press Colorado.

O'Gara, B. W. and J. D. Yoakum. 2004*b*. Group sizes and composition. In *Pronghorn: Ecology and Management*, 154-163. Boulder, CO: University Press Colorado..

O'Gara, B. W. and J. D. Yoakum. 2004*c*. Activities. In *Pronghorn: Ecology and Management*, 163-166. Boulder, CO: University Press Colorado.

Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel: Population genetic software for teaching and research. *Molecular Ecology Notes* 6:288-295.

Pritchard, J. K., M. Stephens, and P. J. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.

Proctor, M. F, B. N. McLellan, C. Strobeck, and R. M. R. Barclay. 2005. Genetic analysis reveals demographic fragmentation of grizzly bears yielding vulnerably small populations. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 272:2409-2416.

Raymond, M. and F. Rousset. 1995. GENEPOP version 1.2: population genetics software for exact tests and ecumenism. *Journal of Heredity* 86:248-249.

Reh, W. and A. Seiz. 1990. The influence of land use on the genetic structure of populations of the common frog *Rana temporaria*. *Biological Conservation* 54: 239-249.

Rhodes, O. E. Jr., R. N. Williams, J. R. Heffelfinger, L. K. Page, E. P. Reat, and J. C. deVos, Jr. 1999. Genetic variation in pronghorn from Arizona. *Proceedings of the 18th Biennial Pronghorn Antelope Workshop* 18:53-63.

Rhodes, O. E. Jr., E. P. Reat, J. R. Heffelfinger, and J. C. deVos, Jr. 2001. Analysis of reintroduced pronghorn populations in Arizona using mitochondrial DNA markers. *Proceedings of the 19th Biennial Pronghorn Antelope Workshop* 19:45-54.

Riley, S. P. D., J. P. Pollinger, R. M. Sauvajot, E. C. York, C. Bromley, T. K. Fuller, and R. K. Wayne. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology* 15:1733-1741.

Sawyer, H. and B. Rudd. 2005. *Pronghorn Roadway Crossings: A Review of Available Information and Potential Options.* Cheyenne, WY: Wyoming Department of Transportation and Wyoming Game and Fish Department.

Stephen, C. L., D. G. Whittaker, D. Gillis, L. L. Cox, and O. E. Rhodes, Jr. 2005. Genetic consequences of reintroductions: an example from Oregon pronghorn antelope (*Antilocapra americana*). *Journal of Wildlife Management* 69:1463-1474.

Vandergast, A. G., A. J. Bohonak, D. B. Weissman, and R. N. Fisher. 2007. Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: Stenopelmatus). *Molecular Ecology* 16: 977-992

Van Riper, C. III. and R. Ockenfels. 1998. The influence of transportation corridors on the movement of a pronghorn antelope over a fragmented landscape in northern Arizona. Pages in Proceedings of the International Conference on Wildlife Ecology and Transportation, ed. G. L. Evink, P. Garrett, D. Zeigler, and J. Berry, 241-248. . Tallahassee, FL: Florida Department of Transportation.