Received: 29 August 2023 | Revised: 19 November 2023 | Accepted: 24 November 2023
DOI: 10.1111/mec.17237

ORIGINAL ARTICLE

Effects of hunting on mating, relatedness, and genetic diversity in a puma population

John A. Erwin[1] | Kenneth A. Logan[2] | Daryl R. Trumbo[3] | W. Chris Funk[4,5] | Melanie Culver[6,7]

[1]Florida International University College of Law, Miami, Florida, USA
[2]Colorado Parks and Wildlife, Montrose, Colorado, USA
[3]Department of Biology, Colorado State University Pueblo, Pueblo, Colorado, USA
[4]Department of Biology, Colorado State University, Fort Collins, Colorado, USA
[5]Graduate Degree Program in Ecology, Colorado State University, Fort Collins, Colorado, USA
[6]U.S. Geological Survey, Arizona Cooperative Fish and Wildlife Research Unit, University of Arizona, Tucson, Arizona, USA
[7]School of Natural Resources and the Environment, University of Arizona, Tucson, Arizona, USA

Correspondence
John A. Erwin, Florida International University College of Law, 11200 SW 8th Street, Miami, FL 33199, USA.
Email: jerwin@fiu.edu

Funding information
Summerlee Foundation

Handling Editor: Andrew DeWoody

Abstract

Hunting mortality can affect population abundance, demography, patterns of dispersal and philopatry, breeding, and genetic diversity. We investigated the effects of hunting on the reproduction and genetic diversity in a puma population in western Colorado, USA. We genotyped over 11,000 single nucleotide polymorphisms (SNPs), using double-digest, restriction site-associated DNA sequencing (ddRADseq) in 291 tissue samples collected as part of a study on the effects of hunting on puma population abundance and demography in Colorado from 2004 to 2014. The study was designed with a reference period (years 1–5), during which hunting was suspended, followed by a treatment period (years 6–10), in which hunting was reinstated. Our objectives were to examine the effects of hunting on: (1) paternity and male reproductive success; (2) the relatedness between pumas within the population, and (3) genetic diversity. We found that hunting reduced the average age of male breeders. The number of unique fathers siring litters increased each year without hunting and decreased each year during the hunting period. Mated pairs were generally unrelated during both time periods, and females were more closely related than males. Hunting was also associated with increased relatedness among males and decreased relatedness among females in the population. Finally, genetic diversity increased during the period without hunting and decreased each year when hunting was present. This study demonstrates the utility of merging demographic data with large-scale genomic datasets in order to better understand the consequences of management actions. Specifically, we believe that this study highlights the need for long-term experimental research in which hunting mortality is manipulated, including at least one non-harvested control population, as part of a broader adaptive, zone management scheme.

KEYWORDS
dispersal, genetic diversity, harvest, predator hunting, *Puma concolor*, relatedness

1 | INTRODUCTION

When setting harvest targets for carnivore management, state wildlife managers are tasked with balancing competing interests, including conservation, public opinion, hunting opportunity, reducing predation on wild and domestic animals, and mitigating human-carnivore conflict (Logan, 2019; Whittaker, 2011). Under the North American Model of Wildlife Conservation, the organization and direction of wildlife harvest must be driven by science (Organ et al., 2012). As such, managers and policymakers have long

 © 2023 John Wiley & Sons Ltd.

examined the effects of anthropogenic mortality on numerical responses of populations to harvest, including changes in age and sex structures (Fryxell et al., 2010; Jensen, 1996). Recently, an increased emphasis has been placed on understanding the effects of harvest on social and mating structures (Frank et al., 2018, 2021; Naude et al., 2020), as well as genetic diversity and other metrics of genetic vulnerability (Ausband, 2022; Ausband & Waits, 2020; Frank et al., 2021; Harris et al., 2002).

Harvest can reduce animal abundance while altering social systems and population structure. These effects are especially pronounced when harvest is highly selective (Leclerc et al., 2019; Leclerc, Zedrosser, et al., 2017; Milner et al., 2007) and mortality is additive, as is the case with most puma hunting (Cooley et al., 2009; Elbroch et al., 2020; Logan & Runge, 2021; Wolfe et al., 2015). For example, harvest creates spatial vacancies by removing established males, releasing surviving individual from competition or other social constraints (Frank et al., 2018; Loveridge et al., 2016). This destabilization of social structure can also result in an increase of intraspecific conflict and infanticide (Gosselin et al., 2017; Leclerc, Frank, et al., 2017; Leclerc, Zedrosser, et al., 2017; Loveridge et al., 2007; Whitman et al., 2004). Alternatively, in some species, harvest restrictions designed to protect females with dependent young can actually promote slower life histories by increasing selective pressure on mothers to stay with their cubs for longer periods of time (van de Walle et al., 2018). In some species, selective harvest based on sex-linked phenotypes, such as horn size or body weight, has been associated with a reduction in the value of those traits (Allendorf et al., 2008; Allendorf & Hard, 2009; Coltman et al., 2003; Festa-Bianchet, 2017; Festa-Bianchet & Mysterud, 2018; Mysterud, 2011; Pigeon et al., 2016; Uusi-Heikkilä et al., 2015).

High levels of harvest-associated mortality can also reduce dispersal and modify patterns of philopatry and kin-clustering (Fattebert et al., 2015; Loveridge et al., 2007; Naude et al., 2020). Sex-biased dispersal is believed to be the primary mechanism of inbreeding avoidance in most species of polygamous mammals (Greenwood, 1980), including pumas (Biek et al., 2006; Laundré & Hernández, 2003; Logan & Sweanor, 2001). For example, female pumas are more philopatric and generally disperse significantly less distance than males (Biek et al., 2006; Logan & Runge, 2021; Newby et al., 2013; Stoner et al., 2013; Sweanor et al., 2000). However, significant harvest can cause dispersal patterns to break down, increasing kin-clustering, male philopatry, and the risk of inbreeding depression (Blyton et al., 2015; Naude et al., 2020; Perrin & Mazalov, 2000). Similar patterns of relatedness and male philopatry have also been seen in populations where dispersal and immigration are severely limited by habitat fragmentation and urbanization (de Oliveira et al., 2023; Gustafson et al., 2017, 2019; Riley et al., 2014; Wultsch et al., 2023).

From a genetic standpoint, harvest can reduce effective population size and decrease migration, causing a loss of genetic diversity in subpopulations (Allendorf et al., 2008; Harris et al., 2002). Decline in genetic variation can reduce individual fitness and hamper long-term potential to evolve (Allendorf et al., 2008). Removal of established adult animals may allow subadults to settle locally rather than disperse, reducing gene flow between subpopulations and increasing inbreeding (Blyton et al., 2015). On the other hand, harvest can increase genetic diversity in some cases; in species, like pumas, where breeding opportunities are dominated by just a few individuals, harvest of these individuals may allow others to breed and contribute to the gene pool (Ausband & Waits, 2020; Frank et al., 2021; Harris et al., 2002).

To further examine how harvest might affect the breeding structure and genetic diversity of a population of pumas, we generated genetic data from tissue samples collected as part of a study on puma demography and hunting (Logan & Runge, 2021). From 2004 to 2014, researchers with Colorado Parks and Wildlife investigated the effects of regulated hunting on the abundance and demography of a puma population on the Uncompahgre Plateau in western Colorado, USA (Logan & Runge, 2021). That study was designed with a five-year reference period, where puma hunting was suspended, followed by a 5-year treatment period, when regulated hunting resumed. During the treatment period, the harvest quota was set at eight pumas per year (a target of 15% of estimated independent pumas on the study area) for the first 3 years, and then reduced to five pumas per year (11% of estimated independent pumas) during years 4 and 5. Prior to the beginning of this study, pumas were subjected to annual hunting, with an average of 12 pumas killed on the study area each year (Logan & Runge, 2021). During the reference period, independent puma abundance more than doubled and survival of adults was high. When hunting was reinstated, annual harvest rates, averaging an estimated 22% of marked independent pumas (i.e. adults and subadults) at the population scale, resulted in a 35% decline in abundance in the study area over 3 years. Survival for adult and subadult males declined by more than half, while survival of adult females declined by 12%. Subadult female survival, however, was constant in both periods. Hunting-caused deaths were not fully compensated by reproduction and immigration. The hunting quota was not sex-specific; however, hunters were highly selective for males. Hunters normally hunted using dogs, allowing hunters to usually determine the sex of the treed puma. Males made up 69% of the total harvest on the study area, and adult males declined in abundance by 59% after three hunting seasons. Furthermore, the age distribution for independent pumas became skewed towards younger individuals when the population was hunted (Logan & Runge, 2021).

In this study, our objectives were to examine the effects of hunting on: (1) paternity and male reproductive success; (2) the relatedness between the pumas within the population and within mated pairs, and (3) genetic diversity. To address these objectives, here, we harnessed the power of genome-wide markers to uncover the effects of hunting on a natural population of carnivores. By manipulating sport hunting as an independent variable, we were able to more directly explore the behavioural and genetic consequences of managed harvest than purely descriptive studies have achieved in the past. This 'science via regulation' experimental design—combining demographic insights collected in the field with genomic analyses to explore ecological, behavioural, and genetic changes to

populations in response to the modification of a regulatory regime by a governmental agency—serves as a model for future work aimed at understanding the effects of management actions on wild animal populations.

2 | MATERIALS AND METHODS

2.1 | Sampling and sequencing

We collected blood and tissue samples from pumas captured and harvested between 2004 and 2014 on a 2996 km^2 study area on the Uncompahgre Plateau and its surrounding area in southwestern Colorado (Figure 1; see Logan & Runge, 2021 for details). Pumas were captured by Colorado Parks and Wildlife Department personnel in accordance with approved CPW Animal Care and Use Committee (ACUC) capture and handling protocols (ACUC file #08-2004 and ACUC protocol #03-2007). Pumas were captured using a variety of methods including trained dogs, cage traps, and by hand for litters of cubs. Life history information including sex, life stage (i.e. juvenile, subadult, or adult), and any observed familial associations were recorded at the time of capture for each individual. The ages of pumas were estimated by the gum-line recession model (Laundré et al., 2000) and dental characteristics of known-age pumas. While live animals were restrained under anaesthesia, a 6 mm^2 biopsy punch was used to make a hole in the pinnas into which ear tags were inserted. When an animal was unable to be safely captured because of dangerous trees or cliffs, a Pneu Dart Type P biopsy dart fired from a pneumatic pistol (Pneu Dart, Inc., Williamsport, PA) was used to collect a skin sample. Additionally, samples from each puma harvested on the study area and surrounding Game Management Units (GMUs) were acquired by using 6 mm^2 biopsy punches or excising a small piece of muscle or integument (about 5 mm^2). Each tissue sample was deposited into a plastic vial containing 70% ethanol, exclusively marked to identify the individual, and stored in a freezer at −20°C until extraction.

We extracted DNA using the Qiagen DNeasy blood and tissue extraction kit following the manufacturer's protocol (Qiagen Ltd., Valencia, CA). Samples with low DNA yield were extracted a second time and concentrated using Microcon centrifugal filters (Merck KGaA, Darmstadt, Germany).

We prepared libraries for genotyping using the double-digest restriction-site associated DNA sequencing (ddRADseq) method described in Peterson et al. (2012). The restriction enzymes, EcoRI-HF (6 bp cut site) and NlaIII (4 bp cut site), and the target fragment size range of 300–400 bps (excluding adapters) were chosen based on optimization performed by Trumbo et al. (2019). Additionally, size

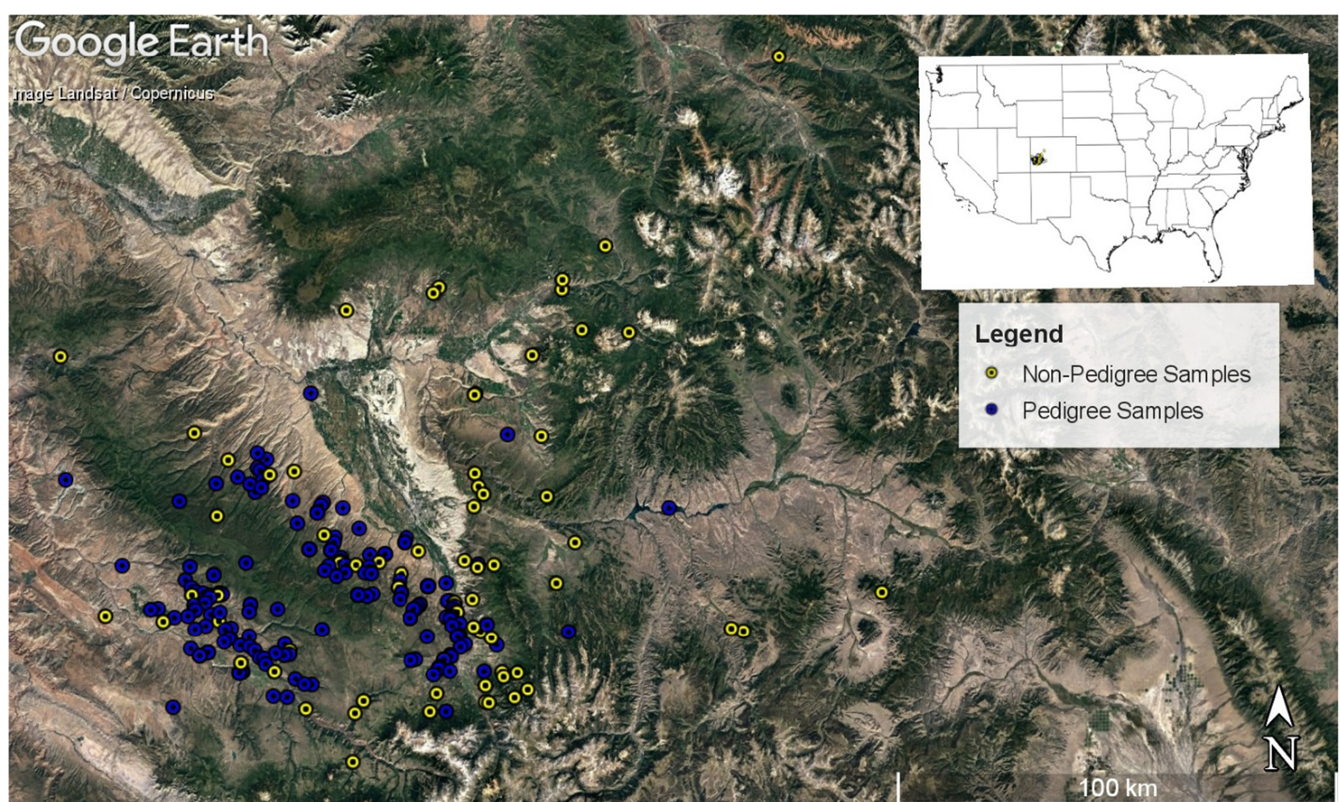


FIGURE 1 Representative map of individual pumas genotyped from the Uncompahgre Plateau study area and surrounding game management units (GMUs) during the study period (2004–2014). Blue dots represent samples that were included in the pedigree and yellow dots were samples that were not linked to the population via the pedigree. Only individuals from the pedigree were included in analyses on mating, relatedness, and genetic diversity.

selection was performed using either a Pippin Prep or a Blue Pippin with internal standards and a 100–600 bp 2% agarose gel cassette (Sage Scientific; Beverly, MA). Polymerase chain reaction (PCR) was performed for 12 cycles and six reactions for each pool of individuals.

Libraries contained between 58 and 74 samples, to maximize multiplexing while aiming for >12× coverage per locus, and were multiplexed using the flex adaptors from Peterson et al. (2012). Each library contained at least three within library and three between library sample replicates to help calculate error rates during downstream analyses. One library was sequenced on a HiSeq4000 machine using 100 bp single-end sequencing at the University of Oregon Genomics Facility (https://gc3f.uoregon.edu/; Eugene, OR). Three additional libraries were sequenced on a HiSeq4000 machine using 150 bp paired-end sequencing by Novogene (https://en.novogene.com/; Beijing, China). Additionally, raw sequences were downloaded from Dryad (https://datadryad.org/stash/dataset/doi:10.5061/dryad.12jm63xsr) for relevant puma samples that were previously sequenced as part of an earlier study looking at the effects of urbanization on puma gene flow (Trumbo et al., 2019). We genotyped a total of 343 samples, including duplicates and 61 sequences from Trumbo et al. (2019).

2.2 | Bioinformatic pipeline and filtering

We evaluated read quality for each lane of sequencing using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We demultiplexed each lane of sequencing into two separate pools, based on the barcode added during the PCR step, using BBMap v. 38.87 (https://sourceforge.net/projects/bbmap/). We used the program process_radtags in Stacks v. 2.4 (Catchen et al., 2013; Rochette et al., 2019) to further demultiplex each pool of reads into separate individuals and to trim and filter the reads.

In order to combine the 150 bp paired-end reads produced in our second, third, and fourth libraries with the 100 bp single-end reads produced in our first library and in Trumbo et al. (2019), reverse reads were discarded prior to demultiplexing with process_radtags. Additionally, all reads were trimmed down to 95 base-pairs using Trimmomatic v. 0.39 (Bolger et al., 2014). Due to high sequencing error in the 96th position, Trumbo et al. (2019) removed that site from their analyses; in order to incorporate the individuals sequenced for that project, we therefore trimmed all reads down to 95 base pairs. Also, for our final dataset, replicate individual reads were concatenated prior to alignment.

We then used the BWA-Mem algorithm (Li, 2013) to align our reads to a recently published puma reference genome (Ochoa et al., 2019; Saremi et al., 2019). We used SAMtools (Li et al., 2009) to sort the reads and convert them to bam files. Then we used the ref_map.pl pipeline in Stacks v. 2.4 (Rochette et al., 2019) to assemble loci, call SNPs, and generate population-level summary statistics. The --write-random-snp flag was used to ensure only one randomly chosen SNP per locus was retained. We exported the SNP matrix with the populations program in Stacks v. 2.4 (Rochette et al., 2019).

The majority of SNP filtering was performed in Plink v. 1.9 (Purcell et al., 2007). We created two datasets with varying levels of filtering stringency based on the downstream analyses we intended to run. For our full dataset, used in all analyses other than our paternity analyses, we removed loci that were sequenced in less than 75% of individuals (-- geno 0.25). Then we removed samples that had more than 50% missing data (-- mind 0.5). Finally, we filtered out all loci with a minor allele frequency less than 0.01 (-- maf 0.01).

For our paternity analyses, we applied overall stricter filtering parameters based on other similar SNP-based relatedness studies (Andrews et al., 2018; vonHoldt et al., 2020). Specifically, we removed loci that were sequenced in less than 90% of individuals (-- geno 0.1), samples that had more than 70% of missing data (-- mind 0.7), and loci with a minor allele frequency less than 0.3 (-- maf 0.3). Additionally, we removed loci that were not in Hardy–Weinberg proportions (--hwe 0.005 using the p midpoint adjustment). Finally, we used Plink to create a blacklist and subsequently remove loci that were found to be in statistical linkage disequilibrium ($r > .2$) using a proxy filter (--indep-pairwise 50 5 0.2).

While the majority of our samples were collected from collared and marked individuals, some samples were collected via biodart and hunter harvest. As such, there was a possibility that some individuals were unknowingly sequenced multiple times. We screened for these unintentional duplicates using the detect_duplicate_genomes function in the radiator R package (Gosselin et al., 2020). These duplicates were then blacklisted and removed via Plink. We additionally used radiator to estimate the genotyping error rate and heterozygote miscall rate.

2.3 | Paternity and relatedness analyses

To aid in the paternity analyses, data tables for each year of the study (2004–2014) were created detailing which pumas were alive, reproductively mature, and not known to have emigrated from the study area. Pumas were binned by their birth year and were considered to be potential parents if they were at least one calendar year older. Pumas typically reach sexual maturity between 18 and 24 months old (Logan & Sweanor, 2001), making a single calendar year a possible minimum age difference.

We used the R package Sequoia (Huisman, 2017) to determine paternity and to construct a pedigree using the reduced SNP set filtered specifically for paternity analyses. Sequoia assigns parents, clusters half-siblings sharing an unsampled 'dummy' parent, and assigns grandparents to half-sibships in order to build a multigenerational pedigree. Birth years were input along with genotype data to aid in the parentage assessment. Based on our analysis of the duplicate individuals, the heterozygote miscall rate calculated in radiator, and known error rates in RAD-seq studies (Bresadola et al., 2020; Luca et al., 2011; Mastretta-Yanes et al., 2015), we set the genotyping error rate at 5×10^{-2}. All mated pairs were compared against field data to ensure that all pairings were biologically, spatially, and temporally possible. The field data included

radio-telemetry locations of marked pumas, harvest locations of non-marked pumas, and capture locations of non-marked pumas that were bio-darted. Using this field data, we also matched dummy individuals, when possible, with pumas that were captured and radio-monitored but were not successfully genotyped. Based on this pedigree and the estimated birth dates for each individual, we separated births into discreet litters. We used these litters to examine male reproductive success by analysing the number of litters each male sired each year and the age of that male when the litter was born. We additionally qualitatively plotted networks of all pumas included in our pedigree using NETDRAW 2.119 (Borgatti, 2002). A node (the polygons) represents each individual. Edges (the lines) connect either parents and offspring or pairs that successfully mated.

We used the R package RELATED (Pew et al., 2014) to calculate pairwise relatedness coefficients between all pairs of pumas that temporally coexisted. Using the coancestry function, we calculated relatedness values using both the full SNP dataset and the more strictly filtered dataset from the paternity analyses. We chose the dyadic likelihood estimator (dyadml = 1) with allowance for inbreeding (allow.inbreeding = TRUE) described by Milligan (2003). We selected this metric due to its inbreeding allowance and low error rate in recent studies that used SNP datasets (Hall et al., 2020; vonHoldt et al., 2020). Additionally, we used the coancestry function to calculate five different moment-based relatedness estimators, in order to include a second estimator with the range of −1 to 1. We ultimately chose the Wang relatedness estimator (Wang, 2002). This estimator produced relatedness values for kinship classes closest to their expected values, with acceptably low variation, and has proven to have low sensitivity to error and low sampling variance that decreases asymptotically with increasing numbers of loci (Blouin, 2003; Naude et al., 2020). A two-tailed, one sample *t*-test was used to determine if mated pair values were significantly different from zero, and we used two-tailed Wilcoxon rank-sum tests to test hypotheses on relatedness across the two study periods (Onorato et al., 2011).

2.4 | Population genetic analyses

To analyse how genetic diversity has changed over time, we grouped individuals into cohorts based on birth year (Juarez et al., 2016). We utilized a two-year sliding window approach to avoid including parents and offspring in the same cohort. We removed any sample that did not have at least one link in the reconstructed pedigree. We created separate population maps for each cohort and then calculated summary statistics based on the full SNP dataset. We used the POPULATIONS program in STACKS to calculate observed heterozygosity (H_o), expected heterozygosity (H_e), and the inbreeding coefficient (F_{IS}) for each generational cohort. We estimated allelic richness (A_r) for each cohort with HP-RARE 1.0 (Kalinowski, 2005), which uses rarefaction to correct for differences in sample size. We performed a one-way ANOVA on each summary statistic and performed Tukey–Kramer HSD post-hoc tests on each pair of means to determine significance.

We also estimated the effective number of breeders for each cohort. When applied to a single cohort, effective population size (N_e) can be used as a metric to estimate the effective number of breeding individuals (N_B) that are genetically contributing to the population (Schwartz et al., 1998; Waples, 2005). We calculated N_B using the linkage disequilibrium method as implemented in NEESTIMATOR V.2.1 (Do et al., 2014). We used the minimum allele frequency of 0.05 and the non-parametric jackknifed 95% confidence intervals for all subsequent analyses of N_B. As large numbers of SNP markers are known to suffer from a downward bias when calculating effective population size, we adjusted the estimates of N_B and confidence intervals based on the haploid number of chromosomes (19; Hsu et al., 1963) per equation 1(a) in Waples et al. (2016).

3 | RESULTS

3.1 | Genotyping and filtering SNP matrices

After calling SNPs using the REF_MAP pipeline in STACKS, our matrix contained 207,635 SNPs and 309 samples. From here, we developed two separate SNP matrices for use in different analyses: our full dataset and our paternity analyses dataset (see Table S1). For our full dataset (Table S1), we retained 43,334 SNPs, after removing loci that were sequenced in less than 75% of individuals. Then we removed individuals with more than 50% missing data, resulting in 11 individuals being lost. We also filtered out all loci with a minor allele frequency less than 0.01, yielding an SNP matrix of 11,377 SNPs across 298 individuals. After removing any duplicate individuals, our final sample size (*N*) was 291 individual pumas.

For our paternity analyses dataset (Table S1), we removed loci that were sequenced in less than 90% of individuals and retained 20,412 SNPs. Then we removed individuals with more than 70% missing data, resulting in seven individuals being lost. We filtered out all loci with a minor allele frequency less than 0.3. After additional filtering for loci out of Hardy–Weinberg proportions and to account for potential linkage disequilibrium, we retained a final SNP matrix of 743 SNPs. This consisted of 295 individuals, after discovered duplicate individuals were removed.

3.2 | Paternity

Using SEQUOIA, we assigned fathers to 156 genotyped individuals (52.3%) and mothers to 141 genotyped individuals (47.3%); when dummy individuals (parents that were not sequenced but can be inferred by the program) were included, we were able to assign fathers for 188 pumas (62.5%) and mothers to 180 pumas (59.8%) (Figure S1a). All maternal parentage assignments were concordant with field data from captured litters and collared mothers. Field data was used to match four of the dummy individuals with pumas that were captured and radio-monitored but were not successfully genotyped.

Sequoia also assigned individuals to full-sibships (groups of individuals that share the same parents) and half-sibships (groups of individuals that share either a mother or father) (Figure S1b). The largest full-sibship was seven individuals (from 2 separate litters), while half-sibships included up to 12 individuals for maternal half-sibships and 26 individuals for paternal half-sibships.

When we qualitatively plotted the pedigree as a network, we found that all of the pumas that were included within the pedigree were part of the same connected network, with the exception of one pair of siblings (Figure 2). As such, we used this network to define our population, and we removed an additional 71 genotyped individuals from all downstream analyses. Nearly all of the individuals removed were from hunter harvest in adjacent game management units, and they were not linked by the pedigree to our study population.

When we restricted the network to mated pairs only, two separate networks emerged (Figure 3a). We plotted the individuals in each network onto a map using each puma's sampling location. The clusters were found to localize on each slope (east or west) of the Uncompahgre Plateau (Figure 3b). One individual from the eastern slope network was captured on the western slope. Upon further investigation, this individual, F40, was only captured once, as a 1-month-old kitten. Her mate and offspring were both captured on the eastern slope, not the western slope where she was born, suggesting she likely bred on the same slope as the rest of her breeding network. Additionally, at least six females and three males were born on one slope and dispersed to the opposite slope where they reproduced.

Using this reconstructed pedigree, we matched both parents to 73 separate litters born to 62 different breeding pairs. For 13 additional litters, we determined one parent paired with a dummy individual that we were not able to match back to a collared puma using field data (seven litters had a dummy father and six litters had a dummy mother). Forty-two litters were born during the reference period, and 43 were born during the treatment period (Table S2). We identified 22 unique fathers across the full study (Figure S2): 12 sired offspring during the reference period and 14 sired offspring during the treatment period. Four males sired litters in both the reference and treatment periods. There were 40 unique mothers identified across the study: 20 of which birthed litters in the reference period and 31 birthed litters in the treatment period. We did not find instances of multiple paternity in any of the litters.

The number of litters born per year fluctuated from year to year, averaging 9.13 litters a year (SD = 1.45) exclusive of the first and last study years when sample sizes were low. The average litter size was 1.98 kittens (SD = 0.98). Individual successful males sired 3.30 litters (SD = 3.25) on average in the reference period and 2.71 litters (SD = 2.15) on average in the treatment period, though this difference was non-significant ($t_{19} = 0.54$, $p = .596$). Additionally, we

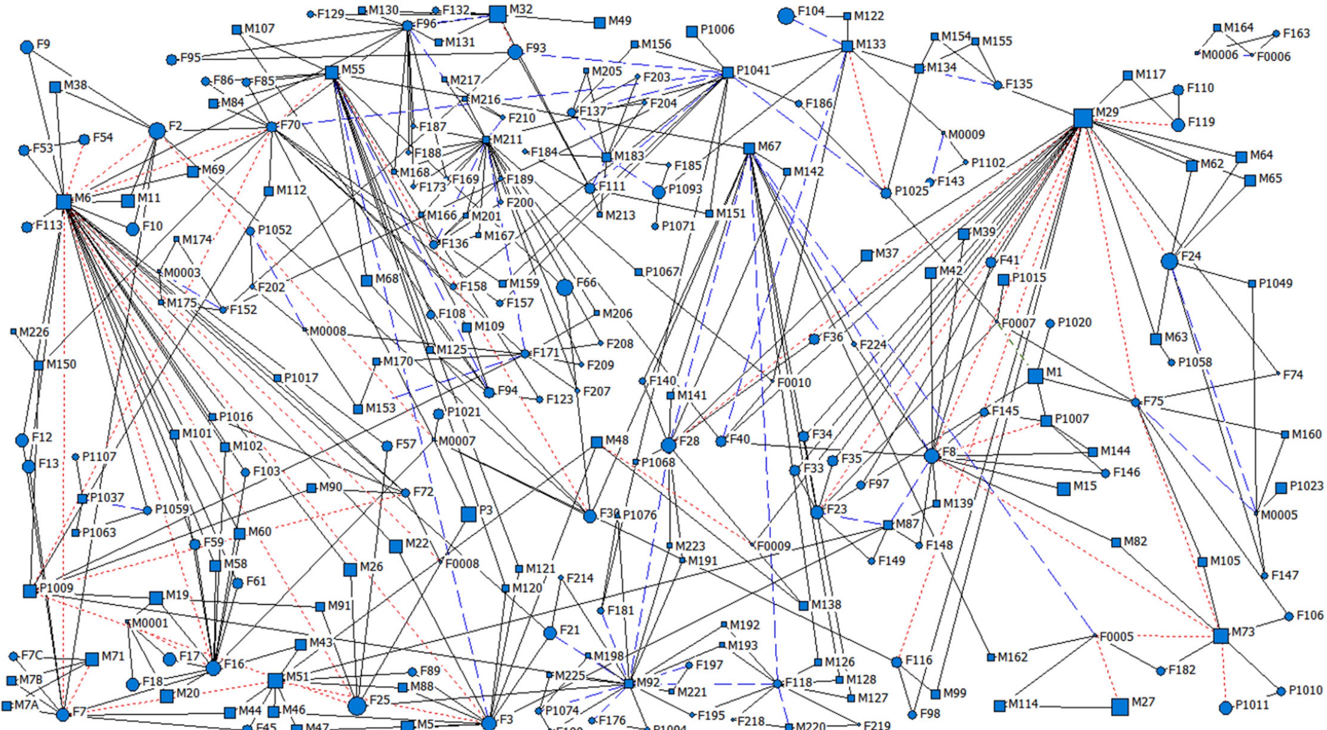
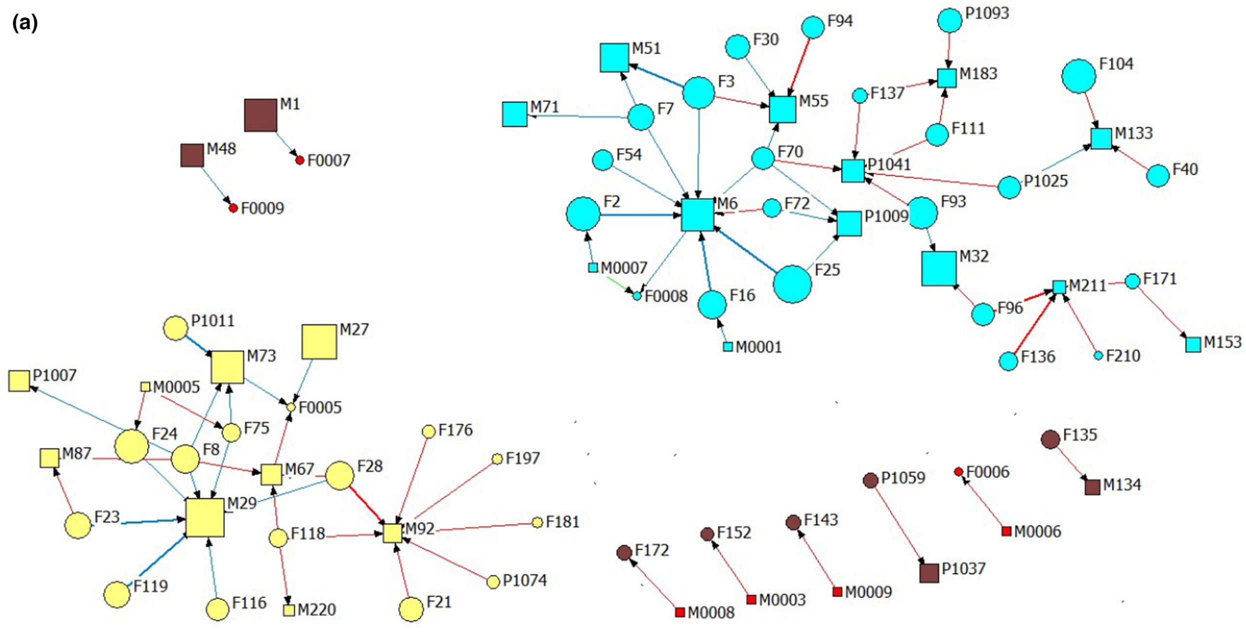


FIGURE 2 Social network was drawn in Netdraw based on the pedigree generated in sequoia. Males are represented with squares and females with circles. Individuals of unknown sex are depicted as triangles. The node size depicts birth year, with earlier birth years being represented by larger nodes. Individuals that are affiliated are connected by lines. Solid black lines represent parent-offspring relationships. Dotted red lines connect pairs that mated during the reference period, and dashed blue lines represent pairs that mated during the treatment period. The dot-dash green line represents a pair that mated prior to the study period. Line distances have no meaning.



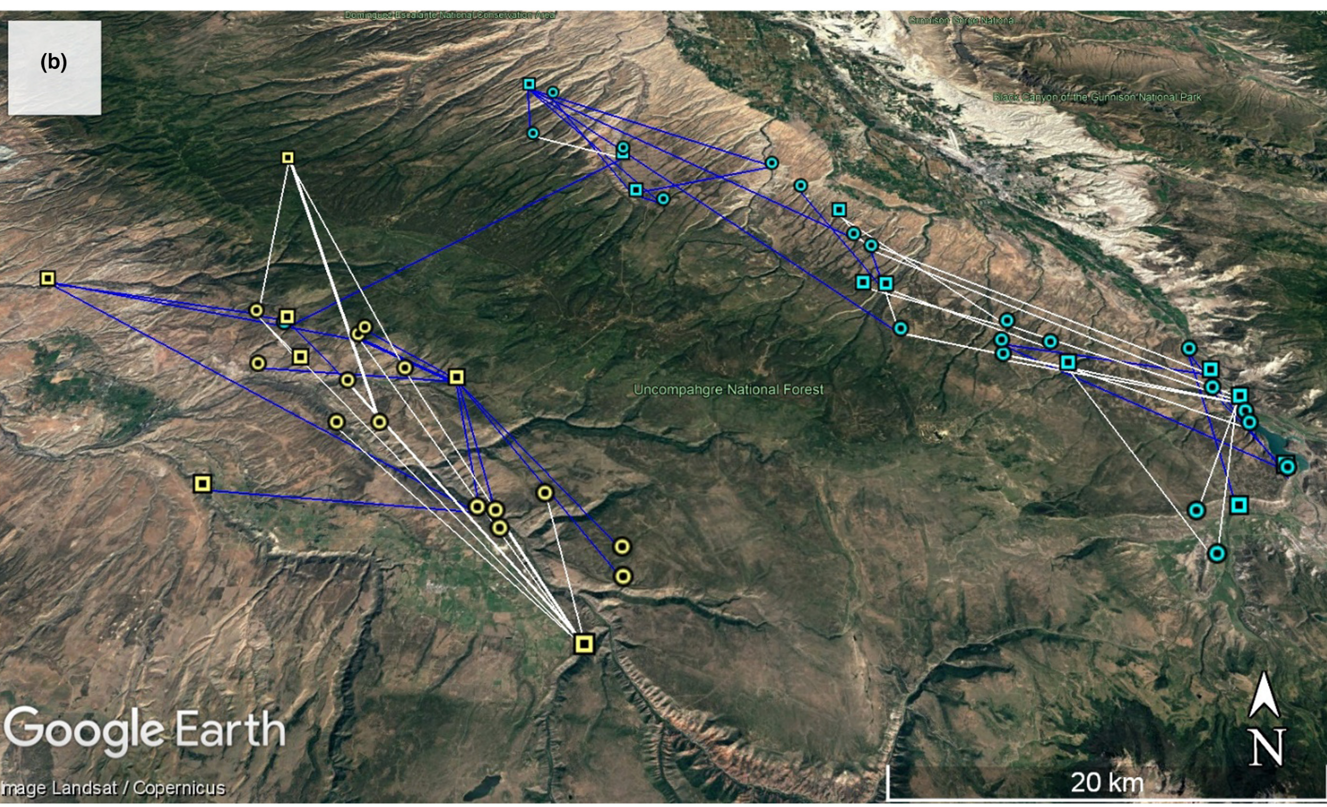


FIGURE 3 Panel (a) depicts networks of mated pairs. As with Figure 2, red lines connect pairs that mated during the reference period. Blue lines connect pairs that mated during the treatment period. The green line represents a pair that mated prior to the study. The two large networks are coloured separately as yellow and blue nodes. Red nodes are dummy individuals created in SEQUOIA and brown nodes are individuals not connected to either of the large networks. Node shape denotes sex—males are squares and females are circles. Node size represents birth year, with larger nodes for earlier birth years. Panel (b) is a map of the two large breeding networks identified by the pedigree. We used the location where individual pumas were last sampled to plot each point. Males are represented with squares and females are circles. The yellow network is localized on the western slope of the Uncompahgre Plateau, and the blue network is localized on the eastern slope of the Uncompahgre Plateau. A single individual, F40, appears on the western slope despite being in the eastern slope network. This individual was first sampled as a 1-month-old kitten; both its mate and offspring were located on the eastern slope. Lines connect mated pairs, with white lines for the reference period and blue lines for the treatment period.

saw a trend where the number of unique fathers increased each year of the reference period and subsequently declined each year of the treatment period (Figure 4).

Using the ages assigned to individual fathers during the birth year for each litter they sired (Figure 5), we found differences between the average age of fathers in the two periods (t_{69}=2.57, p=.012). Fathers were older on average in the reference period, 5.13 years (SD=2.18), than the treatment period, 4.03 years (SD=1.50). While we had males as old as 10 years having litters in the reference period, after the first year of the treatment period, we detected no fathers over the age of 5 years. The age of the father increased each year of the reference period, while it tended to decline in the treatment period years.

3.3 | Relatedness

In the reference and treatment periods, kinship pairs showed average relatedness coefficients similar to their expected distributions (Figure 6; Figure S3). Parent-offspring (PO) pairs and full-siblings (FS) are expected to have relatedness values around 0.5. Half-siblings (HS) are expected to have relatedness values around 0.25. Differences between the average relatedness during the reference period (RP) and the treatment period (TP) were minimal for pairs of full-siblings (RP_{av}=0.549, TP_{av}=0.546, t_{133}=0.324, p=.75), half-siblings (RP_{av}=0.261, TP_{av}=0.266, t_{579}=−0.953, p=.34), and mated pairs (RP_{av}=0.017, TP_{av}=0.0.058, t_{28}=−1.5, p=.146). Average pairwise relatedness of mated pairs during both periods were also not greater than 0 (RP: t_{18}=−0.289, p=.39; TP: t_{25}=0.084, p=.93). Parent-offspring pairs (RP_{av}=0.507, TP_{av}=0.518, t_{265}=−2.59, p=.01) and unrelated pairs (RP_{av}=0.019, TP_{av}=0.020, t_{11617}=−2.18, p=.029) were slightly more related during the treatment period than the reference period.

While the average pairwise relatedness of mated pairs was not different during the reference and treatment periods, we observed three instances of mating between close relatives in the treatment period and none in the reference period (Figure S4). One instance that we identified from the reconstructed pedigree (Litter 60; see Table S2) was mating between a father and his daughter (r=.523). For the other two instances (r=.274 for Litter 21 and r=.437 for Litter 51), we were unable to confidently identify grandparents for these litters, making the exact relationships between the mated individuals unclear.

When we examined relatedness between pairs of independent males (MM) and females (FF) that coexisted during each period (Figure S5), we found that female–female dyads were more related on average than male–male dyads during both the reference (FF_RP_{av}=0.027, MM_RP_{av}=−0.013, z=12.86, p=0) and treatment periods (FF_TP_{av}=0.016, MM_TP_{av}=−0.002, z=7.77, p=7.55e-15). Also, the average male–male dyad became more closely related during the treatment period than they were during the reference period (MM_RP_{av}=−0.013, MM_TP_{av}=−0.002, z=−3.34, p=.0008). Female–female dyads demonstrated the opposite pattern, becoming less related in the treatment period than they were during the reference period (FF_RP_{av}=0.027, MM_TP_{av}=0.016, z=2.17, p=.03).

3.4 | Genetic diversity

We calculated observed heterozygosity (H_O), expected heterozygosity (H_E), the inbreeding coefficient (F_{IS}), and allelic richness (A_r) for each cohort (Table 1). Cohorts differed in observed heterozygosity (ANOVA: F=6.40, p=1.74E-08), expected heterozygosity (F=6.77, p=4.80E-09), and allelic richness (F=33.17, p=3.95E-43). Inbreeding (F_{IS}) was the only summary statistic that did not differ across the cohorts (F=0.09, p=1).

When we plotted the various metrics of genetic diversity over time, a clear trend emerged. Based on the H_E and A_R, genetic diversity was low during the earliest cohorts and gradually increased up until the 2008–2009 cohort at the end of the reference period (Figure 7). Both then decreased, with the final cohort in 2013–2014, at the end of the treatment period, reduced below levels seen in 2004–2005, at the start of the reference period. H_O also peaked in 2008–2009 before decreasing throughout the treatment period, yet H_O was higher in the first two cohorts than the second two cohorts of the reference period (Figure 7a).

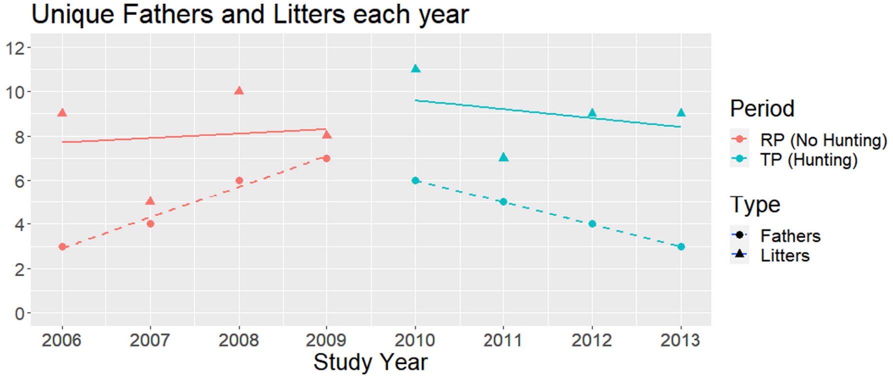


FIGURE 4 Number of litters and unique fathers that sired those litters during each study year. The number of fathers increased during the reference (non-hunting) period (RP) and decreased during the treatment (hunting) period (TP). Red represents the reference period and blue represents the treatment period. The circles and dashed trendlines represent the number of unique fathers that sired a litter born that year. The triangles and solid trendlines represent the number of litters born during that year.

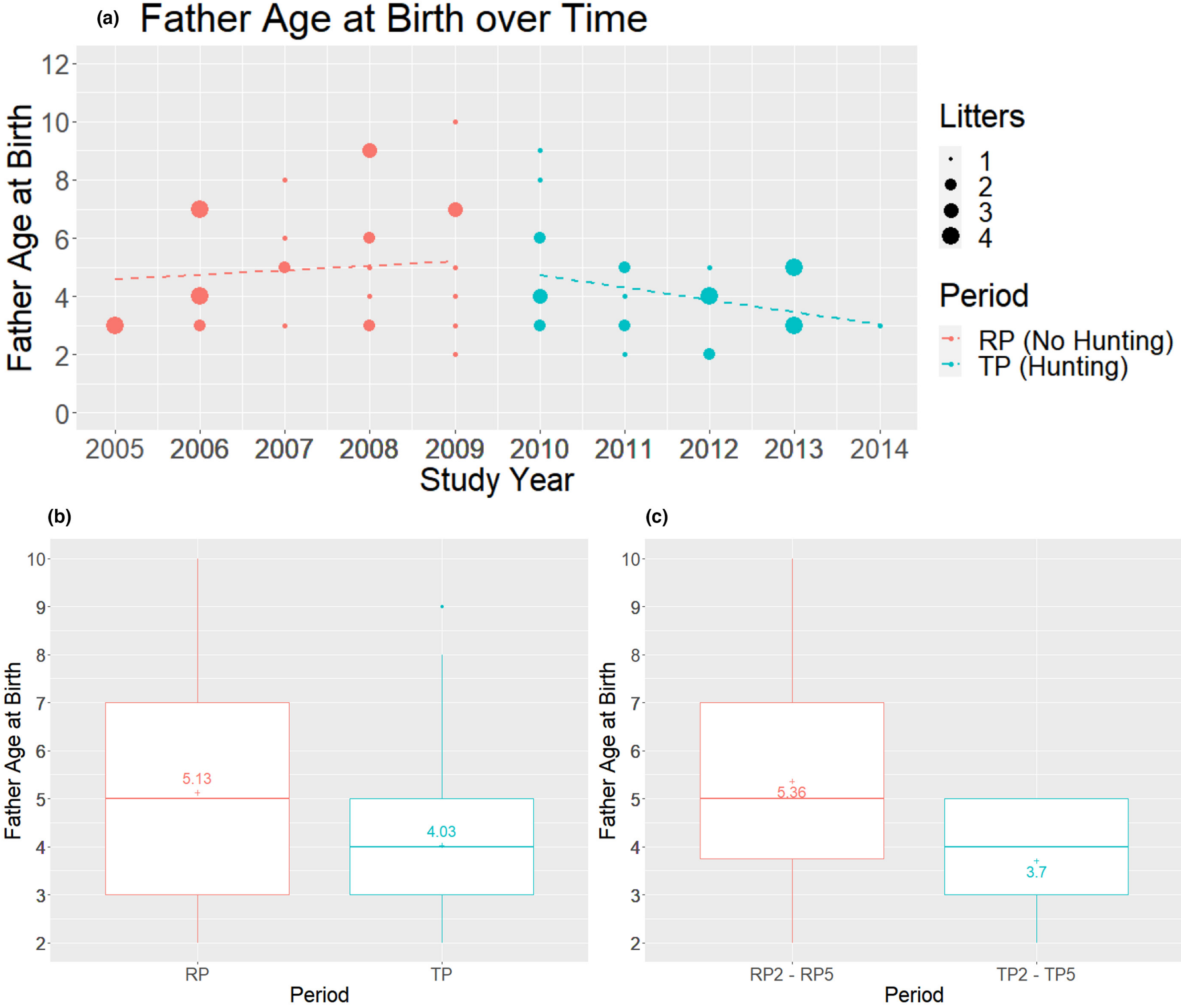


FIGURE 5 In panel (a), we plotted the age of the father at the birth of the litter each year. The age of fathers on average was higher during the reference (non-hunting) period (RP) compared to the treatment (hunting) period (TP). Litters born during the reference period are red and litters born during the treatment period are blue. The size of the dot represents the number of litters that an individual male sired in that given year. Dashed lines represent trend lines. In panel (b), box plots depict the distribution of ages of fathers during the reference (red) and treatment (blue) periods. Additionally, panel (c) depicts box plots of ages of fathers during the second through fifth years of the reference (red) and treatment (blue) periods. Averages are denoted with a + and printed on the figure.

The effective number of breeders (N_B) varied from 19.92 (95% CI: 12.25–35.95) to 57.62 (95% CI: 39.71–94.37) (Table 1). N_B increased steadily each year before peaking in the 2011–2012 cohort (Figure 7e). A sharp decline in N_B was observed for the 2012–2013 and 2013–2014 cohorts, returning N_B back to level below those observed during 2004–2005.

4 | DISCUSSION

Harvest can affect population structure and genetic diversity in complex and complicated ways. Harvest can destabilize population structures and modify a range of behaviours and traits, from infanticide to dispersal. Harvest has also been shown to both increase and decrease genetic diversity across different populations and scales. Critically assessing these impacts and linking changes in behaviour or ecology back to harvest is difficult. Much of what we have learned about the effects of harvest on wild populations has come from post-hoc observational studies rather than controlled experiments, thus obfuscating the researchers' ability to make causal inferences. Our experimental design, however, with hunting suspended for 5 years (the reference period) and then reinstated for 5 years (the treatment period), allowed us to control harvest as a variable and to parse out the effects of hunting on the sexual structure and genetic diversity of a puma population. We believe this approach, experimenting through regulation, will be useful for

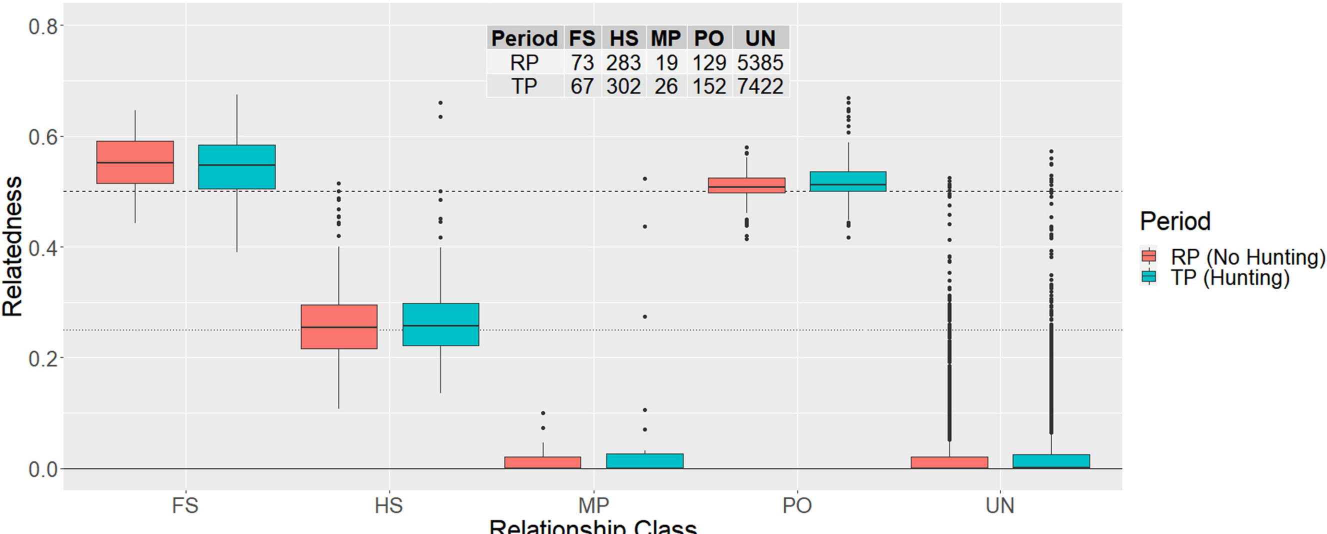


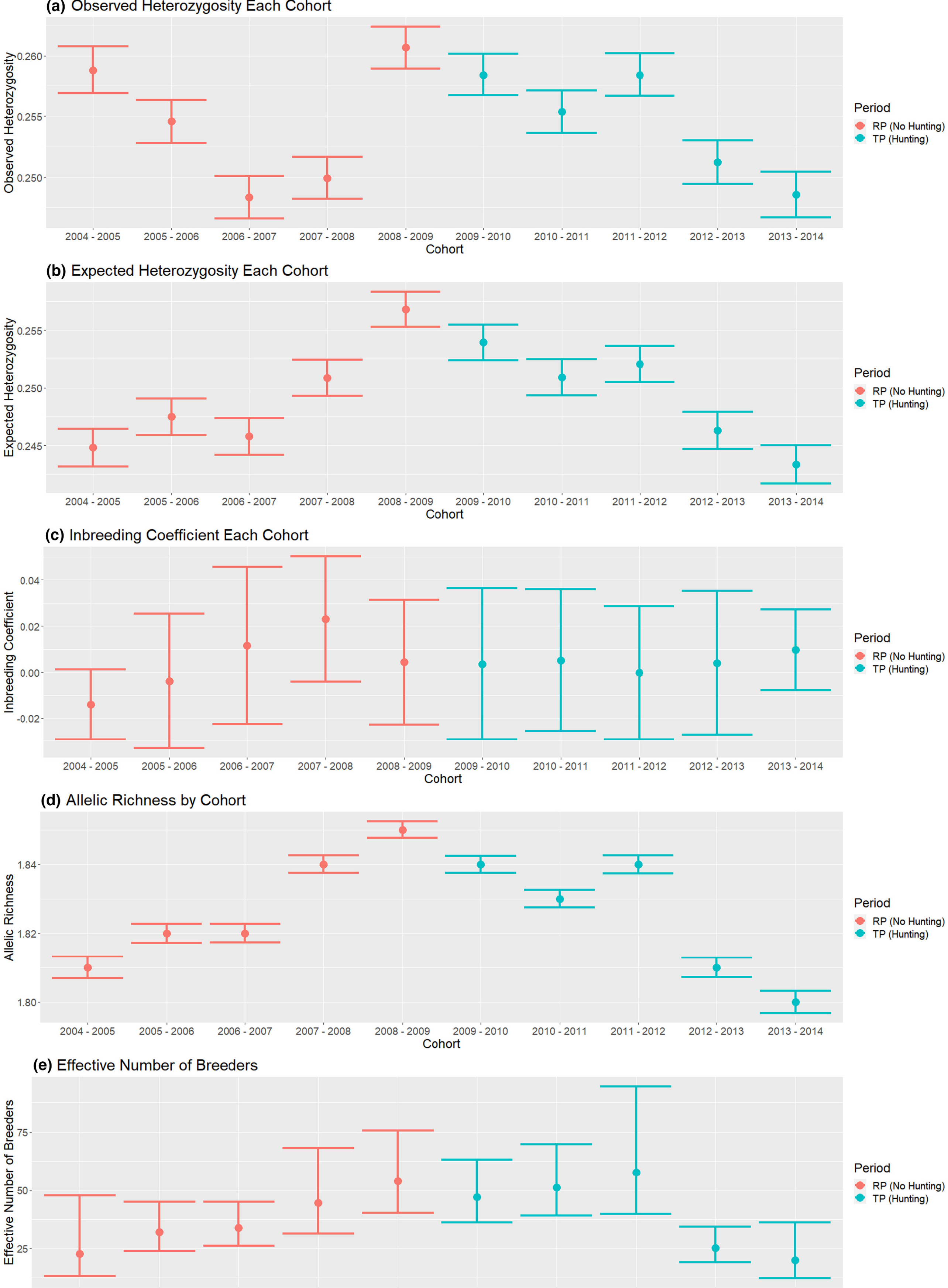
FIGURE 6 Pairwise relatedness estimates of relationship classes determined from field data and a pedigree reconstructed with SEQUOIA. Males and females were generally unrelated across the reference (non-hunting, RP) and treatment (hunting, TP) periods. All relatedness estimates were calculated using the RELATED package in R using the dyadic likelihood estimator. The expected theoretical relatedness coefficient for parent-offspring (PO) and full sibling (FS) relationships is indicated by the dashed line at 0.5. The expected theoretical relatedness coefficient for half-siblings (HS) is indicated by the dotted line at 0.25. The expected theoretical relatedness coefficient for unrelated/random pairs (UN) is indicated by the solid line at 0. MP stands for mated pairs. The red boxplots represent pairs that coexisted during the reference period. The blue boxplots represent pairs that coexisted during the treatment period. Statistics for the number of dyads for each relationship class are given in the imbedded chart.

TABLE 1 Summary statistics for each generational cohort.

Cohort	Period	N	H_O	H_E	F_{IS}	A_r	N_B (95% CI)
2004–2005	RP	21	0.259	0.245	−0.014	1.812	22.62 (13.19–47.66)
2005–2006	RP	44	0.255	0.247	−0.004	1.818	32.04 (23.69–44.96)
2006–2007	RP	46	0.248	0.246	0.012	1.819	33.79 (26.12–44.96)
2007–2008	RP	43	0.250	0.251	0.023	1.836	44.56 (31.37–67.99)
2008–2009	RP	46	0.261	0.257	0.004	1.849	53.72 (40.25–75.53)
2009–2010	TP	50	0.258	0.254	0.004	1.842	46.85 (36.08–63.00)
2010–2011	TP	45	0.255	0.251	0.005	1.832	51.02 (39.04–69.74)
2011–2012	TP	38	0.258	0.252	0.000	1.838	57.62 (39.71–94.37)
2012–2013	TP	42	0.251	0.246	0.004	1.808	25.18 (18.98–34.19)
2013–2014	TP	24	0.249	0.243	0.010	1.799	19.92 (12.25–35.95)

Note: We used a 2-year sliding window approach to avoid parents and offspring being included in the same cohort. Period refers to either RP for the non-hunted 'reference' period or TP for the hunted 'treatment' period. Hunting during the treatment period began in 2009, and, as such, the 2008–2009 and 2009–2010 cohorts in part span each period. N is the number of pumas included in the cohort. H_O is the observed heterozygosity. H_E is the expected heterozygosity. F_{IS} is the inbreeding coefficient. These summary statistics were generated using the POPULATIONS program in STACKS. Allelic richness (A_r) was calculated in HP-RARE in order to account for sample size variation between cohorts. The number of effective breeders (N_B) was calculated using NEESTIMATOR v.2.1 and an adjustment based on chromosome number. 95% jackknifed confidence intervals are displayed in parentheses.

FIGURE 7 Summary statistics plotted for each 2-year cohort of the study. Genetic diversity increased each year during the reference (non-hunting) period, and decreased during the treatment (hunting) period. The reference period (RP) is represented in red while the treatment period (TP) is represented in blue. Hunting during the treatment period began in 2009, and, as such, the 2008–2009 and 2009–2010 cohorts in part span each period. Panel (a) is the observed heterozygosity (H_O). Panel (b) is the expected heterozygosity (H_E). Panel (c) is the inbreeding coefficient (F_{IS}). Panel (d) is allelic richness (A_r). Panel (e) is the effective number of breeders (N_B).


(a) Observed Heterozygosity Each Cohort
Observed Heterozygosity
0.260
0.255
0.250
2004 - 2005
2005 - 2006
2006 - 2007
2007 - 2008
2008 - 2009
2009 - 2010
2010 - 2011
2011 - 2012
2012 - 2013
2013 - 2014
Cohort
Period
RP (No Hunting)
TP (Hunting)
(b) Expected Heterozygosity Each Cohort
Expected Heterozygosity
0.255
0.250
0.245
(c) Inbreeding Coefficient Each Cohort
Inbreeding Coefficient
0.04
0.02
0.00
-0.02
(d) Allelic Richness by Cohort
Allelic Richness
1.84
1.82
1.80
(e) Effective Number of Breeders
Effective Number of Breeders
75
50
25

examining how wildlife management policies affect the populations being managed and whether policies achieve the desired goals of management.

4.1 | Pedigree reconstruction

Pedigree reconstruction offered us an empirical method for delineating our population of interest. We sequenced unmarked pumas that were harvested in surrounding GMUs to maximize our chances of determining paternity for the pumas in our study area. Some of our radio-collared independent pumas had home ranges that overlapped inside and outside the study area, and, thus, we assumed other unmarked pumas had home ranges that did as well (Logan & Runge, 2021). Using the kinship network built from the pedigree and the collected field data, we were able to exclude pumas that were unlikely to have been a part of our population of interest.

By utilizing this methodology, we were able to link fine-scale population structure to individual behaviour. We found two separate breeding networks associated with the eastern and western slopes of the Uncompahgre Plateau. Pumas mated on our study area from December to June with a peak from February to May (Logan & Runge, 2021). During winter and early spring, the Uncompahgre Plateau higher elevation areas are covered in deep snow, thus hindering puma movements across them. Trumbo et al. (2019) found that pumas on the Western Slope of the Rocky Mountains in Colorado preferred to disperse through forests, which were correlated with lower elevation and precipitation. Furthermore, migratory mule deer and elk, the pumas' primary prey, confine their distribution to low elevation ranges with less snow and more available forage (Alldredge et al., 2008; Coe et al., 2018; Gilbert et al., 1970). Field data on movements of marked pumas and our full pedigree network indicated that puma dispersal occurred, and some individuals immigrated onto opposite slopes where they established their adult home ranges and reproduced. Thus, gene flow was occurring between the breeding networks. These inferences highlight the utility of creating kinship networks from reconstructed pedigrees in order to explore dispersal and barriers to gene flow through examining assortative mating (Escoda et al., 2017, 2019).

4.2 | Paternity

Pumas have a polygamous and promiscuous mating system (Logan & Sweanor, 2010; Seidensticker et al., 1973), where multiple territorial males compete to breed with adult females that apparently choose their mates (Logan & Sweanor, 2001; Murphy, 1998). In non-hunted populations, reproductive success in males is highly variable, with the oldest males generally exhibiting the highest reproductive success (Logan & Sweanor, 2001; Murphy, 1998). This social structure was observed during the reference period in our population where just two males sired 53% of the litters. Studies have found upwards of 70%–80% of litters were sired by males over 3 years of age in non-hunted populations (Logan & Sweanor, 2001; Murphy, 1998). Similarly, we found that 67% of litters in the reference period were sired by males at least 3 years old, although this is likely downwardly biased because hunting before the study reduced the age structure of the population at the beginning of the reference period (Logan & Runge, 2021).

In hunted populations, where age structure declines, mating is expected to be constrained to younger adult males with each having low reproductive success (Logan & Sweanor, 2010). The most productive males during the treatment period were far less successful than the most productive males during the reference period, siring only 38% of the litters. Similarly, in a heavily hunted population in Montana, no males over the age of 6 were observed and the majority of litters were sired by males 3 years or younger (Onorato et al., 2011; Robinson & DeSimone, 2011). We found that the average age of fathers was lower in the treatment period than in the reference period, and no males older than 5 years old sired litters after the first year that hunting was reinstated.

Our results suggest that hunting mortality reduced lifetime reproductive success and provided breeding opportunities for other, often younger, males. However, our data also suggests that the unique number of breeding males per year increased during the reference period and decreased during the treatment period (Figure 4). One explanation is that the removal of hunting increased abundance and survival of adult males and females (Logan & Runge, 2021); this led to an increase in successful individual male breeders each year. When hunting was reinstated, however, instead of breeding opportunities increasing per adult male because dominant ones were removed, male abundance and survival declined overall. Logan and Runge (2021) found that recruitment during the treatment period did not compensate for the loss of adult males, but partially compensated for the loss of adult females. In fact, the ratio of adult males to adult females declined greatly in the treatment period (Logan & Runge, 2021), and this probably increased breeding opportunities for the few surviving adult males. Moreover, we expect lifetime reproductive success of those males to be relatively low because their annual survival rates were less than half that of non-hunted adult males (Logan & Runge, 2021).

4.3 | Relatedness

We found no differences in relatedness of mated pairs between the non-hunting and hunting periods, and mean relatedness values among mated pairs in each period indicated that pairs were generally unrelated. Similarly, in a hunted puma population in the Garnett Mountains of Montana, relatedness of parents was not significantly different than zero (Onorato et al., 2011). Furthermore, neither estimates of relatedness nor observations of marked individuals in our puma population indicate a breakdown in dispersal or male kin-clustering as reported in a hunted leopard population in South Africa (Naude et al., 2020).

We observed, however, 3 out of 37 (8%) unique mated pairs during the treatment period with relatedness coefficients over 0.25,

which is equivalent to half-siblings or even more closely related. All three instances involved males between the ages of 5 and 8 years old. In contrast, no mated pairs during the reference period had relatedness values over 0.1. In the heavily hunted puma population in Garnet Mountains of Montana, 29% of mating pairs exhibited pairwise relatedness values over 0.215 (Onorato et al., 2011). On the other hand, in a protected population in New Mexico, researchers observed 8% of the litters derived from fathers and their philopatric daughters (Logan & Sweanor, 2001). Thus, while all of the instances of inbreeding arose during the treatment period of our study, it remains unclear whether or not harvest played a role in this finding or if some background levels of inbreeding are to be expected in any puma population irrespective of hunting pressure.

Variation in relatedness among each sex of independent pumas was associated with hunting. Onorato et al. (2011) found higher relatedness levels for males than females in a puma population in the Garnet Mountains of Montana. This was the opposite of what was found in puma populations in the Northern Yellowstone Ecosystem and in Arizona where females were more closely related on average than males (Biek et al., 2006; Nicholson et al., 2011). Onorato et al. (2011) suggested the difference was possibly due to differences in hunting pressure, as the Garnet Mountains population was subject to heavy harvest and the Northern Yellowstone Ecosystem pumas were mostly protected. In our population, females were more closely related than males during both the reference and treatment periods. This is a result that would be expected in a system with male-biased dispersal and female philopatry (Biek et al., 2006). In the treatment period, however, independent males were more closely related than in the reference period, and independent females became less closely related during the treatment period than they were during the reference period. These results are consistent with the findings from Onorato et al. (2011) that harvest promotes an increase in relatedness among males and decreased relatedness among females.

It is unclear what drove the increase in male relatedness in the population during the hunting period. The increase in relatedness was not due to male kinship clustering, as the field collected data did not find an increase of male philopatry or a decrease in male emigration. Male emigration was similar in the two periods: 39% of the marked male cubs that survived to the subadult stage in the reference period emigrated from the study area; 42% of the marked male cubs that survived to the subadult stage in the treatment period emigrated from the study area. In our population, Logan and Runge (2021) found two potential examples of independent male philopatry during the hunting period. However, both pumas were harvested as young adults (i.e. 28 and 30 months old) and potentially might have dispersed later had they survived. Neither sired offspring.

The reduction in relatedness between females during the treatment period is likely best explained by immigration of females into the population. Logan and Runge (2021) found that female recruitment in our population partially compensated for the loss of adult females, and reproduction rates did not increase during the treatment period. Additionally, observed emigration from the study area of marked female offspring that survived to the subadult stage was the same in both periods (i.e. 1 in each). Therefore, the partial compensation in recruitment of females was more likely from immigration, leading to lower relatedness between female–female dyads.

4.4 | Genetic diversity

While there have been a plethora of studies on puma genetics across the western United States (for a few examples see Anderson et al., 2004; Juarez et al., 2016; Loxterman, 2011; Mcrae et al., 2005; Wultsch et al., 2023), all but two of these studies utilized microsatellite markers rather than large SNP sets, making comparisons using traditional genetic diversity statistics difficult (Sunde et al., 2020; Zimmerman et al., 2020). However, our population does appears to have similar genetic diversity (H_O=0.248–0.261; A_r=1.799–1.849) to pumas found on the Front Range of Colorado (H_O=0.243; A_r=1.89; Trumbo et al., 2019) as well as populations in California (H_O=0.24–0.31; A_r=1.67–1.79; Gustafson et al., 2022). Previous studies have suggested that contemporary patterns of genetic diversity in pumas are a relict of a post-Pleistocene expansion (Gustafson et al., 2022; Mcrae et al., 2005), when pumas recolonized North America from South America (Culver et al., 2000). As a result of this range expansion, genetic diversity is often greater in lower latitudes, and our population displays similar diversity to those in Colorado and California found at similar latitudes.

In our study, genetic diversity (i.e. observed heterozygosity, expected heterozygosity, and allelic richness) tended to increase over the years when hunting was removed and decrease when hunting was reinstated. These results, in relation to temporal variation and hunting, are in contrast to what some previous studies reported (Ausband & Waits, 2020; Juarez et al., 2016). In the Black Hills of South Dakota, a study of genetic diversity in a puma population over a 10-year period in which hunting mortality varied (5 years of no harvest followed by 3 years of moderate harvest followed by 2 years of heavy harvest) found no significant differences in observed heterozygosity, expected heterozygosity, allelic richness, or the effective number of alleles across the different hunting regimes (Juarez et al., 2016). In Idaho wolves, researchers using microsatellites found harvest to have no effect on allelic richness or observed heterozygosity, although they did find a net gain of alleles into groups in subpopulations, and a reduction in private alleles in subpopulations (Ausband & Waits, 2020). However, our use of hundreds to thousands of SNP markers offers statistical power beyond that of a panel of microsatellites, and this difference could be one explanation for our finding of statistically significant differences where previous studies have not.

The calculated effective number of breeders (N_B) for each cohort in our puma population broadly reflected N_C, the estimated number of independent pumas each winter on the study site (Logan & Runge, 2021; Figure S6). Like some of the other genetic diversity metrics, N_B increased as the population expanded during the

reference period and decreased as the population declined during the treatment period. Interestingly, the 2011–2012 cohort has a high N_B with a relatively large confidence interval. We speculate this may be because of the high turnover in male breeders between 2011 and 2012. Only one of six males and one of seven females that successfully bred in 2011 also sired or birthed a litter in 2012. This amount of turnover of male breeders did not occur in any other year of the study (Figure S2). Additionally, the ratio of N_B/N_C was much lower during the final two cohorts (2012–2013 and 2013–2014). This is likely because of the reduction in the number of males that successfully sired litters those years. During 2012 and 2013 when the abundance of adult males was low (Logan & Runge, 2021), the most successful two males sired 67% and 89% of all litters, while in 2010 and 2011 when the abundance of adult males was still high, the most successful two males sired 45% and 57% of all litters.

5 | CONCLUSIONS AND IMPLICATIONS

Our investigation revealed that hunting reduced the age of male breeders and reduced the lifetime reproductive success of males. We found that mated pairs were generally unrelated during each time period, and that females were more closely related than males, as would be expected in a system with male-biased dispersal and female philopatry. We also found that harvest increased relatedness among males and decreased relatedness among females. Finally, genetic diversity, across multiple metrics, increased during the period without hunting and decreased each year when hunting was present.

While the effects we documented were statistically significant, it is far more difficult to determine the biological significance of the changes we observed in genetic diversity and relatedness. These changes, though statistically significant, were often slight. For example, the difference in A_r between the highest and lowest cohorts was 0.05; for H_E the difference was 0.014. Similarly, for relatedness, though we observed changes in how related the pumas in the population were to each other, these changes did not implicate concerns about inbreeding nor does our data suggest large-scale changes in dispersal or philopatry.

However, nearly every phenomenon we observed trended one direction during the period without hunting and another during the period with hunting. Given sufficient time and a continued trend, the changes we observed may have become far more biologically significant. At 5 years each, neither of these periods was likely long enough to fully understand the effects of harvest on the breeding processes and genetics of this puma population. Though the selective effects of hunting have been seen in populations in as little as three to four generations (Pigeon et al., 2016), our study was still much shorter than that. While pumas may reach sexual maturity before 2 years of age, the generation time in pumas has been estimated at 4.45 years (Hostetler et al., 2012). As such, each of our periods extended for barely a single generation.

Our study highlights the need for additional, long-term (i.e. over several puma generations) experimental research in which hunting mortality is manipulated, including at least one control population (i.e. with no harvest), and where population-level responses are monitored in each condition. Further, future research can be performed on a broader, subnational- or region-wide scale to better understand how the effects we observed relate to broader source-sink dynamics (Benson et al., 2023; Wultsch et al., 2023). Scientific understanding of the effects of harvest on the affected populations is necessary for making reliable management decisions, and these effects can only be truly understood through these long-term, adequately controlled studies.

The North American Model mandates that science is the proper tool for making wildlife policy decisions (Organ et al., 2012), yet despite significant developments in wildlife management as a science, there is still much we do not know when it comes to cause and effect in wildlife management (Logan, 2012). In recent years, there have been challenges to harvest as a management tool, questioning both its scientific underpinning as well as its efficacy in achieving desired management outcomes (Artelle et al., 2018; Laundré & Papouchis, 2020). Wildlife agencies are in a unique position—they have the legal authority to manipulate hunting laws to create the experimental conditions necessary to empirically test management decisions. Further, they are the only bodies capable of performing these experiments. Academic scientists alone cannot experimentally manipulate wildlife populations over the scales necessary to draw appropriate conclusions. Science and law in wildlife management can benefit from a bidirectional relationship. Wildlife science can be used to develop management policy, but law can also be utilized to improve the scientific framework for achieving reliable knowledge.

One way to structure this type of relationship is through the incorporation of experimental hunting regulations as part of a broader adaptive, zonal management scheme (Logan & Sweanor, 2001; Logan, 2012; Logan & Runge, 2021; Mech, 2017). A zone management strategy varies harvest regimes across different localities in order to meet the different management priorities set for each zone (Logan & Sweanor, 2001; Mech, 2017). For example, in regions where managers choose to prioritize natural population dynamics or desire to grow the population, harvest quotas and highly selective hunting methods may be limited or entirely eliminated. Alternatively, in regions where minimizing human-carnivore conflict is the priority, high quotas and more effective hunting techniques may be utilized. Critically, regions where harvest is experimentally manipulated and controlled are invaluable to best understand how regulatory regimes affect specified priorities and patterns in nature, as we were able to do in this study. The generation of scientific knowledge that is used to adaptively update management policy can inform the development of management zones.

AUTHOR CONTRIBUTIONS

John A. Erwin developed the project ideas, performed laboratory work, analysed genomic data, and wrote the manuscript. Kenneth A. Logan initially conceived the project, acquired funding, directed fieldwork, collected field data, and contributed to data interpretation. Daryl R. Trumbo performed laboratory work and directed

the bioinformatic portions of data analysis. W. Chris Funk contributed reagents, provided equipment, and directed research. Melanie Culver initially conceived the project, acquired funding, contributed reagents, provided equipment, and directed research. All authors contributed input to the draft and final versions of the manuscript.

ACKNOWLEDGEMENTS

Funding was provided by the Summerlee Foundation. Samples were collected by Colorado Parks and Wildlife. We also thank Bob Fitak, Brenna Forester, Patricia Salerno, and Karla Vargas for bioinformatic and technical assistance. We are grateful to Mike Worobey and Tom Watts for allowing us to use the Pippin Prep DNA size selection system. We are grateful to the editor, Andrew DeWoody, as well as the three reviewers, Marco Festa-Bianchet, Dave Onorato, and Seth Riley, for their incredibly helpful comments during the review process.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

ddRADseq data and necessary metadata used in genomic analyses are available on Dryad at the following DOI: https://doi.org/10.5061/dryad.bnzs7h4hm.

ETHICS STATEMENT

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Vertebrates were captured by Colorado Parks and Wildlife Department personnel in accordance with approved CPW Animal Care and Use Committee (ACUC) capture and handling protocols (ACUC file #08-2004 and ACUC protocol #03-2007).

BENEFIT-SHARING STATEMENT

Benefits from this research include the sharing of our data and results on public databases as described herein.

ORCID

John A. Erwin https://orcid.org/0000-0002-3544-7086
Daryl R. Trumbo https://orcid.org/0000-0002-8438-2856
W. Chris Funk https://orcid.org/0000-0002-6466-3618

REFERENCES

Alldredge, M. W., Bergman, E. J., Bishop, C. J., Freddy, D. J., & Logan, K. A. (2008). Pilot evaluation of predator-prey dynamics on the Uncompahgre plateau. *Federal aid in wildlife restoration report*, project W-185-R.

Allendorf, F. W., England, P. R., Luikart, G., Ritchie, P. A., & Ryman, N. (2008). Genetic effects of harvest on wild animal populations. *Trends in Ecology & Evolution*, *23*(6), 327–337. https://doi.org/10.1016/j.tree.2008.02.008

Allendorf, F. W., & Hard, J. J. (2009). Human-induced evolution caused by unnatural selection through harvest of wild animals. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(Suppl), 9987–9994. https://doi.org/10.1073/pnas.0901069106

Anderson, C. R., Lindzey, F., & Mcdonald, D. B. (2004). Genetic structure of cougar populations across the Wyoming Basin: Metapopulation or megapopulation. *Journal of Mammalogy*, *85*(6), 1207–1214.

Andrews, K. R., Adams, J. R., Cassirer, E. F., Plowright, R. K., Gardner, C., Dwire, M., Hohenlohe, P. A., & Waits, L. P. (2018). A bioinformatic pipeline for identifying informative SNP panels for parentage assignment from RADseq data. *Molecular Ecology Resources*, *18*(6), 1263–1281. https://doi.org/10.1111/1755-0998.12910

Artelle, K. A., Reynolds, J. D., Treves, A., Walsh, J. C., Paquet, P. C., & Darimont, C. T. (2018). Hallmarks of science missing from North American wildlife management. *Science Advances*, *4*, 1–7. https://doi.org/10.1126/sciadv.aao0167

Ausband, D. E. (2022). Genetic diversity and mate selection in a reintroduced population of gray wolves. *Scientific Reports*, *12*, 535. https://doi.org/10.1038/s41598-021-04449-4

Ausband, D. E., & Waits, L. (2020). Does harvest affect genetic diversity in grey wolves? *Molecular Ecology*, *29*(17), 3187–3195. https://doi.org/10.1111/mec.15552

Benson, J. F., Dougherty, K. D., Beier, P., Boyce, W. M., Cristescu, B., Gammons, D. J., Garcelon, D. K., Higley, J. M., Martins, Q. E., Nisi, A. C., Riley, S. P. D., Sikich, J. A., Stephenson, T. R., Vickers, T. W., Wengert, G. M., Wilmers, C. C., Wittmer, H. U., & Dellinger, J. A. (2023). The ecology of human-caused mortality for a protected large carnivore. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(13), e2220030120. https://doi.org/10.1073/pnas.2220030120

Biek, R., Akamine, N., Schwartz, M. K., Ruth, T. K., Murphy, K. M., & Poss, M. (2006). Genetic consequences of sex-biased dispersal in a solitary carnivore: Yellowstone cougars. *Biology Letters*, *2*(2), 312–315. https://doi.org/10.1098/rsbl.2005.0437

Blouin, M. S. (2003). DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology & Evolution*, *18*(10), 503–511. https://doi.org/10.1016/S0169-5347(03)00225-8

Blyton, M. D. J., Banks, S. C., & Peakall, R. (2015). The effect of sex-biased dispersal on opposite-sexed spatial genetic structure and inbreeding risk. *Molecular Ecology*, *24*(8), 1681–1695. https://doi.org/10.1111/mec.13149

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Borgatti, S. P. (2002). *NetDraw software for network visualization*. Analytic Technologies.

Bresadola, L., Link, V., Buerkle, C. A., Lexer, C., & Wegmann, D. (2020). Estimating and accounting for genotyping errors in RAD-seq experiments. *Molecular Ecology Resources*, *20*(4), 856–870. https://doi.org/10.1111/1755-0998.13153

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140. https://doi.org/10.1111/mec.12354

Coe, P. K., Clark, D. A., Nielson, R. M., Gregory, S. C., Cupples, J. B., Hedrick, M. J., Johnson, B. K., & Jackson, D. H. (2018). Multiscale models of habitat use by mule deer in winter. *Journal of Wildlife Management*, *82*(6), 1285–1299. https://doi.org/10.1002/jwmg.21484

Coltman, D. W., O'Donoghue, P., Jorgenson, J. T., Hogg, J. T., Strobeck, C., & Festa-Bianchet, M. (2003). Undesirable evolutionary consequences of trophy hunting. *Nature*, *426*(December), 655–658. https://doi.org/10.1038/nature02187.1

Cooley, H. S., Wielgus, R. S., Koehler, G. M., Robinson, H. S., & Maletzke, B. T. (2009). Does hunting regulate cougar populations? A test of

the compensatory mortality hypothesis. *Ecology*, *90*, 2913–2921. https://doi.org/10.1890/08-1805.1

Culver, M., Johnson, W. E., Pecon-Slattery, J., & O'Brien, S. J. (2000). Genomic ancestry of the American puma (*Puma concolor*). *Journal of Heredity*, *91*(3), 186–197. https://doi.org/10.1093/jhered/91.3.186

de Oliveira, M. E., Saranholi, B. H., Miotto, R. A., Souza, A. S. M. C., Dirzo, R., & Galetti, P. M., Jr. (2023). Female philopatry and unsuccessful male dispersal of a top predator in a human-modified landscape revealed by relatedness analysis. *European Journal of Wildlife Research*, *69*, 95. https://doi.org/10.1007/s10344-023-01726-7

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, *14*(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Elbroch, L. M., Ferguson, J. M., Quigley, H., Craighead, D., Thompson, D. J., & Wittmer, H. U. (2020). Reintroduced wolves and hunting limit the abundance of a subordinate apex predator in a multi-use landscape. *Proceedings of the Royal Society B: Biological Sciences*, *287*, 20202202. https://doi.org/10.1098/rspb.2020.2202

Escoda, L., Fernández-González, Á., & Castresana, J. (2019). Quantitative analysis of connectivity in populations of a semi-aquatic mammal using kinship categories and network assortativity. *Molecular Ecology Resources*, *19*(2), 310–326. https://doi.org/10.1111/1755-0998.12967

Escoda, L., González-Esteban, J., Gómez, A., & Castresana, J. (2017). Using relatedness networks to infer contemporary dispersal: Application to the endangered mammal Galemys pyrenaicus. *Molecular Ecology*, *26*(13), 3343–3357. https://doi.org/10.1111/mec.14133

Fattebert, J., Balme, G., Dickerson, T., Slotow, R., & Hunter, L. (2015). Density-dependent natal dispersal patterns in a leopard population recovering from over-harvest. *PLoS ONE*, *10*(4), 1–15. https://doi.org/10.1371/journal.pone.0122355

Festa-Bianchet, M. (2017). When does selective hunting select, how can we tell, and what should we do about it? *Mammal Review*, *47*(1), 76–81. https://doi.org/10.1111/mam.12078

Festa-Bianchet, M., & Mysterud, A. (2018). Hunting and evolution: Theory, evidence, and unknowns. *Journal of Mammalogy*, *99*(6), 1281–1292. https://doi.org/10.1093/jmammal/gyy138

Frank, S. C., Leclerc, M., Pelletier, F., Rosell, F., Swenson, J. E., Bischof, R., Kindberg, J., Eiken, H. G., Hagen, S. B., & Zedrosser, A. (2018). Sociodemographic factors modulate the spatial response of brown bears to vacancies created by hunting. *Journal of Animal Ecology*, *87*, 247–258. https://doi.org/10.1111/1365-2656.12767

Frank, S. C., Pelletier, F., Kopatz, A., Bourret, A., Garant, D., Swenson, J. E., Eiken, H. G., Hagen, S. B., & Zedrosser, A. (2021). Harvest is associated with the disruption of social and fine-scale genetic structure among matrilines of a solitary large carnivore. *Evolutionary Applications*, *14*, 1023–1035. https://doi.org/10.1111/eva.13178

Fryxell, J. M., Packer, C., McCann, K., Solberg, E. J., & Saether, B. E. (2010). Resource management cycles and the sustainability of harvested wildlife populations. *Science*, *328*, 903–906. https://doi.org/10.1126/science.1185802

Gilbert, P. F., Wallmo, O. C., & Gill, R. B. (1970). Effect of snow depth on mule deer in Middle Park, Colorado. *Journal of Wildlife Management*, *34*(1), 15–23.

Gosselin, J., Leclerc, M., Zedrosser, A., Steyaert, S. M. J. G., Swenson, J. E., & Pelletier, F. (2017). Hunting promotes sexual conflict in brown bears. *Journal of Animal Ecology*, *86*, 35–42. https://doi.org/10.1111/1365-2656.12576

Gosselin, T., Lamothe, M., Devloo-Delva, F., & Grewe, P. (2020). *radiator: RADseq data exploration, manipulation and visualization using R*. R package version 1.1.8. https://thierrygosselin.github.io/radiator/. https://doi.org/10.5281/zenodo.3687060

Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, *28*(4), 1140–1162. https://doi.org/10.1016/S0003-3472(80)80103-5

Gustafson, K. D., Gagne, R. B., Buchalski, M. R., Vickers, T. W., Riley, S. P. D., Sikich, J. A., Rudd, J. L., Dellinger, J. A., LaCava, M., & Ernest, H. B. (2022). Multi-population puma connectivity could restore genomic diversity to at-risk coastal populations in California. *Evolutionary Applications*, *15*, 268–299. https://doi.org/10.1111/eva.13341

Gustafson, K. D., Gagne, R. B., Vickers, T. W., Riley, S. P. D., Wilmers, C. C., Bleich, V. C., Pierce, B. M., Kenyon, M., Drazenovich, T. L., Sikich, J. A., Boyce, W. M., & Ernest, H. B. (2019). Genetic source–sink dynamics among naturally structured and anthropogenically fragmented puma populations. *Conservation Genetics*, *20*(2), 215–227. https://doi.org/10.1007/s10592-018-1125-0

Gustafson, K. D., Vickers, T. W., Boyce, W. M., & Ernest, H. B. (2017). A single migrant enhances the genetic diversity of an inbred puma population. *Royal Society Open Science*, *4*(5). https://doi.org/10.1098/rsos.170115

Hall, D., Zhao, W., Wennström, U., Andersson Gull, B., & Wang, X. R. (2020). Parentage and relatedness reconstruction in *Pinus sylvestris* using genotyping-by-sequencing. *Heredity*, *124*(5), 633–646. https://doi.org/10.1038/s41437-020-0302-3

Harris, R. B., Wall, W. A., & Allendorf, F. W. (2002). Genetic consequences of hunting: What do we know and what should we do? *Wildlife Society Bulletin*, *30*(2), 634–643.

Hostetler, J. A., Onorato, D. P., Jansen, D., & Oli, M. K. (2012). A cat's tale: The impact of genetic restoration on Florida panther population dynamics and persistence. *Journal of Animal Ecology*, *82*(3), 608–620. https://doi.org/10.1111/1365-2656.12033

Hsu, T. C., Rearden, H. H., & Luquette, G. F. (1963). Karyological studies of nine species of Felidae. *The American Naturalist*, *97*(895), 225–234.

Huisman, J. (2017). Pedigree reconstruction from SNP data: Parentage assignment, sibship clustering and beyond. *Molecular Ecology Resources*, *17*(5), 1009–1024. https://doi.org/10.1111/1755-0998.12665

Jensen, A. J. (1996). Density-dependent matrix yield equation for optimal harvest of age-structured wildlife populations. *Ecological Modelling*, *88*, 125–132. https://doi.org/10.1016/0304-3800(95)00075-5

Juarez, R. L., Schwartz, M. K., Pilgrim, K. L., Thompson, D. J., Tucker, S. A., Smith, J. B., & Jenks, J. A. (2016). Assessing temporal genetic variation in a cougar population: Influence of harvest and neighboring populations. *Conservation Genetics*, *17*(2), 379–388. https://doi.org/10.1007/s10592-015-0790-5

Kalinowski, S. T. (2005). HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, *5*(1), 187–189. https://doi.org/10.1111/j.1471-8286.2004.00845.x

Laundré, J. W., & Hernández, L. (2003). Factors affecting dispersal in young male pumas. In S. A. Becker, D. D. Bjornlie, F. G. Lindzey, & D. S. Moody (Eds.), *Proceedings of the seventh mountain lion workshop* (pp. 151–160). Lander.

Laundré, J. W., Hernández, L., Streubel, D., Altendorf, K., & Gonzalez, C. L. (2000). Aging mountain lions using gum-line recession. *Wildlife Society Bulletin*, *28*(4), 963–966.

Laundré, J. W., & Papouchis, C. (2020). The elephant in the room: What can we learn from California regarding the use of sport hunting of pumas (*Puma concolor*) as a management tool? *PLoS ONE*, *15*(2), 1–26. https://doi.org/10.1371/journal.pone.0224638

Leclerc, M., Frank, S. C., Zedrosser, A., Swenson, J. E., & Pelletier, F. (2017). Hunting promotes spatial reorganization and sexually selected infanticide. *Scientific Reports*, *7*, 45222. https://doi.org/10.1038/srep45222

Leclerc, M., Zedrosser, A., & Pelletier, F. (2017). Harvesting as a potential selective pressure on behavioral traits. *Journal of Applied*

Ecology, *54*, 1941–1945. https://doi.org/10.1111/1365-2664.12893

Leclerc, M., Zedrosser, A., Swenson, J. E., & Pelletier, F. (2019). Hunters select for behavioral traits in a large carnivore. *Scientific Reports*, *9*, 12371. https://doi.org/10.1038/s41598-019-48853-3

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *ArXiv:1303.3997*. http://arxiv.org/abs/1303.3997

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Logan, K. A. (2012). Structuring felid management for science. *Wild Felid Monitor*, *Summer 2012*, 11–14.

Logan, K. A. (2019). Puma population limitation and regulation: What matters in puma management? *The Journal of Wildlife Management*, *83*(8), 1652–1666. https://doi.org/10.1002/jwmg.21753

Logan, K. A., & Runge, J. P. (2021). Effects of hunting on a puma population in Colorado. *Wildlife Monographs*, *209*, 1–35. https://doi.org/10.1002/wmon.1061

Logan, K. A., & Sweanor, L. L. (2001). *Desert puma: Evolutionary ecology and conservation of an enduring carnivore*. Island Press.

Logan, K. A., & Sweanor, L. L. (2010). Behavior and social organization of a solitary carnivore. In M. Hornocker & S. Negri (Eds.), *Cougar ecology and conservation* (pp. 105–117). University of Chicago Press.

Loveridge, A. J., Searle, A. W., Murindagomo, F., & Macdonald, D. W. (2007). The impact of sport-hunting on the population dynamics of an African lion population in a protected area. *Biological Conservation*, *134*(4), 548–558. https://doi.org/10.1016/j.biocon.2006.09.010

Loveridge, A. J., Valeix, M., Chapron, G., Davidson, Z., Mtare, G., & Macdonald, D. W. (2016). Conservation of large predator populations: Demographic and spatial responses of African lions to the intensity of trophy hunting. *Biological Conservation*, *204*, 247–254. https://doi.org/10.1016/j.biocon.2016.10.024

Loxterman, J. L. (2011). Fine scale population genetic structure of pumas in the intermountain west. *Conservation Genetics*, *12*(4), 1049–1059. https://doi.org/10.1007/s10592-011-0208-y

Luca, F., Hudson, R. R., Witonsky, D. B., & di Rienzo, A. (2011). A reduced representation approach to population genetic analyses and applications to human evolution. *Genome Research*, *21*(7), 1087–1098. https://doi.org/10.1101/gr.119792.110

Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piñero, D., & Emerson, B. C. (2015). Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. *Molecular Ecology Resources*, *15*(1), 28–41. https://doi.org/10.1111/1755-0998.12291

Mcrae, B. H., Beier, P., Dewald, L. E., Huynh, L. Y., & Keim, P. (2005). Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. *Molecular Ecology*, *14*, 1965–1977. https://doi.org/10.1111/j.1365-294X.2005.02571.x

Mech, L. D. (2017). Where can wolves live and how can we live with them? *Biological Conservation*, *210*, 310–317. https://doi.org/10.1016/j.biocon.2017.04.029

Milligan, B. G. (2003). Maximum-likelihood estimation of relatedness. *Genetics*, *163*(3), 1153–1167.

Milner, J. M., Nilsen, E. B., & Andreassen, H. P. (2007). Demographic side effects of selective hunting in ungulates and carnivores: Review. *Conservation Biology*, *21*(1), 36–47. https://doi.org/10.1111/j.1523-1739.2006.00591.x

Murphy, K. M. (1998). *The ecology of the cougar (Puma concolor) in the northern Yellowstone ecosystem: Interactions with prey, bears, and humans* (Dissertation). University of Idaho.

Mysterud, A. (2011). Selective harvesting of large mammals: How often does it result in directional selection? *Journal of Applied Ecology*, *48*(4), 827–834. https://doi.org/10.1111/j.1365-2664.2011.02006.x

Naude, V. N., Balme, G. A., Riain, J. O., Hunter, L. T. B., Fattebert, J., Dickerson, T., & Bishop, J. M. (2020). Unsustainable anthropogenic mortality disrupts natal dispersal and promotes inbreeding in leopards. *Ecology and Evolution*, *10*(8), 3605–3619. https://doi.org/10.1002/ece3.6089

Newby, J. R., Scott Mills, L., Ruth, T. K., Pletscher, D. H., Mitchell, M. S., Quigley, H. B., Murphy, K. M., & DeSimone, R. (2013). Human-caused mortality influences spatial population dynamics: Pumas in landscapes with varying mortality risks. *Biological Conservation*, *159*, 230–239. https://doi.org/10.1016/j.biocon.2012.10.018

Nicholson, K. L., Krausman, P. R., Munguia-Vega, A., & Culver, M. (2011). Spatial and temporal interactions of sympatric mountain lions in Arizona. *European Journal of Wildlife Research*, *57*(6), 1151–1163. https://doi.org/10.1007/s10344-011-0528-8

Ochoa, A., Onorato, D. P., Fitak, R. R., Roelke-Parker, M. E., & Culver, M. (2019). De novo assembly and annotation from parental and F1 puma genomes of the Florida Panther genetic restoration program. *G3: Genes*, *Genomes*, *Genetics*, *9*(11), 3531–3536. https://doi.org/10.1534/g3.119.400629

Onorato, D., Desimone, R., White, C., & Waits, L. P. (2011). Genetic assessment of paternity and relatedness in a managed population of cougars. *The Journal of Wildlife Management*, *75*(2), 378–384. https://doi.org/10.1002/jwmg.43

Organ, J. F., Geist, V., Mahoney, S. P., Williams, S., Krausman, P. R., Batcheller, G. R., Decker, T. A., Carmichael, R., Nanjappa, P., Regan, R., Medellin, R. A., Cantu, R., McCabe, R. E., Craven, S., Vecellio, G. M., & Decker, D. J. (2012). *The North American model of wildlife conservation*. The Wildlife Society Technical Review 12-04. The Wildlife Society.

Perrin, N., & Mazalov, V. (2000). Local competition, inbreeding, and the evolution of sex-biased dispersal. *The American Naturalist*, *155*(1), 116–127. https://doi.org/10.1086/303296

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Pew, J., Muir, P. H., Wang, J., & Frasier, T. R. (2014). related: An R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, *15*, 557–561. https://doi.org/10.1111/1755-0998.12323

Pigeon, G., Festa-Bianchet, M., Coltman, D. W., & Pelletier, F. (2016). Intense selective hunting leads to artificial evolution in horn size. *Evolutionary Applications*, *9*(4), 521–530. https://doi.org/10.1111/eva.12358

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, *81*(3), 559–575. https://doi.org/10.1086/519795

Riley, S. P. D., Serieys, L. E. K., Pollinger, J. P., Sikich, J. A., Dalbeck, L., Wayne, R. K., & Ernest, H. B. (2014). Individual behaviors dominate the dynamics of an urban mountain lion population isolated by roads. *Current Biology*, *24*(17), 1989–1994. https://doi.org/10.1016/j.cub.2014.07.029

Robinson, H., & DeSimone, R. (2011). *The Garnet Range mountain lion study: Characteristics of a hunted population in West-Central Montana*.

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*, *28*(21), 4737–4754. https://doi.org/10.1111/mec.15253

Saremi, N. F., Supple, M. A., Byrne, A., Cahill, J. A., Coutinho, L. L., Dalén, L., Figueiró, H. V., Johnson, W. E., Milne, H. J., O'Brien, S. J., O'Connell, B., Onorato, D. P., Riley, S. P. D., Sikich, J. A., Stahler, D.

R., Villela, P. M. S., Vollmers, C., Wayne, R. K., Eizirik, E., … Shapiro, B. (2019). Puma genomes from North and South America provide insights into the genomic consequences of inbreeding. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-12741-1

Schwartz, M. K., Tallmon, D. A., & Luikart, G. (1998). Review of DNA-based census and effective population size estimators. *Animal Conservation*, *1*(4), 293–299. https://doi.org/10.1017/S1367943098000274

Seidensticker, J. C., Hornocker, M. G., Wiles, W. V., & Messick, J. P. (1973). Mountain lion social organization in the Idaho primitive area. *Wildlife Monographs*, *35*, 3–60.

Stoner, D. C., Wolfe, M. L., Mecham, C., Mecham, M. B., Durham, S. L., & Choate, D. M. (2013). Dispersal behaviour of a polygynous carnivore: Do cougars Puma concolor follow source-sink predictions? *Wildlife Biology*, *19*(3), 289–301. https://doi.org/10.2981/12-124

Sunde, J., Yıldırım, Y., Tibblin, P., & Forsman, A. (2020). Comparing the performance of microsatellites and RADseq in population genetic studies: Analysis of data for pike (*Esox lucius*) and a synthesis of previous studies. *Frontiers in Genetics*, *11*(March), 1–17. https://doi.org/10.3389/fgene.2020.00218

Sweanor, L. L., Logan, K. A., & Hornocker, M. G. (2000). Cougar dispersal patterns, metapopulation dynamics, and conservation. *Conservation Biology*, *14*(3), 798–808. https://doi.org/10.1046/j.1523-1739.2000.99079.x

Trumbo, D. R., Salerno, P. E., Logan, K. A., Alldredge, M. W., Gagne, R. B., Kozakiewicz, C. P., Kraberger, S., Fountain-Jones, N. M., Craft, M. E., Carver, S., Ernest, H. B., Crooks, K. R., VandeWoude, S., & Funk, W. C. (2019). Urbanization impacts apex predator gene flow but not genetic diversity across an urban-rural divide. *Molecular Ecology*, *28*(22), 4926–4940. https://doi.org/10.1111/mec.15261

Uusi-Heikkilä, S., Whiteley, A. R., Kuparinen, A., Matsumura, S., Venturelli, P. A., Wolter, C., Slate, J., Primmer, C. R., Meinelt, T., Killen, S. S., Bierbach, D., Polverino, G., Ludwig, A., & Arlinghaus, R. (2015). The evolutionary legacy of size-selective harvesting extends from genes to populations. *Evolutionary Applications*, *8*(6), 597–620. https://doi.org/10.1111/eva.12268

van de Walle, J., Pigeon, G., Zedrosser, A., Swenson, J. E., & Pelletier, F. (2018). Hunting regulation favors slow life histories in a large carnivore. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-03506-3

vonHoldt, B. M., DeCandia, A. L., Heppenheimer, E., Janowitz-Koch, I., Shi, R., Zhou, H., German, C. A., Brzeski, K. E., Cassidy, K. A., Stahler, D. R., & Sinsheimer, J. S. (2020). Heritability of interpack aggression in a wild pedigreed population of North American grey wolves. *Molecular Ecology*, *29*(10), 1764–1775. https://doi.org/10.1111/mec.15349

Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics*, *160*(3), 1203–1215.

Waples, R. K., Larson, W. A., & Waples, R. S. (2016). Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, *117*(4), 233–240. https://doi.org/10.1038/hdy.2016.60

Waples, R. S. (2005). Genetic estimates of contemporary effective population size: To what time periods do the estimates apply? *Molecular Ecology*, *14*(11), 3335–3352. https://doi.org/10.1111/j.1365-294X.2005.02673.x

Whitman, K., Starfield, A. M., Quadling, H. S., & Packer, C. (2004). Sustainable trophy hunting of African lions. *Nature*, *428*(6979), 175–178. https://doi.org/10.1038/nature02395

Whittaker, D. (2011). Managing cougars in North America. In J. A. Jenks (Ed.), *Managing cougars in North America* (pp. 9–13). Berryman Institute Press.

Wolfe, M. L., Koons, D. N., Stoner, D. C., Terletsky, P., Gese, E. M., Choate, D. M., & Aubry, L. M. (2015). Is anthropogenic cougar mortality compensated by changes in natural mortality in Utah? Insight from long-term studies. *Biological Conservation*, *182*, 187–196. https://doi.org/10.1016/j.biocon.2014.12.008

Wultsch, C., Zeller, K. A., Welfelt, L. S., & Beausoleil, R. A. (2023). Genetic diversity, gene flow, and source-sink dynamics of cougars in the Pacific Northwest. *Conservation Genetics*, *24*, 793–806. https://doi.org/10.1007/s10592-023-01532-3

Zimmerman, S. J., Aldridge, C. L., & Oyler-McCance, S. J. (2020). An empirical comparison of population genetic analyses using microsatellite and SNP data for a species of conservation concern. *BMC Genomics*, *21*, 382. https://doi.org/10.1186/s12864-020-06783-9

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Erwin, J. A., Logan, K. A., Trumbo, D. R., Funk, W. C., & Culver, M. (2023). Effects of hunting on mating, relatedness, and genetic diversity in a puma population. *Molecular Ecology*, *00*, 1–18. https://doi.org/10.1111/mec.17237