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Pronghorn population genomics show connectivity in the core of their range

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Preserving connectivity in the core of a species' range is crucial for long-term persistence. However, a combination of ecological characteristics, social behavior, and landscape features can reduce connectivity among wildlife populations and lead to genetic structure. Pronghorn (*Antilocapra americana*), for example, exhibit fluctuating herd dynamics and variable seasonal migration strategies, but GPS tracking studies show that landscape features such as highways impede their movements, leading to conflicting hypotheses about expected levels of genetic structure. Given that pronghorn populations declined significantly in the early 1900s, have only partially recovered, and are experiencing modern threats from landscape modification, conserving connectivity among populations is important for their long-term persistence in North America. To assess the genetic structure and diversity of pronghorn in the core of their range, we genotyped 4,949 genome-wide single-nucleotide polymorphisms and 11 microsatellites from 398 individuals throughout the state of Wyoming. We found no evidence of genetic subdivision and minimal evidence of isolation by distance despite a range that spans hundreds of kilometers, multiple mountain ranges, and three interstate highways. In addition, a rare variant analysis using putatively recent mutations found no genetic division between pronghorn on either side of a major highway corridor. Although we found no evidence that barriers to daily and seasonal movements of pronghorn impede gene flow, we suggest periodic monitoring of genetic structure and diversity as a part of management strategies to identify changes in connectivity.

Key words: gene flow, genotyping by sequencing, landscape barriers, population genetics, ungulate

Characterizing the genetic structure of wildlife populations and identifying the mechanisms driving those genetic patterns are important tools used by biologists to manage populations. Many factors can influence genetic structure, including landscape features (Manel et al. 2003), social behavior, and ecology (Storz 1999; Ross 2001). Understanding genetic patterns and their drivers can be used to delineate populations and management units (Palsbøll et al. 2007; Funk et al. 2012). Because gene flow can promote population persistence (Frankham 2015), knowledge of barriers to gene flow can be used to maintain connectivity in managed species.

Ungulates are highly vagile animals that use contiguous habitat across large geographic areas, and the degree of genetic structure varies significantly among species. Many ungulates exhibit female philopatry to natal ranges, creating social structure that contributes to distinct genetic clustering (Coltman et al. 2003; Frantz et al. 2008; Miller et al. 2010). Some species with social structure, however, do not cluster into distinct genetic groups; instead, they exhibit isolation by distance, where individuals in close geographic proximity are more related to each other than to individuals farther away (Colson et al. 2013; Crawford et al. 2018). Solitary species also can exhibit

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patterns of isolation by distance, without distinct genetic clusters (Schmidt et al. 2009). Nomadic behavior combined with lack of familial social structure can lead to no detectable genetic structure nor isolation by distance (Okada et al. 2015).

Natural and human-constructed features on the landscape also can influence genetic structure. Natural features, including mountains, rivers, unsuitable habitat, and many others, have been demonstrated to influence ungulate distributions and genetic structure across the globe (Worley et al. 2004; Pérez-Espona et al. 2008; Locher et al. 2015). In addition to natural landscape features, human-constructed features such as highways, roads, fences, canals, and cities, are increasingly altering the genetic structure of many ungulates by impeding gene flow (Epps et al. 2005; Coulon et al. 2006; Yang et al. 2011). In some cases, however, anthropogenic modifications to the landscape appear to have little-to-no detectable impact on genetic connectivity among ungulate populations (Epps et al. 2013; Okada et al. 2015; Budd et al. 2018). These contrasting results highlight the need to undertake investigations on a per-species basis into how natural and human-constructed landscape features influence genetic structure.

The pronghorn (*Antilocapra americana*) is the only extant species in the North American family Antilocapridae and has experienced human-induced population fluctuations (O'Gara and Yoakum 2004a, 2004b). Historical documents from the 1800s suggest that roughly 30–40 million pronghorn inhabited North America before westward settlement of the continent led to range-wide declines of as low as 13,000 individuals in the early 1900s (Nelson 1925; O'Gara and Yoakum 2004b). Decades of harvest restrictions allowed populations to recover to the current range-wide estimate of 750,000 individuals, over half of which reside in the state of Wyoming (IUCN 2016). Wyoming continues to be the stronghold for remaining pronghorn due to the presence of preferred sagebrush and grassland habitat and lower density of human development relative to other parts of the range (O'Gara and Yoakum 2004b). Over the past several decades, however, Wyoming has experienced rapid habitat loss and fragmentation due to increasing energy development, rural development, and associated roads and fencing (Gates et al. 2012). Global Positioning System (GPS) tracking studies indicate that roads, fences, and energy extraction infrastructure, significantly affect the daily and seasonal movements of pronghorn (van Riper et al. 2001; Beckmann et al. 2012; Seidler et al. 2015). In some cases, alterations to daily and seasonal behavior increase mortality risk and ultimately cause population declines (Christie et al. 2015; Taylor et al. 2016). The impact of these demographic and landscape changes on genetic connectivity and diversity of pronghorn largely is unknown. To date, only one study has investigated the genetic impacts of human disturbance on pronghorn: the authors found significant genetic structure associated with an interstate highway in northern Arizona (Theimer et al. 2012). While this small-scale study provides one case of altered genetic connectivity, drawing conclusions about the effects of human activities on pronghorn across their North American range requires evidence from additional and larger-scale studies.

We generated a data set of genome-wide single-nucleotide polymorphisms (SNPs) and a second data set of microsatellites to characterize patterns of genetic structure and genetic diversity of pronghorn in the core of their range, the state of Wyoming. We hypothesized that pronghorn exhibit genetic population structure in response to landscape features. Pronghorn prefer flat, open habitat with short vegetation, so natural landscape features such as mountain ranges could reduce genetic connectivity among pronghorn populations (Ockenfels et al. 1994; Beckmann et al. 2012; Reinking et al. 2019). Human-constructed landscape features, including highways and fencing, impede the daily and seasonal movements of pronghorn and increase the risk of mortality (van Riper et al. 2001; Seidler et al. 2015; Jones et al. 2019). Such features therefore may contribute to genetic structure by reducing connectivity (Theimer et al. 2012). However, the permeability of highways and fencing for pronghorn varies based on traffic levels and fence types (Seidler et al. 2015), so the potential impact to genetic connectivity is unclear. In addition, pronghorn may not exhibit population structure because of their flexible social group composition and variability in seasonal migration strategies. Pronghorn social groups frequently fluctuate in size and composition, leading to low rates of relatedness within groups (Byers 1998). Also, pronghorn employ more variable seasonal migration strategies than other North American ungulates, varying from migratory to resident to more nomadic (O'Gara and Yoakum 2004c), and these flexible behavioral patterns could influence connectivity across the state. Characterizing the genetic structure of Wyoming pronghorn in the context of landscape features, social behavior, and ecology provides an important additional resource for monitoring and managing pronghorn in the core of their range.

Materials and Methods

Sample collection and DNA extraction.—Muscle samples were collected from harvested male and female pronghorn by the Wyoming Game and Fish Department (WGFD) from August to December in 2015–2017 under WGFD Chapter 33 Scientific/Educational/Special Purpose Permit #1035. The Wyoming State Veterinary Laboratory donated additional muscle samples from pronghorn necropsies performed in 2014–2016. The Wyoming Cooperative Fish and Wildlife Research Unit and the University of Wyoming Department of Ecosystem Science and Management donated blood samples from pronghorn captured from 2014 to 2018. Sample collection followed the guidelines of the American Society of Mammalogists (Sikes et al. 2011, 2016). The study area spanned the state of Wyoming, excluding Yellowstone National Park and Grand Teton National Park (Fig. 1). Date of sample collection, sex, hunt area, and location of collection were recorded at the time of sample collection (available in Dryad repository: https://doi.org/10.5061/dryad.8931zcrmb—LaCava et al. 2020). For samples that lacked a precise GPS location, we used the centroid of the hunt area where they were collected to represent their location (see map of hunt areas in Supplementary Data SD1).

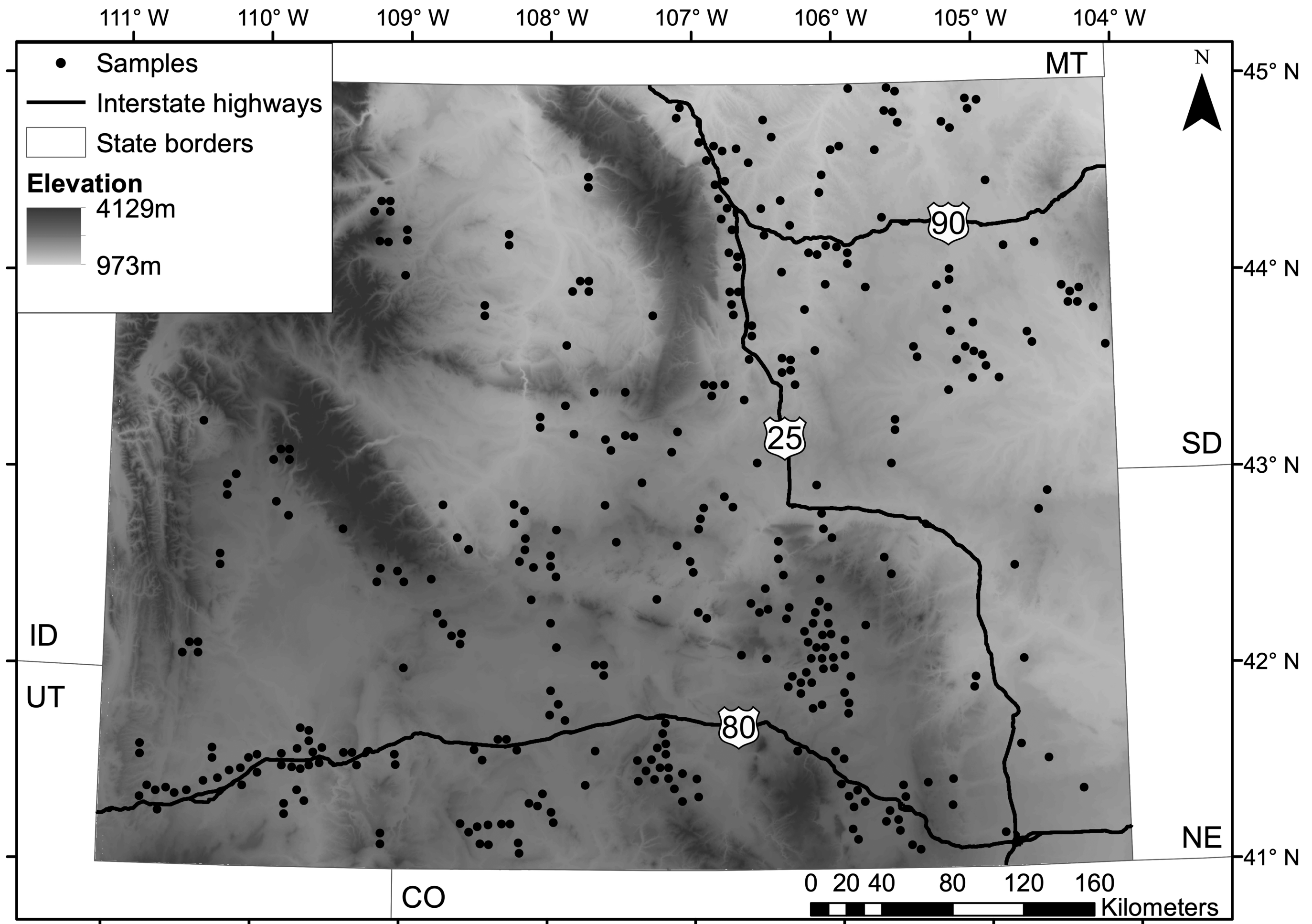

Fig. 1.—Map of study area and sample collection sites for Wyoming pronghorn from 2014 to 2018 (excluding Yellowstone and Grand Teton National Parks partially covered by figure legend). Surrounding states are labeled by their two-letter code (CO = Colorado, ID = Idaho, MT = Montana, NE = Nebraska, SD = South Dakota, UT = Utah). Samples without precise GPS locations were assigned the centroid of the hunt area from which they were collected (see Supplementary Data SD1). The three interstate highways in the state are represented by black lines and are labeled.

Muscle samples from harvested animals were stored in 1.5-ml microcentrifuge tubes with silica desiccant beads to preserve samples at room temperature until they could be stored at −80°C. Samples from necropsied carcasses were immediately stored at −80°C. Blood was stored in EDTA tubes at 4°C. Within 1 week of collection, we fractionated the blood by centrifuging samples at 1,000 × *g* for 10 min, then isolated the buffy coat layer for use in DNA extraction.

We selected samples for extraction based on geographic location, precision of harvest location data, and the sex of the animal. We aimed to represent male and female pronghorn equally to ensure that any differences in behavior between the sexes did not alter our findings. We extracted DNA using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Inc., Valencia, California) or Omega Bio-tek Mag-Bind Blood & Tissue DNA HDQ Kits (Omega Bio-tek, Inc., Norcross, Georgia), using the manufacturer's specifications. We quantified DNA concentrations using a Qubit 3 Fluorometer either with high sensitivity or broad range assays (Invitrogen, Carlsbad, California). We normalized DNA concentrations across samples to 240–600 ng of starting product for library preparation. We diluted high-concentration samples with Qiagen elution buffer AE and concentrated low-concentration samples with a Thermo Savant DNA120 SpeedVac Concentrator or with AMPure XP beads (Beckman Coulter, Inc., Brea, California).

Illumina sequencing.—We prepared our samples for reduced representation genotyping by sequencing using the protocol described in Parchman et al. (2012), with the addition of an AMPure bead cleanup. Briefly, we digested the DNA with two restriction enzymes, EcoRI and MseI, ligated Illumina adaptors and unique barcodes to the digested fragments, then amplified the fragments using PCR. We used AMPure XP beads at a ratio of 1.5× to clean and concentrate the PCR product before using a BluePippin to select for 300–500 base pair fragments (Beckman Coulter, Inc., Brea, California; Sage Science, Inc., Beverly, Massachusetts). A detailed library preparation protocol can be found in the Dryad repository: https://doi.org/10.5061/dryad.8931zcrmb (LaCava et al. 2020). We submitted a total of 718 samples for sequencing on four lanes (196 samples per lane, some samples sequenced multiple times to

obtain more data) on an Illumina HiSeq4000 (Illumina, San Diego, California) with 150 base pair reads at the University of Texas at Austin Genomic Sequencing and Analysis Facility or the University of Oregon Genomics and Cell Characterization Core Facility. The 718 samples sequenced include 392 female pronghorn and 326 male pronghorn.

SNP filtering.—We used bowtie2 to remove reads that aligned to Illumina adapters or the PhiX control added to the sequencing lane to increase diversity at the beginning of the reads (Langmead and Salzberg 2012). We then used a custom script (available in the Dryad repository: https://doi.org/10.5061/dryad.8931zcrmb—LaCava et al. 2020) to demultiplex reads, matching samples to sequencing data based on the unique appended barcodes. We used the dDocent pipeline for de novo assembly (i.e., assembly of reads without a reference genome—Puritz et al. 2014), using a percent match parameter of 0.9. We then aligned reads to the artificial reference genome using bwa (Li and Durbin 2009). We identified SNPs and filtered these loci with Samtools (Li et al. 2009). We discarded loci with a mapping quality score below 20 and with a Phred quality score below 19 to avoid potential sequencing errors. To address the issue of paralogs being grouped into any single locus, we discarded loci with more than two alleles at a site or with a maximum depth greater than 100 (Willis et al. 2017). We used only the first SNP per read to avoid linkage disequilibrium, and we retained loci with a minor allele frequency above 0.01, with a minimum depth of 3 reads per individual, and with genotypes for at least 50% of individuals. After finalizing the SNP data set, we discarded samples with more than 60% missing data. We calculated depth of coverage (i.e., number of reads at a locus) averaged across individuals and loci using the vcftools --depth function (Danecek et al. 2011). In order to eliminate library effects (i.e., differences among sequencing lanes), we used BayeScan to identify outlier SNPs while treating sequencing lanes as populations and using a false discovery rate of 0.05 (Foll and Gaggiotti 2008). We then used custom scripts to generate genotype likelihoods and convert likelihoods into a genotype matrix used in population genetic analyses (available in the Dryad repository: https://doi.org/10.5061/dryad.8931zcrmb—LaCava et al. 2020). To ensure that our locus filtering settings did not affect our results, we analyzed an alternative SNP data set that prioritized retaining individuals over loci (see Supplementary Data SD2). We also evaluated the impact of potential paralogs by removing loci with excess heterozygosity (i.e., loci that were heterozygous in more than 95% of samples) and comparing results to the unfiltered data set (Willis et al. 2017; see Supplementary Data SD2 and Dryad repository: https://doi.org/10.5061/dryad.8931zcrmb—LaCava et al. 2020).

Microsatellite sequencing and genotyping.—We included a microsatellite dataset to compare with our SNP results because these markers evolve at different rates (Haasl and Payseur 2011). We genotyped individuals at 34 microsatellite loci (Carling et al. 2003; Dunn et al. 2010; Munguia-Vega et al. 2013). Forward primers were labeled with fluorescent dyes 6-FAM, VIC, NED, or PET, and then coordinated into multiplexes based on pre-established groupings or sequence size (Supplementary Data SD3). Genotypes were scored independently by two individuals using Genemapper 5 (Applied Biosystems, Foster City, California), with genotype calls compared to ensure consistency. We discarded monomorphic loci and loci with inconsistent single base pairs shifts. We used the R package PopGenReport (Adamack and Gruber 2014) to test remaining loci for Hardy–Weinberg equilibrium (HWE) using all samples in the study, as well as subsets of smaller geographic areas, and discarded loci consistently found to be out of HWE. To ensure that removing loci out of HWE did not affect our results, we also analyzed a data set with these loci retained to compare findings (see Supplementary Data SD2).

Genetic structure analysis.—We carried out all structure and diversity analyses on both the SNP and microsatellite data sets. We used principal components analysis (PCA) to visually assess genetic structure using the R package Adegenet for the microsatellite data (Jombart 2008), and used a custom R script for the SNP data that accounts for missing data by only using loci each pair of individuals share in common to calculate genetic covariance (available in the Dryad repository: https://doi.org/10.5061/dryad.8931zcrmb—LaCava et al. 2020). We further investigated genetic structure using the Bayesian clustering program STRUCTURE (Pritchard et al. 2000). We ran STRUCTURE for the SNP data set on the University of Wyoming "Teton Computing Environment, Intel x86_64 cluster" (Advanced Research Computing Center 2018) using StrAuto (Chhatre and Emerson 2017). Our STRUCTURE analyses used the admixture and correlated allele frequencies model and with a burn-in of 100,000 iterations followed by 500,000 Markov chain Monte Carlo (MCMC) iterations. We ran 20 replicates for each of K from 1 to 10, then used Structure Harvester 06.94 to check for model convergence and compare likelihood values to gauge support for the alternative number of clusters (Earl and vonHoldt 2012).

We tested for isolation by distance using the individual-based spatial autocorrelation test in GenAlEx (Peakall and Smouse 2012). We calculated pairwise genetic distances using Smouse and Peakall's individual genetic distance metric (Smouse and Peakall 1999). The test generated 95% confidence intervals (*CI*s) using 999 bootstrap iterations, and generated a null model (i.e., no spatial autocorrelation) using 999 permutations. Samples pairs were binned into 12 distance classes of 50 km each based on geographic distance between them (Supplementary Data SD4). This spatial autocorrelation test produces a correlation coefficient (r) that ranges from +1 to −1, where +1 is complete positive correlation, 0 is no correlation, and −1 is complete negative correlation (Peakall and Smouse 2012).

Finally, we explicitly tested the hypothesis that highways affect the genetic structure of Wyoming pronghorn by analyzing rare nucleotide variants (hereafter rare variants). Rare variants are mutations that occur at low frequency in a population, and theoretically represent more recent mutations compared to more common variants (Gravel et al. 2011). Rare variant analyses can detect more recent genetic structure than analyses

using common, theoretically older, variants (Mathieson and McVean 2012; O'Connor et al. 2015). We evaluated the spatial distribution of rare variants in relation to Interstate-80, a highway corridor in southern Wyoming that was built in the 1960s (Hepworth 1965). This four-lane highway corridor is bounded by impermeable fences for long distances and is a known barrier to daily and seasonal movements of pronghorn (Deblinger et al. 1984; Beckmann and Hilty 2010). We used a subset of 173 samples close to Interstate-80 for this analysis (see map of samples tested in Supplementary Data SD5). We determined whether rare variants (i.e., a rare allele that only is found in between 2–10 pronghorn) were found in individuals on the same side of the highway or on different sides. If Interstate-80 is a barrier to gene flow, we expected to find more rare variants shared among pronghorn on the same side of the highway rather than shared across the highway barrier. We compared the proportion of rare variants found on only one side of the highway (i.e., private variants) to a null expectation of randomly distributed rare variants.

Genetic diversity and effective population size.—We used the R package Adegenet (Jombart 2008) to calculate mean observed heterozygosity and mean expected heterozygosity for the SNP data set, and R package PopGenReport (Adamack and Gruber 2014) to calculate number of alleles, observed heterozygosity, and expected heterozygosity for the microsatellite data set. We calculated effective population size (N_e) using the linkage disequilibrium method in NeEstimator 2.1 (Do et al. 2014). Effective population size is an estimate of the size of an ideal Wright–Fisher population that experiences genetic drift at the same rate as the study population (Allendorf et al. 2013). N_e is a metric separate from census estimates that provides additional information on a population's evolutionary potential and susceptibility to inbreeding depression. Because the SNP data are genome-wide, we employed a correction to the linkage disequilibrium estimate of N_e based on the haploid number of chromosomes pronghorn have (n = 29—O'Gara and Yoakum 2004d) following equation 1a in Waples et al. (2016).

Results

SNP filtering.—We sequenced 718 samples on four Illumina lanes (196 samples per lane, some samples sequenced multiple times to obtain more data). After initial filtering and demultiplexing, samples had an average of 908,000 reads each (range 100–6.8 million). Some of the muscle samples collected from harvested animals had low DNA concentrations (i.e., below 10 ng/µl), and despite efforts to increase these concentrations using a vacuum centrifuge or magnetic beads, many of these low-quality samples produced few sequenced reads and were removed from the study. We dropped 320 samples that did not pass filtering steps from our SNP data set. We removed 88 outlier SNPs associated with large allele frequency differences among sequencing lanes to reduce artificial signals of genetic structure. Our final SNP data set included 4,949 SNPs from 398 individuals (Fig. 1), with a mean depth of coverage of 7.86 at each SNP within an individual. Our evaluation of potential paralogs revealed 32 loci that were heterozygous in more than 95% of samples. We found a correlation of > 99.9% between the genetic covariance among individuals in the data set containing 4,949 SNPs and the subset of 4,917 SNPs after removal of the 32 loci with improbable heterozygosity, and therefore proceeded to analyze the complete 4,949 SNP dataset (see Supplementary Data SD2).

Microsatellite sequencing and genotyping.—Of the 34 microsatellites tested, 18 were monomorphic or had single base pair shifts. An additional five loci did not meet expectations of HWE across the entire study area as well as in a smaller geographic subset. In total, we genotyped 274 individuals at the 11 remaining loci that met our filtering criteria (Supplementary Data SD3). We did, however, analyze the 16 loci (11 final loci plus the five loci out of HWE) to ensure that we did not obtain different results based on HWE filtering (Supplementary Data SD2).

Genetic structure.—Both the SNP and microsatellite PCAs showed no evidence for geographic subdivisions of the samples (Fig. 2). For the SNP data, the first two PC axes explained 3.6% of the total variance among individuals (Fig. 2B), whereas the first two PC axes for the microsatellite data explained 5.7% of the total variance in the dataset (Fig. 2C). Our Bayesian Structure analyses for the SNP and microsatellite data sets both provided no evidence for genetic subdivision. The Structure analysis using microsatellites indicated that $K = 1$ had the highest likelihood, with declining likelihood as the number of clusters (K) increased (Supplementary Data SD6). The Structure analysis using SNPs indicated that $K = 2$ had a slightly higher likelihood than $K = 1$; however, with $K = 2$, all individuals were assigned to both genetic clusters, suggesting poor evidence for two clusters, leading us to conclude that $K = 1$ is the most probable biologically (Supplementary Data SD6). Likewise, our alternatively filtered data sets also supported a single genetic cluster rather than subdivision (see Supplementary Data SD2).

We detected weak isolation by distance using the spatial autocorrelation test. Specifically, we observed positive spatial autocorrelation at distances up to 150 km for the SNP data set and distances up to 100 km for the microsatellite data set. However, the autocorrelation coefficient (r) remained close to zero across all distance bins (for SNPs: −0.002 to 0.007, for microsatellites: −0.006 to 0.015; Fig. 3; Supplementary Data SD4 and SD7).

Our rare variant analysis in relation to Interstate-80 found no evidence that this highway acted as a barrier to gene flow (Fig. 4). As expected, the proportion of rare variants found on the same side of Interstate-80 declined as the rare variant count increased (i.e., as the number of copies of an allele in the population increased, it was less likely to be restricted to one side of highway). But the proportion of rare variants found on the same side of Interstate-80 fit the null expectation of a random distribution of rare variants, suggesting that even for presumably the most recent mutations, Interstate-80 has not restricted gene flow.

Genetic diversity and effective population size.—The observed and expected heterozygosity using the SNP data set was

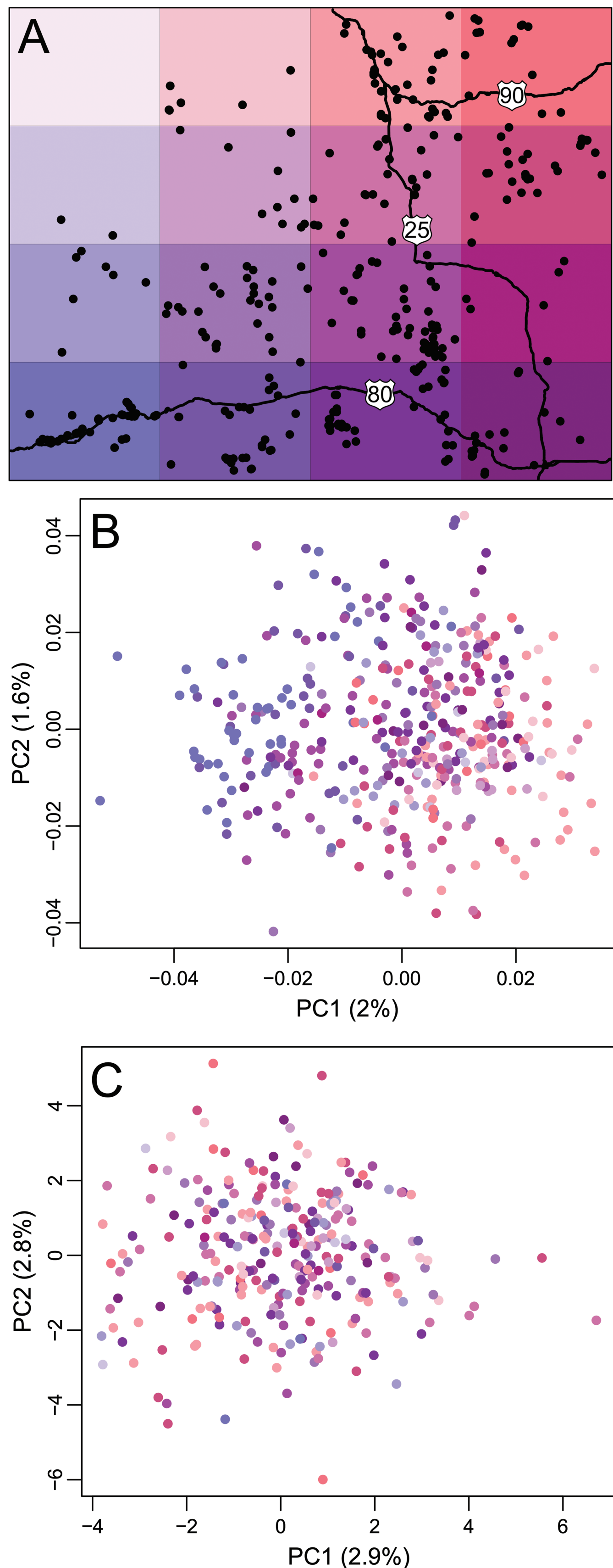

Fig. 2.—Principal components analysis (PCA) for genetic covariances among Wyoming pronghorn collected in 2014–2018. Each point represents one individual pronghorn, colored using a gradient to represent geographic location of collection within the state of Wyoming (A). Interstate highways are represented by black lines and are labeled in (A) to orient readers. Genetic covariances were calculated using (B) 4,949 single-nucleotide polymorphisms (SNPs) and (C) 11 microsatellites. The PC axes represent genetic covariance, so points closer together represent individuals that are more genetically similar.

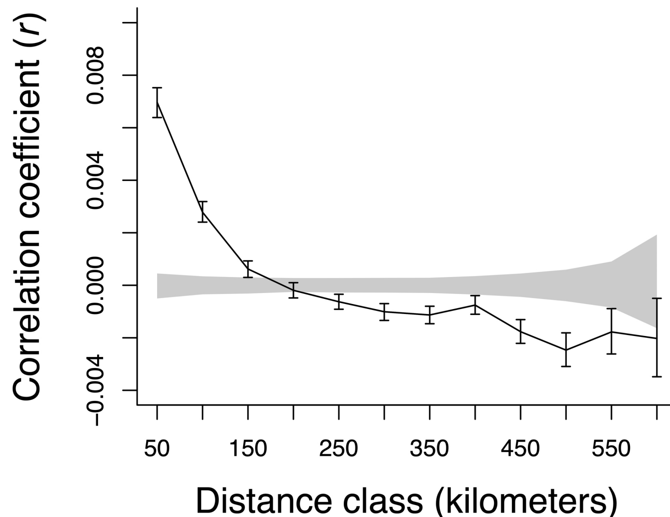

Fig. 3.—Spatial autocorrelation of pairwise genetic distance calculated using 4,949 single-nucleotide polymorphisms (SNPs) for 398 Wyoming pronghorn samples collected from 2014 to 2018. Sample pairs were binned into 12 even-distance bins based on geographic distance between them. In this figure, the correlation coefficient r is represented by the solid line and each r value is bounded by a 95% *CI* determined by 999 iterations of bootstrapping. The null model (i.e., no spatial autocorrelation) was calculated using 999 permutations of the data and is indicated by the shaded area. See Supplementary Data SD4 for r values and sample sizes for each distance class and Supplementary Data SD7 for the microsatellite spatial autocorrelation results.

0.21 and 0.16, respectively. For the microsatellite data set, the observed and expected heterozygosity was 0.63 and 0.65, respectively. The mean number of alleles per microsatellite locus was 9.18 (Supplementary Data SD3).

The estimated effective population size based on the SNP data set was 6,833 after adjusting for the number of chromosomes (95% *CI* = 6,346–7,399). The estimated effective population size based on the microsatellite data set was 2,180 (95% *CI* = 963–infinite).

Discussion

Despite spanning hundreds of kilometers, multiple mountain ranges, and three major interstate highways, our results indicate that Wyoming pronghorn exhibit little-to-no population genetic differentiation at this scale. Pronghorn at distances within 100–150 km were slightly more genetically similar to one another than expected if the same individuals occurred at random locations. Our spatial autocorrelation test for both SNPs and microsatellites produced r values between −0.006 and 0.015.

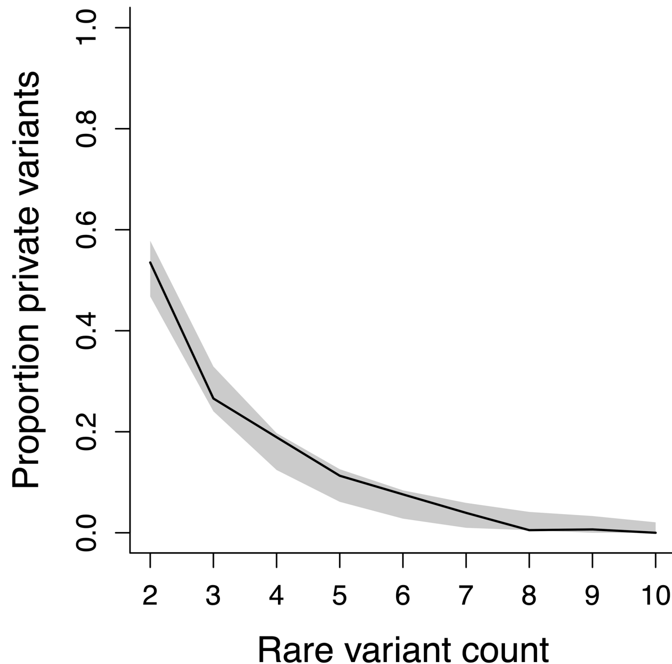

Fig. 4.—Rare variant analysis to test the Interstate-80 highway barrier hypothesis for Wyoming pronghorn collected from 2014 to 2018. We used a subset of samples on either side of Interstate-80 (68 samples south, 105 samples north; see Supplementary Data SD5 for a map of samples included in this analysis) and identified loci with rare variants (i.e., a rare allele found in 2–10 pronghorn) for these samples. For these 2,345 loci, we determined the proportion of rare variants that were found only on one side of the highway (i.e., private variants; black line) and compared this to a null expectation of randomly distributed rare variants (shaded area).

Therefore, although we obtained statistically significant correlations, it is important to recognize the extremely low magnitude of the correlations. The slightly negative values of spatial autocorrelation for the largest distance bins indicate that pronghorn farthest apart in Wyoming are less similar to each other than expected by chance, which likely is a result of slight isolation by distance and genetic drift.

Overall, our findings align with existing evidence that pronghorn exhibit variable social and behavioral strategies (reviewed in O'Gara and Yoakum 2004c; Gates et al. 2012). Despite previous documentation that landscape features (e.g., major highways) restrict the daily and seasonal movements of pronghorn and increase mortality risk (van Riper et al. 2001; Seidler et al. 2015; Jones et al. 2019), we found no evidence that these barriers affect genetic connectivity among Wyoming pronghorn. The time it takes for a barrier to produce a detectable genetic signal varies based on a variety of factors (e.g., population size, generation time, permeability of the barrier—Landguth et al. 2010), which is why we include multiple genetic data sets in our study. Thousands of genome-wide SNPs can detect finer scale structure than other markers (Lah et al. 2016; Vendrami et al. 2017; Aguillon et al. 2018), but microsatellites tend to evolve faster than SNPs and therefore may represent more recent genetic patterns (Haasl and Payseur 2011). Both SNPs and microsatellites indicated no genetic structure in Wyoming pronghorn, and our rare variant analysis using putatively recent mutations showed no evidence that Interstate-80 was a barrier to gene flow. The lack of genetic structure therefore likely results from limited, but sufficient gene flow across the landscape barriers. The interstate highways have underpasses that animals can use to cross under them, and although studies show that pronghorn strongly avoid the use of underpasses (Sawyer et al. 2016), only a few individuals need to cross and successfully breed on the other side to maintain genetic connectivity across these barriers (Wright 1969; Gustafson et al. 2017).

Genetic studies in other ungulate systems also have found high genetic connectivity despite GPS tracking studies that identify barriers to movement. GPS tracking of Mongolian gazelles did not detect a single animal crossing a railroad over a 3-year period, suggesting that the railroad acts as a significant barrier to the movements of these animals (Ito et al. 2013). A genetic study, however, did not detect any genetic differentiation across the railroad, despite its presence on the landscape for 50 years before the genetic samples were collected (Okada et al. 2015). Understanding if and when genetic studies align with tracking studies has important implications for wildlife management. In the only previous study whereof we are aware that investigated pronghorn genetic structure in relation to landscape features, a fenced interstate highway in northern Arizona reduced gene flow (Theimer et al. 2012). This genetic study corroborated an earlier GPS tracking study that found very few instances of pronghorn crossing the fenced highway (Sprague et al. 2013). Although tracking studies have found many barriers to daily and seasonal movements of Wyoming pronghorn (Deblinger et al. 1984; van Riper et al. 2001; Seidler et al. 2015), the permeability of barriers and the length of time barriers have been present on the landscape will influence how these barriers affect genetic connectivity. As the footprint and severity of movement barriers continues to expand, recurrent genetic monitoring will allow the detection of genetic impacts of barriers as they arise. Combining tracking data and genetic data to identify landscape barriers impacting wildlife has the potential to improve mitigation efforts by incorporating multiple lines of evidence into decision-making (e.g., the construction of highway overpasses—Sawyer et al. 2016).

One reason to maintain connectivity among populations is to prevent isolation and subsequent loss of genetic diversity. Our diversity estimates for Wyoming pronghorn are near the average reported for pronghorn: microsatellite-based studies throughout the range of pronghorn using 5–18 loci produced a mean observed heterozygosity (H_O) of 0.59 (see Supplementary Data SD8 for a summary of genetic diversity estimates from the literature—Stephen et al. 2005a, 2005b; Jenks et al. 2006; Barnowe-Meyer and Byers 2008; Keleher 2010; Klimova et al. 2014). The lowest estimate of H_O from microsatellites was 0.36 for the endangered subspecies Peninsular pronghorn (*Antilopcapra americana peninsularis*), which has only 400 animals remaining in a captive population (Klimova et al. 2014). The highest microsatellite estimate of H_O was 0.74 in South Dakota from a 2006 study using five microsatellites (Jenks et al. 2006).

Our microsatellite-based estimate of H_O for Wyoming pronghorn was 0.63, falling close to the mean value throughout the species' range. Note, however, that these are approximate comparisons because not all loci were used in all studies, which can affect diversity calculations. Our SNP-based estimate of H_O was 0.21, but there are no published estimates of heterozygosity for comparison at this time that are based on SNP data. This SNP-based estimate provides a baseline for comparison with future studies.

Comparisons of N_e estimates to the census estimate (N_C) of adults from the previous generation (i.e., the generation that produced the gene pool we sampled from) provide an additional tool to monitor pronghorn populations over time. The estimated number of adult pronghorn in Wyoming in 2011 was 337,000 (Walker 2012), so the approximate N_e/N_C ratio for SNPs is 2% and for microsatellites is 1%. Effective population size is affected by many biological factors including sex ratio, overlapping generations, variable reproductive success, and changes in population size (Allendorf et al. 2013). Pronghorn have an estimated buck:doe ratio of 1:2 (Vore 2016), have overlapping generations, and have a mating system where almost every adult female reproduces, but only a fraction of males reproduce (O'Gara and Yoakum 2004c, 2004e). In addition, pronghorn experienced a dramatic population decline 100 years ago and have fluctuated in population size since then (O'Gara and Yoakum 2004b). These combined factors likely contribute to the low N_e/N_C ratio we detected. Disentangling the weight of each of these potential factors on the N_e/N_C is beyond the scope of this study; however, documenting this ratio in the current pronghorn population provides a benchmark of comparison for future genetic monitoring of the species.

This study documents the current population structure and diversity of pronghorn in the state of Wyoming, providing a reference for future studies. Although Wyoming has a low density of highways, roads, and other forms of development, relative to other parts of the pronghorn range, the human footprint on the landscape is rapidly expanding. Over the past several decades, Wyoming has experienced significant habitat loss and fragmentation associated with land use conversion for energy development, rural development, and associated construction of roads and fences (McDonald et al. 2009; Polfus and Krausman 2012). The pace of habitat loss and fragmentation will be exacerbated by predicted increases in the human population of Wyoming and increasing energy demands (McDonald et al. 2009; Kauffman et al. 2018). In other continuously distributed species, there is a threshold of human development that limits connectivity and can ultimately pose a threat to the persistence of populations. For example, mountain lions (*Puma concolor*) are continuously distributed throughout the western United States, but coastal and southern California populations have become severely isolated by a dense network of anthropogenic development (Gustafson et al. 2019). We found that levels of development at the time of this study did not inhibit pronghorn gene flow in the core of the species' range, despite the documented impacts of development on behavior and mortality risk of Wyoming pronghorn (Sawyer et al. 2019). Conserving landscape connectivity for pronghorn clearly is a management priority, as demonstrated in 2008 by the designation of the first federally protected migration corridor, the "Path of the Pronghorn," in western Wyoming (Hamilton 2008; Berger and Cain 2014). Our study provides fundamental data concerning the genetic connectivity of pronghorn in the core of their range, which will aid in future monitoring and protection of the species. Maintaining healthy core populations supports the long-term persistence of a species by mitigating genetic drift, contributing individuals and genetic diversity to peripheral populations, and reducing extinction risk (Brown 1984; Reinertsen et al. 2016). Understanding the current status of core populations allows for better management and conservation of wildlife species.

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Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Map of hunt areas for pronghorn in Wyoming.

Supplementary Data SD2.—Methods and results for alternatively filtered single-nucleotide polymorphism (SNP) and microsatellite data sets.

Supplementary Data SD3.— Locus-specific information for microsatellite loci including number of alleles, observed heterozygosity, and expected heterozygosity.

Supplementary Data SD4.—Spatial autocorrelation values for single-nucleotide polymorphism (SNP) and microsatellite data sets.

Supplementary Data SD5.—Map of samples used in rare variant analysis to test Interstate-80 barrier hypothesis.

Supplementary Data SD6.—Structure likelihood values for single-nucleotide polymorphism (SNP) and microsatellite analyses, and assignment plots for SNP data set for number of clusters $K = 2–4$.

Supplementary Data SD7.—Spatial autocorrelation plot for microsatellite data set.

Supplementary Data SD8.—Table of genetic diversity estimates from the literature.

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