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ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

Average kinship within bighorn sheep populations is associated with connectivity, augmentation, and bottlenecks

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Abstract

Understanding the influence of population attributes on genetic diversity is important to advancement of biological conservation. Because bighorn sheep (*Ovis canadensis*) populations vary in size and management history, the species provides a unique opportunity to observe the response of average pairwise kinship, inversely related to genetic diversity, to a spectrum of natural and management influences. We estimated average pairwise kinship of bighorn sheep herds and compared estimates with population origin (native/indigenous/extant or reintroduced), historical minimum count, connectivity, and augmentation history, to determine which predictors were the most important. We evaluated 488 bighorn sheep from 19 wild populations with past minimum counts of 16–562 animals, including native and reintroduced populations that received 0–165 animals in augmentations. Using the Illumina High Density Ovine array, we generated a dataset of 7728 single nucleotide polymorphisms and calculated average pairwise kinship for each population. Multiple linear regression analysis determined that connectivity between populations via dispersal, greater number of animals received in augmentations, and greater minimum count were correlated with lower average pairwise kinship at the population level, and whether the population was extant or reintroduced was less important. Thus, our results indicated that genetic isolation of populations can result in increased levels of inbreeding. By determining that natural and human-assisted gene flow were likely the most important influences of average pairwise kinship at the population level, this study can serve as a benchmark for future management of bighorn sheep populations and aid in identifying populations of genetic concern to define priorities for conservation of wild populations.

K E Y W O R D S
gene flow, genetic diversity, inbreeding, kinship, North America, *Ovis canadensis*, population bottleneck

INTRODUCTION

As wild plant and animal populations become increasingly fragmented due to human activities, small and isolated populations gradually lose genetic diversity due to chance (genetic drift) and inbreeding (mating between relatives; Frankham et al., 2017). Inbred individuals and populations can exhibit reduced reproductive fitness, termed inbreeding depression (Charlesworth & Charlesworth, 1987; Keller & Waller, 2002; Wright, 1984). In addition, populations with low genetic diversity have a reduced ability to adapt to environmental change, novel diseases, and evolving resident pathogens (Hoffmann et al., 2003; Hughes & Boomsma, 2004; Kellermann et al., 2006; MacPherson et al., 2018; Spielman, Brook, Briscoe, & Frankham, 2004; Whiteman et al., 2006). As a result, level of inbreeding in a population can influence population persistence (Madsen et al., 1996; Penn et al., 2002; Saccheri et al., 1998; Soulé & Mills, 1998). Thus, understanding the influence of population attributes on genetic diversity is important for the advancement of biological conservation.

Genetic processes are influenced by effective population size, defined as a population that would result in the same amount of loss of diversity associated with genetic drift as an "idealized" population (Frankham et al., 2010). Effective population size is based on the total number of fertile adults, but also accounts for mating system, unequal sex ratios, overlapping generations, and change in actual population size over successive generations (Alcala & Vuilleumier, 2014; Frankham et al., 2010). Other influences on effective population size over time include bottlenecks, founder events, and dispersal. A bottleneck can result from a sudden reduction in population size, such as a die-off event, which can result in loss of genetic variation (England et al., 2003; Leberg, 1992; Spielman, Brook, & Frankham, 2004). Theory and research suggest that historical population minimum is important in determining a population's genetic diversity (Avise et al., 1988; Wright, 1938). Similar to a bottleneck, a founder event refers to loss of genetic variation by chance when a small number of individuals immigrate or are introduced to an unoccupied area to start a new population (Templeton, 1980). Thus, considering a population's origin can be important to distinguish between populations that were recently reintroduced by human intervention and native (indigenous/extant) populations that were not recently founded by a small population (Jamieson, 2011; Mayr, 1963).

Regardless of population origin, gene flow can enhance genetic diversity (Falconer & Mackay, 1996; Spielman & Frankham, 1992). Natural gene flow between populations can be disrupted by human development, such as roads, which can lead to a reduction in genetic diversity (Epps et al., 2005; Holderegger & Di Giulio, 2010). Gene flow via dispersal or augmentation (translocations implemented by managers into existing populations) can enhance genetic diversity (Falconer & Mackay, 1996; Spielman & Frankham, 1992). To specifically address inbreeding depression, managers may add unrelated individuals to an inbred population to enhance its viability and genetic diversity (Frankham, 2015; Hogg et al., 2006; IUCN/SSC, 2013; Tallmon et al., 2004; Whiteley et al., 2015). When considering management efforts to increase gene flow, such as increasing habitat connectivity, reducing barriers to movement, or augmenting populations, managers must decide which populations should receive additional animals and the identity of source populations while considering local adaptation. In addition, when planning an augmentation, managers must decide on the number of animals to translocate. Thus, it would be useful to improve understanding regarding what aspects of augmentation decisions and other influences on populations over time most affect genetic diversity in populations of conservation concern. Research questions that evaluate the determinants of genetic diversity can benefit from recent technical advances in genomics, which greatly increase precision through genotyping thousands of genetic markers (Leffler et al., 2012).

While there are many metrics to assess genetic diversity and inbreeding status of populations, the kinship coefficient is informative for genetic management decisions (Frankham et al., 2017). Kinship, also termed coancestry, represents the probability that two randomly sampled alleles from two individuals are identical by descent (Manichaikul et al., 2010). Kinship can be estimated from pedigrees and genetic marker information (Ballou & Lacy, 1995; Manichaikul et al., 2010). An individual's inbreeding coefficient is equal to the level of kinship between its mother and father (Falconer & Mackay, 1996). Thus, the mean of all pairwise kinship values from a sample of individuals in a population represents the expected level of inbreeding in the next generation (Frankham et al., 2017). In addition, mean kinship of a population is inversely related to genetic diversity, meaning that greater mean kinship indicates lower genetic diversity (Frankham et al., 2010). Kinship metrics estimate identity by descent between individuals relative to that found within individuals, so the estimate is not biased by being measured relative to a reference population generated from all samples included in the calculation (Manichaikul et al., 2010). Thus, the metric is invariant to which samples are included in the calculation and it is not impacted by linkage disequilibrium (LD) among genetic markers (Manichaikul et al., 2010). The metric's maximum value is 1, with higher values meaning that individuals are more related; zero or negative values mean that the individuals are unrelated (Manichaikul et al., 2010).

Minimizing mean kinship is an optimal approach to retain genetic and allelic diversity and minimize

inbreeding in wild populations (Ballou & Lacy, 1995; Fernández et al., 2004; Frankham et al., 2017). Its utility has been verified in *Drosophila* experiments (Montgomery et al., 1997), and biologists use mean kinship to make breeding decisions for threatened species in captivity and rare domestic livestock breeds (Ballou et al., 2010; Falconer & Mackay, 1996; Frankham et al., 2010). Mean kinship is used to make breeding decisions to minimize inbreeding in most, if not all, wildlife captive-breeding programs to support reintroductions, including for the black-footed ferret (*Mustela nigripes*) and California condor (*Gymnogyps californianus*; Ballou & Foose, 1996; Moran et al., 2021; Ralls & Ballou, 2004; Russell et al., 1994). Based on the fact that kinship is expected to be 0.125 for a second-degree relationship (i.e., half-siblings) and 0.0625 for a third-degree relationship (i.e., first cousins), Frankham et al. (2017) recommended that populations have a mean kinship value less than 0.1 to avoid inbreeding depression (Frankham et al., 2017; Manichaikul et al., 2010; Van Dyke, 2008). This may vary slightly based on the genetic marker used and the species life history. Specifically, a meta-analysis of inbreeding in livestock species estimated that beyond the inbreeding threshold of 10%, inbreeding depression corresponds to an average decrease of 0.137% in mean viability of reproduction traits per 1% increase in inbreeding (Leroy, 2014). Researchers can calculate the average of all pairwise kinship values for samples within each population to estimate the level of inbreeding in the next generation (Finger et al., 2011), which we refer to as average pairwise kinship and used to evaluate bighorn sheep populations.

Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) management has experienced many challenges that are common in conservation, including fragmented populations and disease, such that areas of historical range remain unoccupied despite restoration efforts. Many populations are now genetically differentiated after recent fragmentation due to human activities (Epps et al., 2005; Luikart & Allendorf, 1996). Some populations still represent historical conditions and are large and interconnected, whereas others are small and isolated with a history of drastic bottlenecks due to epizootic events that often result in mortality of over half of the population (Cassirer et al., 2018). Since 1922, managers have moved more than 21,500 bighorn sheep in greater than 1460 translocations with the intention of reestablishing populations in historical range and increasing size and genetic diversity of existing populations (Wild Sheep Working Group, 2015). Despite these efforts, many reintroduced populations remain small and isolated, sometimes without a clear explanation, with epizootic events and lack of migratory diversity as likely contributing factors (Cassirer et al., 2018; Lowrey et al., 2020). Recent concerns regarding resident pathogens in existing bighorn sheep populations and disease spillover from domestic sheep have resulted in managers keeping many populations small and isolated to avoid the spread of disease (Butler et al., 2018; Cassirer et al., 2016; Sells et al., 2016). This complex history has resulted in concern that genetic factors could limit population growth and viability of the species, as the species does not seem to exhibit inbreeding avoidance (Hogg et al., 2006; Johnson et al., 2011; Rioux-Paquette et al., 2010; Singer et al., 2000). Because bighorn sheep populations vary greatly in their origin, population size, connectivity, and augmentation history, they provide a unique opportunity to observe the response of average pairwise kinship to a spectrum of natural and management influences.

This study addressed the following objectives: (1) characterize average pairwise kinship of bighorn sheep populations with varied management histories and (2) compare kinship at the population level with population origin (reintroduced vs. native), size, connectivity (natural dispersal), and augmentation history to determine whether there is a correlation. We expected that native (indigenous/extant) origin, large population size, and greater gene flow via augmentations or natural dispersal would result in lower average pairwise kinship than reintroduced origin, small population size, and genetic isolation. We also predicted that the number of animals received in augmentations and origin would be the most important influences, due to observed effects of these variables on genomic similarity among populations (Flesch et al., 2020). This effort to evaluate how different influences on population size over time may affect average pairwise kinship in multiple free-ranging populations can serve to assess the relative importance of these influences to enhance genetic management strategies, identify priorities for conservation of genetic diversity in different types of populations (e.g., native [indigenous/extant] vs. reintroduced), and improve the identification of populations of concern if genetic data are not available.

METHODS

Study populations

We evaluated 19 wild populations of Rocky Mountain bighorn sheep located in the United States and Canada, including the US states of Montana, Wyoming, Colorado, and Utah, and the Canadian provinces of Alberta and British Columbia (Figure 1). In the following descriptions of these populations, we use the terms "population" and "herd" synonymously. We aimed to sample at least 20–25 individuals per herd, based on sample size simulations that indicated lower sample sizes introduced an
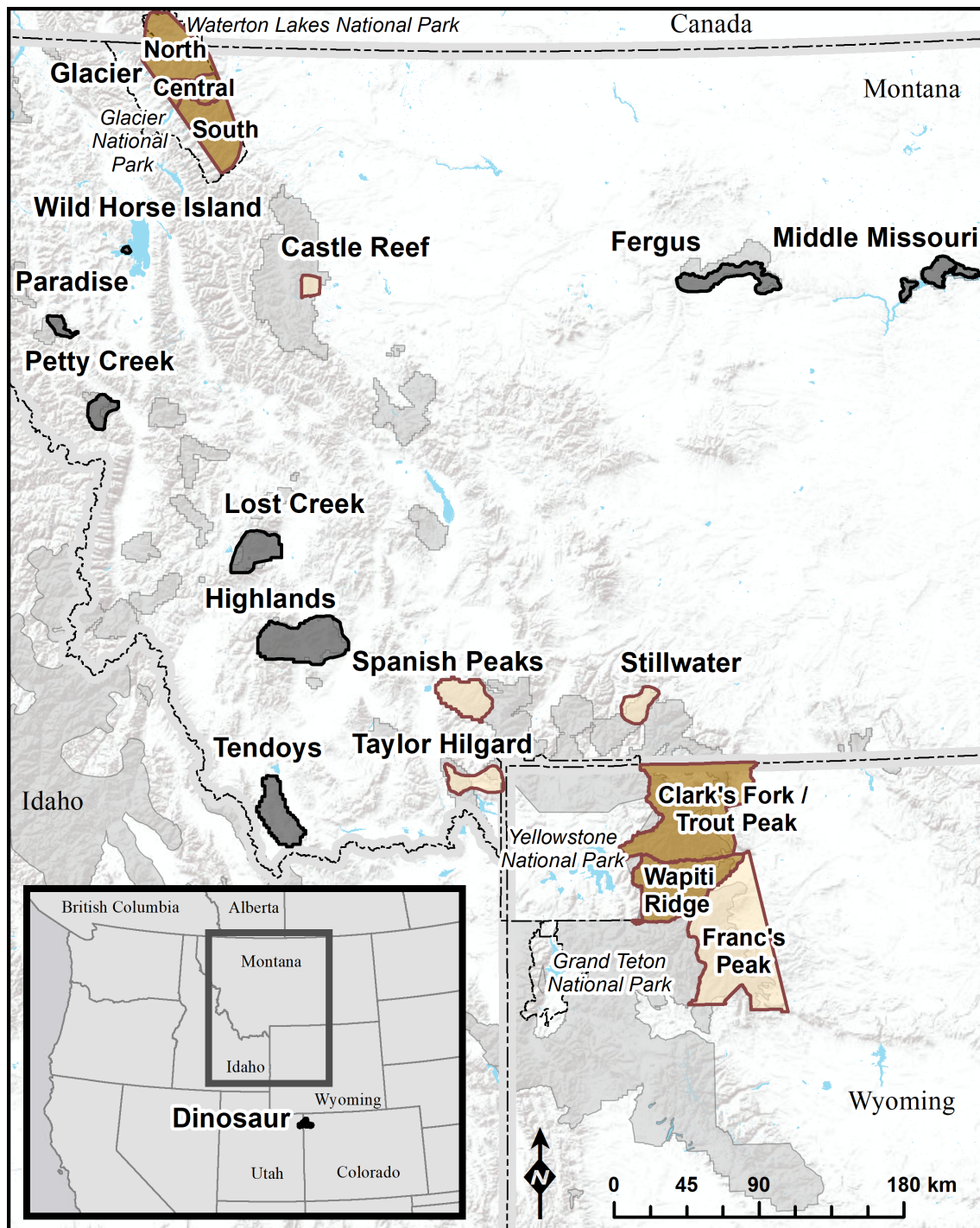
FIGURE 1 Approximate distributions of 19 bighorn sheep (*Ovis canadensis*) populations located in Montana, Wyoming, Utah, and Colorado that were evaluated. Native populations that did not receive animals from other areas through augmentation are dark brown. Native populations that received animals from other areas through augmentation are beige. Reintroduced populations are shown in black. Approximate bighorn sheep ranges, including populations not in this study, are shown in gray polygons for Idaho, Wyoming, and Montana (Montana Fish, Wildlife and Parks, 2008; Thomas, 2019; Wyoming Game and Fish Department, 2012). State boundaries are dashed lines outlined in gray; national park boundaries in the study area are dashed lines. Base layer credits include Environmental Systems Research Institute, Tele Atlas North America, Inc., U.S. Geological Survey, National Oceanic and Atmospheric Administration, HERE Technologies, Garmin, ©OpenStreetMap contributors, and the GIS user community

unacceptable level of uncertainty to average pairwise kinship estimates (Flesch et al., 2018).

We sampled 10 native herds, ranging in size from 70 to 726 animals. Seven native herds represented natural

population structure prior to widespread fragmentation, as they are part of spatially structured populations, defined as a collection of subpopulations that occupy distinct geographic areas but are linked by animal movement. These populations included three units in the eastern front of the Greater Yellowstone Area of Wyoming, three units in Glacier and Waterton Lakes National Parks, and Castle Reef in Montana. The Wyoming units included Clark's Fork/Trout Peak, Wapiti Ridge, and Franc's Peak, with their boundaries defined by administrative units. The Glacier area was split into north, central, and south for our analyses, and the divisions were informed by previous genetic and movement analyses (T. Graves unpublished data; Flesch et al., 2020). South of Glacier National Park, Castle Reef, is assumed to have connectivity across four administrative units in the Rocky Mountain Front of Montana (Montana Department of Fish, Wildlife and Parks, 2010). Two of the remaining native herds were genetically isolated, and one herd had some genetic connectivity with a neighboring population (Flesch et al., 2020; Appendix S1: Table S1).

We sampled nine reintroduced herds that were established by translocations within the past 75 years. Reintroduced herds ranged in size from 70 to 416 animals (Appendix S1: Table S1). Founder size for the reintroduced herds ranged from 8 to 53 animals. We considered a translocation to be part of the original founding group if it occurred within 3 years of the first reintroduction event to an unoccupied area, for all populations except for Wild Horse Island. For this population, we considered two translocations 8 years apart as both founding events, because the first reintroduction effort moved only two animals. Five to eleven generations occurred since establishment of evaluated reintroduced herds, based on a generation time of 6 years (Hogg et al., 2006; Johnson et al., 2011).

Sample collection and DNA extraction

We collected genetic samples from 2002 to 2018. All but two populations were sampled over a time span of 1 day to 4 years (less than one generation). North Glacier and Central Glacier were sampled over a period of 7 and 9 years (about 1.2 and 1.5 generations), respectively. Animal capture and handling protocols were approved by Institutional Care and Use Committees at Montana State University (Permit No. 2011-17, 2014-32), Montana Department of Fish, Wildlife, and Parks (Permit No. 2016-005), Parks Canada (Permit No. WL-2005-638), Wyoming Game and Fish Department (Permit No. 854), U.S. Geological Survey (Permit No. 2004-01, DINO-2008-SCI-0010), and University of Montana (Permit Nos. 024-07MHWB-071807 and 012-16MMMCWRU-022916). We captured live animals using helicopter net gunning, ground darting, and drop netting. The drop net capture method was most likely to capture family groups and was only used in Taylor Hilgard and Castle Reef. To avoid biasing results from capturing family groups, we evaluated animals from three different capture years in Taylor Hilgard (16 animals from 2013, four animals from 2015, and 10 animals from 2016) and used other capture methods in addition to drop netting in Castle Reef (15 animals captured using drop netting, one animal captured using ground darting, and nine animals captured using helicopter net gunning). Genetic samples included Whatman FTA classic cards, biopsy punches from ear cartilage and whole blood samples from live animals, and tissue from hunter-harvested or road-killed animals. We extracted DNA using Maxwell 16 LEV Blood DNA Kit for whole blood and the Maxwell 16 SEV Tissue Kit for biopsy punch and tissue samples per kit instructions. For gene cards, we modified the Promega LEV Blood DNA Kit procedure; we incubated two to three 5-mm gene card punches with proteinase K and lysis buffer in a DNA IQ spin basket (Promega), spun at 3000 XG for 5 min, and loaded the flow-through into the Maxwell 16 LEV Blood DNA cartridge. For genotyping, we used samples with a minimum DNA concentration of 20 ng/μL, minimum of 300 ng of DNA, and 260 nm/280 nm ratio of 1.0–1.5.

Genomic dataset and quality control

We contracted GeneSeek (Lincoln, NE, USA) to genotype genetic samples using the Illumina High Density (HD) Ovine array, also termed a single nucleotide polymorphism (SNP) chip. The SNP chip included 606,006 SNPs at a density of one SNP per 4.279 kb. The Ovine array was originally developed for the evaluation of domestic sheep, but its development included five bighorn sheep and four Dall's sheep (*Ovis dalli*; Kijas et al., 2009, 2014). Species divergence between domestic and bighorn sheep took place about three million years ago, but the two species can interbreed and produce viable offspring (Bunch et al., 2006; Young & Manville, 1960). In addition, the two species have the same number of chromosomes and are expected to have high genomic synteny (Poissant et al., 2010). Thus, about 24,000–33,000 SNPs in the Ovine array are informative for Rocky Mountain bighorn sheep (Flesch et al., 2020; Miller et al., 2015), and the domestic sheep reference genome enables mapping SNPs to chromosomes (Kohn et al., 2006). Because only a select number of individuals were used to create the SNP chip, this genotyping approach can have an ascertainment bias (Albrechtsen et al., 2010). However, differences in mean kinship within populations can be differentiated at a sample size of 20–25 individuals per population (Flesch et al., 2018).

We concatenated Illumina genotype data in Golden Helix SNP and Variation Suite version 8.6.0 (Golden Helix, Inc., 2016). Using Golden Helix software, we mapped genotype data to the domestic sheep reference genome Oar version 4.0, removed samples with a call rate less than 0.85, removed markers on sex chromosomes and with unknown mappings, and exported the genotypes into PLINK version 1.90 for filtering (Golden Helix, Inc., 2016; Purcell et al., 2007). We completed the remaining analyses using R version 4.1.0 and bash code version 4.3.48 in the RStudio environment; all analysis code is provided as supplementary information (Appendix S2; GNU Bash, 2013; R Core Team, 2017; RStudio Team, 2015). We used minimal filtering prior to kinship analysis based on KING software guidelines (Manichaikul et al., 2010). We removed monomorphic and extremely rare markers using a minor allele frequency of less than 0.01 and removed markers with poor performance by requiring a SNP call rate greater than 0.99 (Huisman et al., 2016).

Estimating genomic kinship

We calculated genomic kinship between all possible pairs of individuals within each population using KING version 2.2.6 (Manichaikul et al., 2010). We calculated the average of all pairwise kinship values for samples within each population to estimate the level of inbreeding in the next generation. We termed this approach average pairwise kinship, which is similar to the method employed by Finger et al. (2011). The formal definition of mean kinship includes self-kinship values (Frankham et al., 2017; Van Dyke, 2008), but we excluded self-kinships in calculating a mean for each herd, because uneven sample sizes among populations would bias relative comparisons of mean kinship among herds with the inclusion of self-kinship values. Specifically, with the inclusion of self-kinship, mean kinship of herds with smaller sample sizes would be biased higher than that of herds with larger sample sizes.

After calculating average pairwise kinship, we could not calculate an uncertainty interval around these values, because uncertainty could be influenced by many factors, including the quality of samples, genotyping approach, number of genetic markers, and the specific kinship estimator. There is no known approach to account for all of these influences in a single measure of uncertainty, so we present our average pairwise kinship estimates without uncertainty intervals. To provide context for the average pairwise kinship estimates, we used PLINK to calculate the population mean of method-of-moments F coefficient estimates, which are individual inbreeding coefficients based on observed and expected number of homozygotes (Purcell et al., 2007). Prior to estimating F coefficient estimates, we completed LD pruning to filter non-independent SNPs that informed the presence of nearby variants, using a window size of 100 SNPs, window increment of 25 SNPs, and LD statistic of r^2 (Flesch et al., 2020; Huisman et al., 2016; Purcell et al., 2007).

Evaluating influences on average pairwise kinship using linear models

We used multiple linear regression to evaluate the relationship between average pairwise kinship and four categories of predictor variables, including origin, connectivity, population size, and augmentation history (Appendix S1: Tables S1 and S2). Population origin was defined as native (indigenous/extant) or reintroduced. The predictor variable of origin represented the potential for a recent founder effect within the past 75 years (12.5 bighorn sheep generations). Connectivity represented the potential level of gene flow due to dispersal between the examined population and neighboring populations. Classification of herds into connectivity categories was based on geographic distances between populations, hunter harvest data, and previous research that evaluated movement between populations using GPS data (Appendix S1: Table S3). Herds were classified as "isolated" if there was no known movement between areas within the past 20 years and "connected" if there was indication or some possibility for movement between areas.

We evaluated augmentation history of herds to account for assisted gene flow via translocations. Thirteen out of 19 populations received at least one augmentation (Flesch et al., 2020). Augmentation recommendations are often based on the number of migrants per generation (Mills & Allendorf, 1996; Wright, 1931), but the number of translocation sources can also be associated with heterozygosity in bighorn sheep herds (Jahner et al., 2018). Thus, we evaluated four alternative augmentation history variables: the number of animals received in augmentations, the number of augmentations received, and quantitative and categorical versions of the number of distinct populations that contributed to the current population (Appendix S1: Table S1).

We evaluated population size using count data, based on genetic theory that historical bottlenecks would be the most important influence on genetic diversity (Wright, 1938). We evaluated two alternative variables for population size, including the mean of the most recent counts from five consecutive survey years if available and minimum count (Appendix S1: Tables S1 and S2). Agency staff and reports provided count data (George et al., 2009; Keating, 1985; Montana Department of Fish, Wildlife and Parks, 2010; Paterson

et al., 2021). We expected counts among populations would have different detection probabilities and observer platforms (i.e., aerial vs. ground surveys). However, survey approach was consistent for each population, so we considered the raw counts to be an index for relative abundance across the evaluated populations. Historical minimum count represented the lowest credible count after 1980. This minimum count could be a result of epizootic events, population growth following reintroduction, or other unknown factors. Fourteen out of 19 evaluated herds (74%) experienced a documented all-age die-off suspected to be an epizootic event after 1970, so disease events were a predominant cause of documented bottlenecks.

We assessed the level of correlation between quantitative variables that described augmentation history and population size with Pearson's correlation coefficients. To identify and evaluate the most important variables, we compared the Akaike's information criterion with small-sample bias adjustment (AIC_c) for models with all possible combinations of variables that did not include variables correlated at ≥0.89 ("MuMIn" R package; $n = 60$ models; Barton, 2020; Doherty

et al., 2012). We selected the best model as that with the lowest AIC_c value (Akaike, 1973; Hurvich & Tsai, 1989). The number of animals received in augmentations and minimum count were included in the top ranked model with connectivity, so these variables were used to represent augmentation history and population size categories, respectively, for all further analyses.

In general, we expected that the four covariates of origin, connectivity, number of animals received in augmentations, and minimum count were reasonable to include in a single linear model, due to expected independence of each predictor's influence on average pairwise kinship. We were not able to evaluate two-way interactions due to limited sample size. We summarized the data graphically to visualize the relationships among the predictors and with average pairwise kinship, and we verified that all populations had observations for each predictor (Table 1, Appendix S1: Figure S1). Because inbreeding increases at a faster rate in small populations than large ones, studies can employ a logarithmic transformation to some predictors of genetic diversity (Wang et al., 2017). However, the population size of the

TABLE 1 Data for 19 bighorn sheep herds integrated into linear models that compared the relationship between average pairwise kinship and origin, connectivity, animals contributed in augmentations, and minimum count

Herd	Sample size	Origin	Connectivity	Animals received in augmentations	Minimum count	Average pairwise kinship	Kinship standard deviation
Glacier (South)	26	Native	Connected	0	136	0.015	0.060
Clark's Fork/Trout Peak	27	Native	Connected	0	562	0.018	0.032
Glacier (North) and Waterton	25	Native	Connected	0	106	0.023	0.042
Wapiti Ridge	27	Native	Connected	0	552	0.024	0.033
Franc's Peak	23	Native	Connected	23	270	0.032	0.040
Castle Reef	25	Native	Connected	23	99	0.036	0.032
Stillwater	24	Native	Connected	5	23	0.042	0.073
Glacier (Central)	44	Native	Connected	0	103	0.048	0.047
Taylor Hilgard	30	Native	Isolated	68	25	0.059	0.057
Spanish Peaks	20	Native	Isolated	4	80	0.059	0.057
Dinosaur	20	Reintroduced	Connected	165	100	0.022	0.074
Tendoys	25	Reintroduced	Connected	99	16	0.031	0.091
Highlands	17	Reintroduced	Isolated	140	80	0.037	0.085
Fergus	30	Reintroduced	Connected	28	108	0.039	0.066
Paradise	25	Reintroduced	Isolated	22	93	0.044	0.041
Lost Creek	25	Reintroduced	Connected	2	58	0.047	0.046
Wild Horse Island	25	Reintroduced	Isolated	2	38	0.055	0.048
Petty Creek	25	Reintroduced	Isolated	4	63	0.065	0.055
Middle Missouri	25	Reintroduced	Isolated	0	79	0.081	0.048

Note: Sample size describes the number of individual bighorn sheep genotypes used to estimate average pairwise kinship.

herds in our study ranged from approximately 70 to 416 animals, and this number of animals can result in an approximately linear increase of inbreeding over less than 20 generations (Frankham et al., 2010). Thus, we did not log-transform the explanatory variables. We evaluated all possible subsets of a global model that included all four predictors, because our relatively small sample size of herds ($n = 19$) could cause precision to vary among models, and an all combination analysis is expected to perform better than ad hoc strategies in terms of variable selection (Doherty et al., 2012).

For all evaluated linear models, we ensured that statistical assumptions were met. The first assumption was that the average pairwise kinship response was independent among populations. We considered the possibility for a cluster effect, as some native herds were geographically proximate (Figure 1), but the Durbin–Watson test for all models indicated that the residuals were not autocorrelated (Fox, 2015). The second assumption was that the relationship between average pairwise kinship and the explanatory variables of origin, connectivity, minimum count, and animals received in augmentations was linear. We evaluated this assumption using scatterplots and boxplots of average pairwise kinship versus the predictor variables (Appendix S1: Figure S1) and residual plots. The relationships appeared to be approximately linear, and the residual plot had random scatter, indicating this assumption was met. The third assumption was that for each value of the covariates, the variance of average pairwise kinship was constant. To examine this assumption, we evaluated the residual plots and determined there was even spread around the lowess curve to satisfy this assumption. The fourth assumption was that for each value of the explanatory variables, the average pairwise kinship values were normally distributed. We considered this assumption by examining the Q–Q plot of residuals, and this assumption was reasonably satisfied.

We evaluated additional linear regression diagnostics, including correlation among predictors, outliers, and high leverage points. All models had variance inflation factor values less than 3, indicating low collinearity (Fox, 2015; Fox & Monette, 1992; Fox & Weisberg, 2018). Bonferroni outlier tests for all candidate models indicated there were no studentized residuals with a p value less than 0.05 (Fox, 2015). There were no high leverage points, because all Cook's distance values were less than 0.4 (Cook, 1977).

To evaluate relative model plausibility, we used an information-theoretic approach (Burnham & Anderson, 1998). We used AIC_c to compare models (Akaike, 1973; Hurvich & Tsai, 1989). In addition, relative plausibility of each candidate model was represented by Akaike weights, which range from 0 (lowest plausibility) to 1 (highest plausibility) (Burnham & Anderson, 1998). Because each predictor was included in an equal number of models, we estimated

the importance of individual predictor variables using the sum of Akaike weights for the candidate models that included the predictor (Burnham & Anderson, 1998).

RESULTS

Genomic kinship and herd attributes

We genotyped 488 bighorn sheep samples with a SNP call rate greater than 0.85, including 375 females, 108 males, and five animals of unknown sex. Age classifications included 398 adults, 34 yearlings, 13 lambs, and 43 animals of unknown age. We met our sample size goal of at least 20–25 individuals for 18 Rocky Mountain bighorn sheep populations, excluding Highlands (Table 1). However, we still included the Highlands ($n = 17$) in our analyses due to the limited number of herds in the study. Filtering markers on sex chromosomes and with unknown mappings removed 29,411 SNPs. Filtering based on a minor allele frequency of less than 0.01 removed 530,042 SNPs; requiring a SNP call rate greater than 0.99 removed 38,825 SNPs. After filtering, we used 7728 SNPs to estimate genomic kinship between all individuals in each population. For quantitative predictor values, minimum count per population ranged from 16 to 562 individuals; number of animals received in augmentations ranged from 0 to 165 individuals (Table 1, Appendix S1: Figure S1). Average pairwise kinship per population ranged from 0.015 to 0.081 (Figure 2), and positive values suggested that all populations were in Hardy–Weinberg equilibrium. Mean method-of-moments F coefficient estimates averaged 0.14 and ranged from 0.06 to 0.29 (Appendix S1: Table S5).

Connectivity and other predictors were associated with average pairwise kinship

The Pearson's correlation coefficients between augmentation history variables ranged from 0.90 to 0.94, and the Pearson's correlation coefficient between minimum count and recent mean count was 0.89 (Appendix S1: Table S4). We evaluated 15 linear models to assess the variables of connectivity, minimum count, animals received in augmentations, and origin. We show the eight top-performing linear models with delta AIC_c <9 in Table 2, because the coefficients were less stable for models with delta AIC_c >9 (Appendix S1: Tables S6 and S7). The eight top-performing models had r^2 values that averaged 0.61 and ranged from 0.53 to 0.71, indicating precise fits (Table 2, Appendix S1: Table S6). All top-performing models contained connectivity as a predictor.
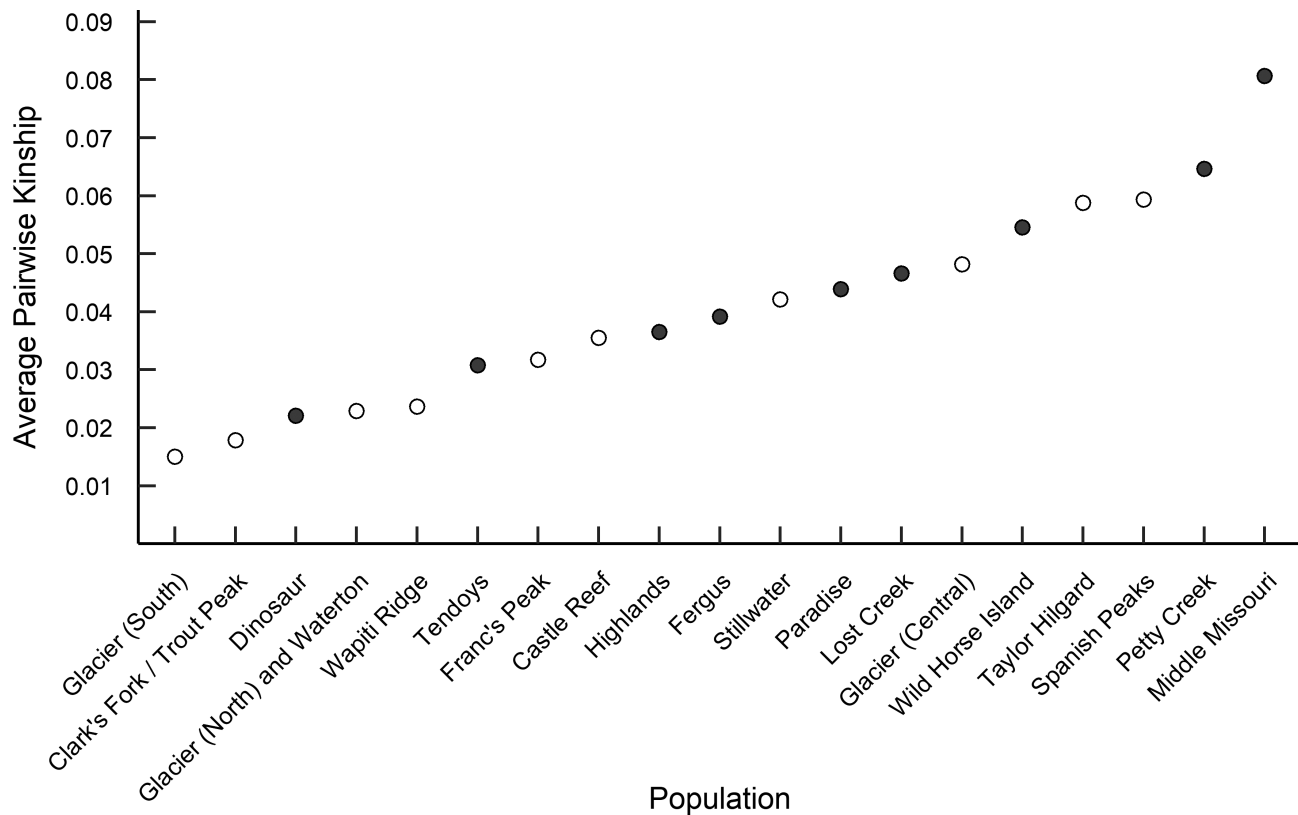
FIGURE 2 Average pairwise kinship for 19 bighorn sheep (*Ovis canadensis*) populations located in Montana, Wyoming, Utah, and Colorado that were evaluated. Native populations are represented by white points. Reintroduced populations are represented by black points. Average pairwise kinship values are sorted from lowest to highest

TABLE 2 Predictor variables, number of parameters (*K*), log-likelihood (Log*L*), Akaike's information criterion with small-sample bias adjustment (AIC$_c$), ΔAIC$_c$, and Akaike weights (*w*) for candidate models (*i*) that predicted bighorn sheep population average pairwise kinship

Candidate model	K	LogL	AIC$_c$	ΔAIC$_c$	w_i
Connectivity, minimum count, and animals received in augmentations	5	61.95	−109.29	0.00	0.395
Connectivity	3	57.56	−107.51	1.78	0.162
Connectivity and animals received in augmentations	4	59.04	−107.23	2.07	0.140
Connectivity and minimum count	4	58.97	−107.09	2.21	0.131
Connectivity, minimum count, animals received in augmentations, and origin	6	62.31	−105.63	3.67	0.063
Connectivity, animals received in augmentations, and origin	5	59.86	−105.11	4.18	0.049
Connectivity and origin	4	57.63	−104.40	4.89	0.034
Connectivity, minimum count, and origin	5	58.98	−103.35	5.95	0.020

Note: Eight models with ΔAIC$_c$ <9 are shown out of 15 evaluated models.

Connectivity had the highest importance weight (0.99), followed by number of animals contributed in augmentations (0.65), minimum historical count (0.61), and origin (0.17). The most plausible model of average pairwise kinship contained connectivity, number of animals received in augmentations, and minimum count. All estimated coefficients in the top two models had 95% confidence intervals (CIs) that did not overlap with 0. Coefficients were relatively stable across models (Appendix S1: Table S6), so we further discuss the results from the full
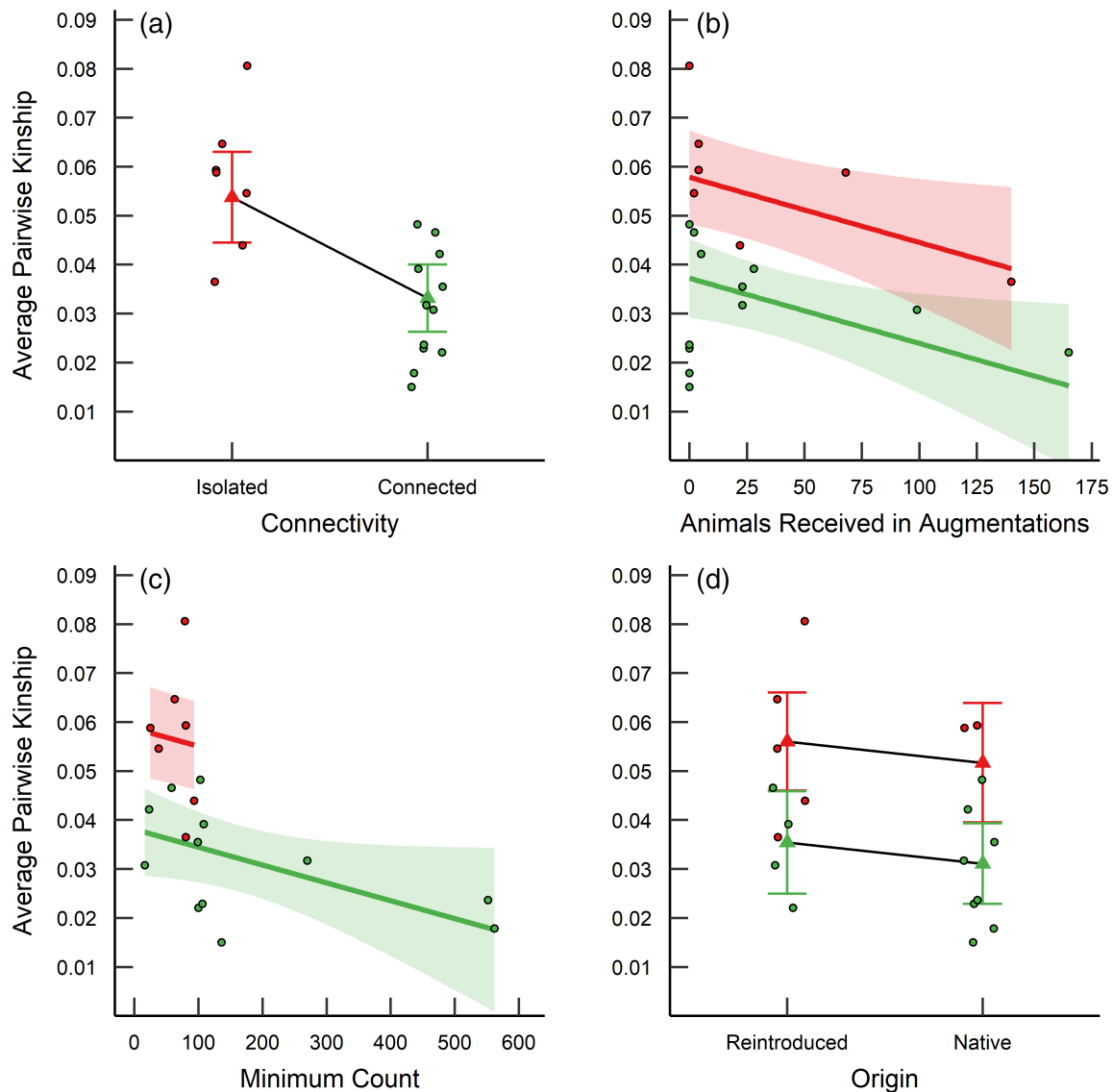
FIGURE 3 Empirical linear model estimates of the relationship between average pairwise kinship for 19 bighorn sheep populations and connectivity (a), total number of animals received in augmentations by connectivity (b), minimum count by connectivity (c), and origin by connectivity (d). Connected is represented by green and isolated is red. Whiskers and bands around coefficient estimates represent the 95% confidence interval for the full model. Observed data are shown as points

model: $\hat{\mu}$ {Average pairwise kinship|Connectivity, Minimum count, Animals received in augmentations, Origin} = 0.06511 − 0.02061 (Connectivity [Connected]) − 0.00004 (Minimum count) − 0.00013 (Animals received in augmentations) − 0.00435 (Origin [Native]).

The relationship between average pairwise kinship and all predictors was in the expected direction. Connected populations generally had lower average pairwise kinship, implying greater genetic diversity, than those that were isolated (Figure 3a). Based on the full model, the mean of average pairwise kinship decreased by 0.02 (95% CI: −0.03 to −0.009) for herds with connectivity, all other variables held constant. Similar to increasing connectivity, increasing the number of animals received in

augmentations was associated with a decrease in average pairwise kinship, suggesting greater genetic diversity (Figure 3b). For an increase of 10 animals received in augmentations, the estimated average pairwise kinship decreased by about 0.001 (95% CI: −0.0025 to −0.0002), controlled for connectivity, minimum historical count, and origin. This result suggests that management intervention to supplement existing populations via augmentation was effective in decreasing average pairwise kinship and potentially increasing genetic diversity. Greater minimum count was associated with decreasing average pairwise kinship in an inverse relationship (Figure 3c), suggesting that bottlenecks consisting of smaller numbers of animals lowered genetic diversity.

Finally, origin was the least important predictor and the model suggested that native herds may have lower average pairwise kinship and thus greater genetic diversity than reintroduced herds, but the CI of this predictor overlapped zero (Figure 3d, Appendix S1: Table S6).

DISCUSSION

We determined that population attributes, including influences from natural processes and management, were correlated with average pairwise kinship for 19 different bighorn sheep populations. Population connectivity via dispersal and animals received in augmentations, closely followed by historical minimum count, were more important predictors of average pairwise kinship than origin. This result suggests that gene flow in more recent generations could reduce inbreeding, overcoming negative influences on effective population size in earlier generations, such as a founder effect in reintroduced populations or a bottleneck of population size resulting from an epizootic event.

Native and reintroduced populations had similar levels of average pairwise kinship

We did not expect that origin of populations (native vs. reintroduced) would be the least important predictor of average pairwise kinship, due to previous research that suggested reintroduced bighorn sheep herds generally have lower genetic diversity due to a founder effect (Fitzsimmons et al., 1997; Hedrick et al., 2001; Olson et al., 2013). Seven out of nine reintroduced populations were established or augmented from multiple population sources, which may have mitigated a founder effect. Thus, our results suggest that gene flow and admixture (interbreeding between differentiated populations) after reintroduction can serve to minimize average pairwise kinship. Similarly, recent studies of bighorn sheep herds in Nevada, Arizona, and Wyoming did not find clear differences in mean heterozygosity between native and reintroduced populations (Gille et al., 2019; Jahner et al., 2018; Love Stowell et al., 2020). Admixture had a greater influence on maximizing genetic diversity within reintroduced populations of Alpine ibex (Capra ibex) than releasing a large number of founders from the same source (Biebach & Keller, 2012). This admixture approach to reintroduction has also been effective in multiple Australian mammal species (McLennan et al., 2020; White et al., 2018). Thus, sourcing reintroductions and augmentations from different populations can be an effective management tool to maximize genetic diversity in reintroduced populations if translocated animals are expected to be adapted to the destination's environmental conditions. Additional variables beyond admixture, such as rate of population growth after population establishment, can also be important to maintaining genetic diversity after reintroduction (Lacy, 1994).

Previous bighorn sheep research has suggested sourcing only from native bighorn sheep populations for translocations due to higher success rates, which was speculated to be in part due to greater genetic diversity in native populations (Fitzsimmons et al., 1997; Singer et al., 2000). In contrast, our research suggests that reintroduced herds could also be a viable option, if reintroduced herds had gene flow to enhance genetic diversity. Considering reintroduced populations as potential sources for translocations would be helpful to translocation planning by providing a larger pool of candidate source populations. However, lack of a clear relationship between origin and average pairwise kinship in our analysis also suggests that this predictor could be explored further with additional observations.

A previous study also evaluated heterozygosity of two reintroduced herds in Montana (Paradise and Middle Missouri; Love Stowell et al., 2020). Love Stowell et al. (2020) detected qualitative evidence for a genetic bottleneck in the Missouri Breaks, consistent with our finding that this population had the highest average pairwise kinship of all evaluated herds. In addition, Barbosa et al. (2021) similarly suggested that isolation, small population size, and reintroduced origin led to loss of genetic diversity in bighorn sheep populations. In regard to population origin, Olson et al. (2013) and Barbosa et al. (2021) reported that reintroduced herds originally founded by other reintroduced populations with low genetic diversity also had low genetic diversity initially. For this study, we did not have genetic samples available from the time of reintroduction. Thus, we could not evaluate whether the reintroduced populations in our study had low inbreeding levels because they were founded by translocations from herds with high genetic diversity.

In contrast to 7728 SNPs used in this study, Olson et al. (2013), Love Stowell et al. (2020), and Barbosa et al. (2021) evaluated 15 to 38 microsatellite markers to estimate heterozygosity. The type, number, and polymorphism of molecular markers used as input data and the metric employed can greatly impact the accuracy of resulting estimates (Blouin, 2003). Microsatellite markers, which are short tandem repeats of DNA motifs, can provide kinship and inbreeding estimates that correlate poorly with those derived from pedigrees (Slate et al., 2004; Taylor et al., 2015; Toro et al., 2002). SNP chips and sequencing data enhance reproducibility of results over time and across different labs to enable comparison within and between populations. Thus, reproducible genomic data will likely

have greater utility in informing management decisions to maintain genetic diversity in conservation programs in future (Fernández et al., 2012; Frankham et al., 2017; Jeffries et al., 2016; Saura et al., 2013).

In our analysis, we were not able to directly evaluate the effects of the number of generations since reintroduction and founder size due to a limited sample size of reintroduced herds. However, our results suggest a weak inverse relationship between the number of founders and average pairwise kinship, as expected (Appendix S1: Figure S2; Aguilar et al., 2008; Templeton, 1980). In reintroduction biology, it is typically recommended to avoid a founder effect by translocating a sufficiently large number of individuals to serve as founders, such that the group will be representative of the source's population genetics (Jamieson & Lacy, 2012). Future research could evaluate the optimal number of founders for bighorn sheep reintroductions, as optimal founder size can vary by reintroduction area and species, based on factors such as carrying capacity and expected survival rate after translocation (Tracy et al., 2011).

Bottlenecks of greater magnitude were associated with greater average pairwise kinship

Lower minimum counts used to index population size were correlated with higher average pairwise kinship, suggesting that past bottlenecks of greater severity negatively affected genetic diversity as expected. Most examined herds experienced bottlenecks due to epizootic events, and these disease events can reduce genetic diversity of bighorn sheep populations (Ramey et al., 2000). Similarly, studies on marine and freshwater fish found a positive relationship between abundance and genetic diversity while accounting for overfishing (McCusker & Bentzen, 2010; Pinsky & Palumbi, 2014). Although minimum count was not one of the most important predictors of average pairwise kinship, small population size or bottlenecks can interact with other influences, such as predation, to negatively affect population trend (Berger, 1990; Rominger et al., 2004). Although minimum count had higher support in models than recent mean count (based on counts from the most recent 5 years), the high correlation (Pearson's $r = 0.89$) prevented disentangling these variables completely, and either or both could drive genetic diversity. A less correlated dataset would be needed to more thoroughly evaluate how these two variables contribute to genetic diversity (Appendix S1: Tables S1 and S4). Future research could further evaluate the influences of bottleneck duration and fluctuations in population size over time on average pairwise kinship.

In general, we observed some variation in average pairwise kinship among herds with similar minimum counts and other population attributes. This observed variation could be due to sampling or stochastic processes. We attempted to account for sampling variation by targeting a sample size of 20–25 animals per population, which can differentiate average pairwise kinship estimates for bighorn sheep herds with different attributes using the applied genetic marker set (Flesch et al., 2018). Stochastic processes and chance effects have a greater effect on the evolution of small populations (Frankham et al., 2017), and the minimum count for most evaluated populations was less than 500 individuals. Thus, random genetic drift could result in differences in average pairwise kinship among small populations with similar attributes.

Augmentation was associated with lower average pairwise kinship

As the number of animals received in augmentations increased, average pairwise kinship within populations decreased, as expected. Thus, translocations to supplement existing populations were generally effective in minimizing inbreeding. A similar association was observed in other bighorn sheep populations between heterozygosity and the number of translocated animals received (Jahner et al., 2018). Gene flow via translocations can also potentially enhance bighorn sheep juvenile survival in the generation following augmentation (Olson et al., 2012; Poirier et al., 2018). Increase in genetic diversity following augmentation has been documented for bighorn sheep and other species, such as the Florida panther (*Puma concolor coryi*) and Swedish adder (*Vipera berus*; Hogg et al., 2006; Johnson et al., 2010; Madsen et al., 1999). This result is consistent with genetic management guidelines that suggest a certain number of migrants (i.e., 1–10 animals per generation) are needed to maintain genetic variation (Mills & Allendorf, 1996; Wright, 1931). Pearson's correlation coefficients for variables that described augmentation history ranged from 0.90 to 0.94. This similarity prevented us from disentangling which aspect of augmentation history most influenced average pairwise kinship (Appendix S1: Tables S1 and S4). Future research could seek to differentiate between the influences of the number of animals moved, number of augmentation events, and number of population sources.

When planning to increase connectivity between populations using either habitat modifications or augmentation, managers can identify optimal source populations by evaluating the level of mean kinship between populations to minimize inbreeding and evaluating other considerations, such as disease and local

adaptation concerns (Ewen et al., 2012; Finger et al., 2011; Flesch et al., 2020; Frankham et al., 2017; Garbe et al., 2016). There has been great concern that bighorn sheep augmentation efforts may spread respiratory pathogens novel to the recipient population or disrupt a stable relationship between resident pathogens and the host population, which could result in epizootic events (Aiello et al., 2014; Cassirer et al., 2016; Cunningham, 1996). Disease concerns have resulted in widespread testing of bighorn sheep herds for a variety of pathogen species and strains associated with epizootic events, but the results of these tests can be inconclusive due to imperfect detection probability and lack of understanding as to what individual test results imply for the overall population (Butler et al., 2017, 2018; Paterson et al., 2020). Thus, when considering translocations between bighorn sheep populations, agencies could recognize the risk of mixing pathogens, consider how to mitigate this risk, and monitor outcomes for adaptive management.

Individual attributes of translocated animals may impact their potential influence on the recipient population's mean kinship. Translocations of a small number of males to bighorn sheep populations may not always be successful, as Flesch et al. (2020) did not find genetic evidence of augmentations of two males into two mainland study populations (Spanish Peaks and Stillwater). Thus, infusion of only a few males may be most useful in island populations with a limited number of existing males, rather than mainland populations, where males may wander away from the targeted recipient population during the breeding season (Geist, 1971). Genomic evaluation of other bighorn sheep populations indicated that augmentations of larger female groups may be more effective in genetically contributing to the target population, similar to translocated desert tortoises (*Gopherus agassizii*; Flesch et al., 2020; Mulder et al., 2017). Longitudinal genetic studies over time could further evaluate the long-term genetic effects of augmentations in island wildlife populations (Hedrick et al., 2014).

Connectivity via dispersal was the most important predictor of average pairwise kinship

Populations with high connectivity had lower average pairwise kinship than isolated populations. Thus, natural dispersal of breeders between populations, likely resulting in gene flow, was the most important influence in minimizing average pairwise kinship. This result is consistent with a previous study that indicated reduction of natural gene flow between bighorn sheep populations due to highways can result in decreased genetic diversity

(Epps et al., 2005). Gene flow was also more important to genetic diversity than decline in population size for Atlantic salmon (*Salmo salar*; Consuegra et al., 2005). Conversely, Wang et al. (2017) found that island size, population size, and time since isolation were more important drivers of genetic diversity of the black-spotted pond frog (*Pelophylax nigromaculatus*) than distance to other populations that could facilitate connectivity, emphasizing the importance of evaluating this research question in different systems. When managing bighorn sheep populations, enabling natural dispersal and gene flow may be of highest priority to avoid negative impacts of inbreeding, when this approach does not conflict with other management considerations. Our study considered categorical variables to describe connectivity based on geographic distance, hunter harvest data, and GPS collar data (Appendix S1: Table S3), but future research could more specifically evaluate the relationship between the number of animals dispersing and average pairwise kinship to establish a more specific target level of natural gene flow between populations.

All examined populations had average pairwise kinship values lower than the concerning mean kinship threshold of 0.1 (Frankham et al., 2017), but this threshold may vary slightly based on the genetic marker used and the species life history. Average pairwise kinship estimates evaluated in this study were a slight underestimate of mean kinship, due to our exclusion of self-kinship values. However, we expect that only one evaluated population (Middle Missouri) might have a mean kinship value of greater than 0.1 with self-kinships included in the calculation. Seven populations had average pairwise kinship values greater than 0.0442, implying a third-degree relationship on average (Manichaikul et al., 2010). This may be due to the fact that populations with common ancestry can have higher than expected levels of kinship, as a result of cumulative effects over time. We did not have pedigree data to evaluate this further, preventing a more thorough interpretation of kinship values beyond relative comparisons between populations. In addition, there is no known approach to account for all of the factors that influence average pairwise kinship to provide an accurate measure of uncertainty for the estimates.

Mean kinship estimates represent the expected level of inbreeding in the next generation but do not necessarily provide information regarding adaptive variation in a population. While greater inbreeding may be correlated with lower disease resistance (Balloux et al., 2004), immune response can also be associated with specific loci (Acevedo-Whitehouse et al., 2003, 2006; Alves et al., 2019). In this case, augmentations that add genetic diversity to populations may not immediately improve

survival of an epizootic event (Boyce et al., 2011; Ewen et al., 2012). High genetic diversity in a population is still an important management goal, as it enables evolution of more resilient populations through natural selection of individuals that survive epizootic events (Alves et al., 2019). Even though gene flow can be beneficial to population genetic diversity, extremely high levels of gene flow can result in the loss of alleles associated with local adaptation (Kovach et al., 2015; Lenormand, 2002). Thus, evaluating if populations are adapted to similar environmental conditions can be useful and translocations should involve moving a minimum number of animals needed to minimize inbreeding in the population (Frankham et al., 2017; Wiedmann & Sargeant, 2014).

CONCLUSIONS

Our study found that greater connectivity, number of animals received in augmentations, and minimum count were correlated with lower average pairwise kinship at the population level, and origin was less important. These results also demonstrated that HD Ovine SNP chip genotype data and the kinship metric can be used to effectively evaluate the level of expected inbreeding in the next generation of bighorn sheep populations. In addition, reproducibility of the same genetic markers assessed through a SNP chip allows for genotype and kinship data to be compared across populations and different sampling times. However, the HD Ovine SNP chip included many monomorphic SNPs for bighorn sheep, and future research could involve developing a SNP chip with variable markers specific to bighorn sheep. In addition, other studies have found that kinship is effective for informing translocation decisions in plants and animals to maximize genetic diversity and minimize inbreeding (Finger et al., 2011; Garbe et al., 2016).

This study also highlights which herd attributes may result in inbreeding at concerning levels. Thus, managers can prioritize herds of highest genetic concern based on kinship or population attributes ranked in importance by our analysis if genetic data are not available. This approach would provide managers with additional information to weigh the costs and benefits of augmentation efforts or management to promote natural dispersal. For example, our results suggested that populations that are isolated with no natural connectivity or augmentations have the highest average pairwise kinship, regardless of origin. However, we were not able to directly assess the role of genetic attributes of translocated animals or their source populations at the time of a reintroduction or augmentation event, because high-quality genetic samples were not available from those capture events. In addition,

we were not able to directly evaluate populations that were extirpated. Whenever animals are captured for translocation or other studies, we suggest that managers collect high-quality genetic samples to enable longitudinal studies that can evaluate the success of these measures and genetic effects in greater detail over time. For example, genetic samples of founders in reintroductions and periodic sampling of the populations would be greatly informative. A long-term monitoring plan that includes assessment and follow-up regarding population genetics and other population attributes would serve to inform future restoration efforts.

An extensive history of augmentation efforts likely played a role in limiting inbreeding in many of the evaluated bighorn sheep populations, such that the observed range of average pairwise kinship values was not at concerning levels. However, there are new concerns that translocations could transmit novel pathogen species and genetic variants associated with respiratory disease from source to recipient populations, which could increase the potential for epizootic events and poor recruitment in populations after augmentation. Due to awareness of the risk of inadvertent spread of pathogens, managers are now more cautious about moving animals. To enhance species restoration, it will be important to learn more about how to address both disease and inbreeding concerns in translocation planning and apply adaptive management by monitoring consequences when augmenting or establishing populations.

Kinship and other genomic analyses alone cannot dictate augmentation and management decisions, as species life history, population demography, disease, and habitat concerns are also important (Van Dyke, 2008). However, increasing the role of genomic analyses and considerations in wildlife management would help biologists identify what circumstances result in genetic attributes that can limit the distribution or population growth for species of conservation concern (Van Dyke, 2008). Thus, our study can help inform genetic management and conservation of other fragmented wild populations to retain genetic diversity for population persistence and evolution into the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Genotype data (Flesch et al., 2019) are available from Figshare: http://doi.org/10.6084/m9.figshare.10394711.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.