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RESEARCH

Gene flow prevents genetic diversity loss despite small effective population size in fragmented grizzly bear (*Ursus arctos*) populations

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Abstract

Genetic monitoring is important in small, fragmented populations that rely on gene flow to maintain genetic diversity. The Selkirk, Yaak, and Cabinet grizzly bear (*Ursus arctos*) populations are among the smallest in North America and are near the southernmost extent of the species' range. These populations received little to no effective migration for generations but have recently experienced increased gene flow through natural migration and a population augmentation program. A long-term dataset of grizzly bear microsatellite genotypes from 1973 to 2021 presented a unique opportunity to examine genetic trends in these populations over time. We used this dataset of 464 bears to evaluate if gene flow affected observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (A_R), and average pairwise relatedness (r) in each of these populations. We also estimated effective population size (N_e) using the temporal and linkage disequilibrium (LD) methods. Post gene flow, A_R increased in the Selkirk and Cabinet populations and r decreased in all three populations. We did not observe any significant changes in H_E or H_O, but H_E values in our populations were significantly higher than those estimated using a model without gene flow. Our N_e estimates were consistent between the temporal and LD methods and ranged from 15.2 to 15.8, 15.4–17.5, and 5.6–8.9 for the Selkirk, Yaak, and Cabinet populations, respectively. Overall, our findings indicate that gene flow is increasing or maintaining genetic diversity in these populations. However, N_e remains low and additional connectivity or augmentation may be needed, particularly in the Cabinet population.

Keywords Genetic monitoring · Grizzly bear · Fragmentation · Effective population size · Genetic diversity

Introduction

Habitat loss and population fragmentation are major contributors to the current global decline in biodiversity (Wilcove et al. 1998; Pinto et al. 2024). Fragmentation interrupts natural movements associated with dispersal and breeding (Frankham 2006), which decreases effective population size (N_e) (England et al. 2010). Isolated populations with low N_e often lose genetic diversity more quickly than larger, more well-connected populations due to the effects of genetic drift (Lande 1988; Segelbacher et al. 2003). Small, isolated populations also experience higher levels of inbreeding, which can lead to reduced fitness (Liberg et al. 2005; Allendorf et al. 2012). Inbreeding and decreased genetic diversity have been shown to elevate extinction risk and reduce a population's ability to adapt to environmental change (Frankham 2005). However, gene flow between populations can help alleviate some of the deleterious effects of fragmentation,

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even if gene flow occurs at relatively low levels (Mills and Allendorf 1996; Gustafson et al. 2017).

Fragmentation affects a wide variety of plant and animal species, but large mammals may be disproportionately impacted due to their large home range requirements and potential for negative interactions with humans (Woodroffe and Ginsberg 1998; Rocha et al. 2018). Grizzly bears (*Ursus arctos*) in North America are one such example. Grizzly bears currently occupy approximately 2% of their historical range in the conterminous United States (U.S.) (Mattson and Merrill 2002) and persist in four recovery areas: the Greater Yellowstone Ecosystem (GYE), the Northern Continental Divide Ecosystem (NCDE), the Selkirk Ecosystem (SE), and the Cabinet-Yaak Ecosystem (CYE) (Fig. 1; U.S. Fish and Wildlife Service 1993). Although populations in these recovery areas have grown in recent years, there is relatively little migration between them (Proctor et al. 2012), resulting in fragmentation of what used to be a contiguous population. Grizzly bears in the conterminous U.S. are known to disperse up to 176 km (km) from their natal range, but highways and human development can be significant barriers to movement (Proctor et al. 2012, 2015). Male bears are more

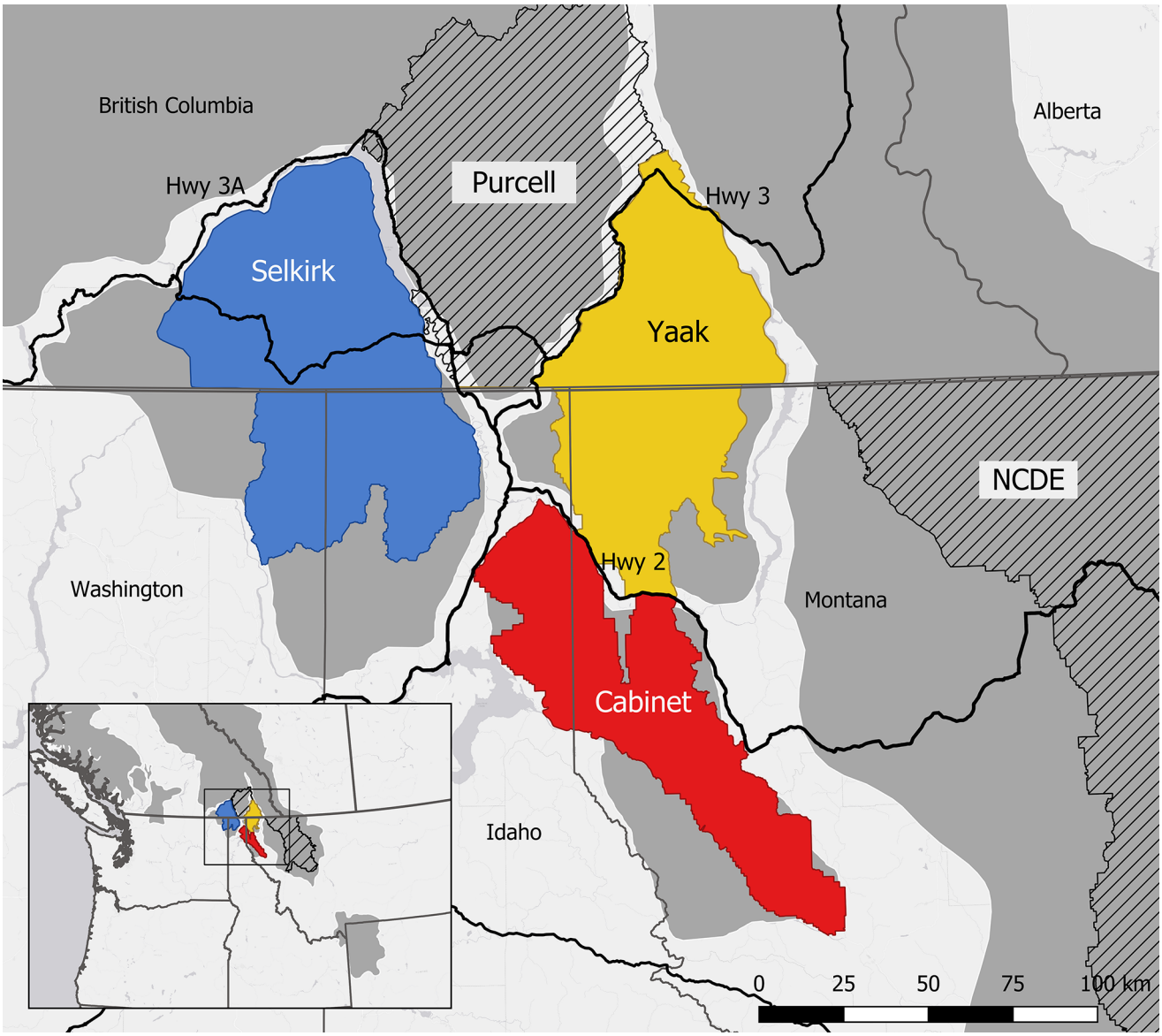

Fig. 1 Map of the study area in the northwestern United States and southern British Columbia. Solid labeled areas represent boundaries for the Selkirk and Cabinet-Yaak grizzly bear recovery zones. Actual grizzly bear distribution is not limited to these recovery zones, and highway boundaries and genetics were used to assign populations to bears detected outside recovery zone boundaries. Hashed lines represent the Northern Continental Divide Ecosystem (NCDE) and Purcell populations, which are the primary sources of migrants and augmentation bears into our study area. Solid black lines are relevant highways and gray shaded areas represent approximate extant grizzly bear distribution

likely than females to make long-distance dispersal movements (McLellan and Hovey 2001) and as a result, males may be the only individuals who introduce novel genetic material in areas with fragmented populations (Proctor et al. 2004). These barriers to movement and tendency for shorter female dispersal exacerbate the fragmentation observed at the southern end of grizzly bear range in North America (Proctor et al. 2012).

The SE and CYE contain some of the smallest grizzly bear populations in North America, with approximately 100 bears in the SE (Proctor et al. 2022), approximately 35 bears in the international Yaak/Yahk portion of the CYE (Proctor et al. 2018, 2023; Kendall et al. 2016), and approximately 29 bears in the Cabinet portion of the CYE (Kendall et al. 2016). Although the Cabinet and Yaak populations are in the same recovery area, they are divided by U.S. Highway 2 and the Kootenai River which limit movement between these populations (Kendall et al. 2016). The SE and CYE became fragmented from other grizzly bear populations in the early 20th century and remained completely isolated to gene flow for multiple bear generations due to highways and settled valleys between populations (Proctor et al. 2005, 2012).

Historically, two of the most problematic barriers were British Columbia (B.C.) Highway 3 A, which runs along the northern edge of the SE, and B.C. Highway 3, which runs along the northern edge of the CYE. These highways and their associated human settlement effectively prevented immigration between these recovery areas and larger, more well-connected grizzly bear populations further north in Canada, particularly the North Purcell population (Proctor et al. 2012). Generations of isolation caused the Selkirk population to have low expected heterozygosity (H_E) compared to other North American grizzly bear populations (Proctor et al. 2012). Although recent H_E estimates in the CYE are more comparable to estimates in other conterminous U.S. grizzly bear populations, the Yaak and Cabinet populations are small and the Cabinet population is inbred, signifying a need for higher levels of connectivity with other populations (Kendall et al. 2016).

In recent years, natural immigration across Highways 3 and 3 A into the SE and CYE from the Purcell Mountains to the north has increased due in part to habitat conservation and conflict prevention along key migration corridors (Proctor et al. 2018, 2022). Movements between the SE, CYE, and NCDE have also been observed in several instances. However, if migrants do not breed, they will not impact long-term genetic diversity of the population. Population genetic diversity, measured by H_E or allelic richness (A_R), is primarily impacted by effective migrants who successfully produce offspring in a recipient population to establish gene flow.

Gene flow into the CYE and SE was first documented in 2000 and 2006, respectively (Kasworm et al. 2021a, b). Including these initial events, nine effective migrants from the Purcell Mountains in B.C. have contributed gene flow to the CYE and five effective migrants from the Purcell Mountains and Yaak have contributed gene flow to the SE (Kasworm et al. 2023a, b). In the CYE, however, gene flow has been limited to the Yaak population, leaving the Cabinet portion of the CYE isolated from naturally occurring gene flow. To date, the only documented gene flow into the Cabinet population has been human-mediated through the Cabinets augmentation program led by the U.S. Fish and Wildlife Service (USFWS) and Montana Fish, Wildlife & Parks (Kasworm et al. 2023a). This program has translocated 22 bears into the Cabinet population from the NCDE since 1990, although not all these bears remained in the population after translocation. The first instance of an augmentation bear reproducing with a native Cabinet bear occurred in 1997. There is some evidence that gene flow has increased genetic diversity in the Selkirk population (Proctor et al. 2018), but definitive analysis of gene flow and augmentation impacts on the Selkirk and Cabinet-Yaak populations is lacking. Additionally, effective population size (N_e) has never been estimated for these populations.

Small grizzly bear populations like those in the SE and CYE are unlikely to reach a demographic or effective population size necessary to maintain genetic diversity in the absence of gene flow (Miller and Waits 2003; Proctor et al. 2015). Therefore, monitoring the impact of gene flow on these populations is critical to ensure that genetic diversity is being maintained. N_e estimation is also important to determine the susceptibility of these populations to genetic drift. Our objectives were to use a long-term dataset of grizzly bear nuclear DNA microsatellite genotypes from the Selkirk and Cabinet-Yaak populations to (i) estimate genetic diversity and average pairwise relatedness (r) over multiple bear generations to evaluate the impacts of recent gene flow and population augmentation, and (ii) estimate N_e of each population. We hypothesized that observed heterozygosity (H_O), H_E, and A_R would increase and r would decrease as a result of gene flow.

Materials and methods

Study area

The boundaries of our study area are largely defined by highways and human developments. The SE is bordered by B.C. Highway 3 A to the north, B.C. Highway 21 and U.S. Highways 95 and 2 to the east, U.S. Highway 2 to the south, and B.C. Highway 22 and Washington Highway 31

to the west (Fig. 1). The core of the SE is the Selkirk grizzly bear recovery area, an international recovery area that includes the B.C. South Selkirk grizzly bear population unit and encompasses approximately 6,700 km^2 in northeastern Washington, northern Idaho, and southern B.C. (U.S. Fish and Wildlife Service 1993). The CYE is bordered by B.C. Highway 3 to the north, Lake Koocanusa and U.S. Highway 2 to the east, Montana Highway 200 to the south, and Highway 95 to the west. The Cabinet-Yaak grizzly bear recovery zone is central to the CYE, but unlike the Selkirk recovery area, the Cabinet-Yaak recovery area does not include the adjacent B.C. Yahk grizzly bear population unit. However, for the purposes of this study the term CYE refers to both the Cabinet-Yaak recovery area and the Yahk grizzly bear population unit in B.C. This aligns with the well-documented observation that bears freely cross the U.S.-Canada international border (Kasworm et al. 2023b). The CYE encompasses approximately 6,800 km^2 in northern Idaho and northwestern Montana and approximately 1,500 km^2 in southern B.C.

Field methods

DNA samples were collected from captured bears, mortalities, and noninvasive hair or fecal samples. Capture and handling of bears followed an approved Animal Use Protocol through the University of Montana, Missoula, MT (061-14CSCFC111714 and 040-20HCCFC-092420).

Grizzly bear captures were performed under Montana, Idaho and Washington state collection permits (MT 2020-066-W, ID 140226, and WA20-074) and a federal permit (TE704930-0). Most samples were obtained through an annual genetic sampling effort using hair corrals and rub objects. Hair corrals consisted of a single strand of barbed wire strung around four or more trees approximately 50 cm from the ground (Woods et al. 1999). A liquid mixture of aged cattle blood and fish oil was poured in the center of the enclosure to attract bears without providing a food reward. Samples were also collected from rub trees and posts with barbed wire attached to increase the probability of collecting high-quality hair samples. Hair corrals and rubs were checked approximately every four weeks during the field season (May–September) to minimize DNA sample degradation due to sunlight and moisture exposure. Samples were stored in paper envelopes, placed in desiccant, and stored at room temperature until they were sent to the lab.

Laboratory methods

Extraction and genotyping were conducted by Wildlife Genetics, International in Nelson, B.C. Extraction followed established protocols using QIAGEN's DNeasy tissue kits as detailed in numerous other studies (Paetkau 2003; Proctor et al. 2012; Kendall et al. 2016). Samples were genotyped at 21 microsatellite loci (G10J, G10B, G1D, G10H, G10M, MU50, G10P, CXX110, G1A, G10C, G10L, G10U, G10X, CXX20, MU59, MU23, REN145P07, MSUT2, CPH9, REN144A06, MU51) and an amelogenin locus to determine sex (Ennis and Gallagher 1994). A few samples were genotyped at only 15 microsatellite loci due to their age or low DNA quantity and a lack of additional genetic material. These 21 loci have been used extensively in brown bear genetic studies and have been described as unlinked, independent, and meeting Hardy-Weinberg expectations (Proctor et al. 2012). After genotyping, all samples were error-checked following protocols detailed in Paetkau (2003) and Kendall et al. (2008).

Dataset

Sampling from 1983 to 2021 yielded a dataset of 464 total bears sampled in the SE and CYE. We analyzed the Selkirk, Yaak, and Cabinet populations separately. Although the Cabinet and Yaak populations are located in the same grizzly bear recovery zone, prior studies have found no evidence of gene flow into the Cabinets, except through augmentation, and Cabinet bears clustered separately from Yaak bears in a recent principal components analysis of microsatellite genotypes (Kendall et al. 2016). Therefore, we analyzed the Cabinet and Yaak populations separately to examine genetic diversity and N_e of this recovery area at a finer scale. The total number of individual bears sampled in each study area was 276 bears in the SE, 135 bears in the Yaak, and 67 bears in the Cabinets, 8 of which were augmentation bears. A few migrant bears were sampled in multiple populations. Birth years for bears spanned from 1973 to 2021 and were obtained through cementum annuli analysis of bears sampled during live capture or mortality events, or by detecting bears as dependent young (Scheffer 1950; Mundy and Fuller 1964).

We used population samples and definite origin samples for different analyses. Population samples included all bears present in a population at any time, regardless of their origin. Definite origin samples came from bears that: (i) were detected as dependent young (age 0–2 years); (ii) have parentage tying them to a mother whose location was known at the time of their birth; or (iii) were genetically assigned based on the GENECLASS assignment methods detailed in Proctor et al. (2005).

Hardy-Weinberg and linkage disequilibrium

We split our dataset into 10-year population samples to match the generation interval of grizzly bears (U.S. Fish

and Wildlife Service 2021). We then tested for deviations from Hardy-Weinberg equilibrium and evidence of linkage disequilibrium (LD) across all loci and 10-year groups in the Selkirk, Yaak, and Cabinet datasets. We used the *pegas* (Paradis 2010) and *genepop* (Rousset 2008) packages in R and implemented a Bonferroni correction for multiple tests.

Genetic diversity and relatedness over time

To study genetic diversity over time and the impact of gene flow, bears from each population were divided into cohorts based on year of birth (YOB). Each cohort spanned 10 years and was designed to align with the first known instance of gene flow in each population to clearly differentiate groups pre- and post-gene flow. To focus on the effects of gene flow on our study populations, only bears with definite origins in one of these populations were included in our analysis of genetic diversity over time and known immigrants were excluded. Sample sizes and YOB for definite origin bears are shown in Table 1.

We estimated H_O and unbiased H_E for each cohort using the *hierfstat* package in R (Goudet 2005) and we estimated A_R for each cohort using the *adegenet* package in R (Jombart 2008), which uses a rarefaction method to correct for unequal sample sizes. We used Wang's estimator, which is robust at small sample sizes, to obtain estimates of r (Wang 2002). We implemented this estimator using the *Demerelate* package in R (Kraemer and Gerlach 2017).

We used a two-sided Fisher's exact test to evaluate if there was a difference in H_O between pre-gene flow cohorts over all 21 loci. If no significant difference between pre-gene flow cohorts was found, we combined them into a single pre-gene flow sample. We then used a two-sided exact test to evaluate changes in H_O across loci in subsequent generations post gene flow. Using the exact test allowed us to test for significant differences in the number of individuals heterozygous at each locus across time periods. A Bonferroni correction was used to correct for testing across all 21 loci.

Fisher's exact test is only suitable for count data, so we used a two-sided paired t-test for H_E and A_R using locus as the paired measure, and a two-sided two sample t-test for r to evaluate if there was a significant difference between cohorts prior to gene flow. If these cohorts were not significantly different, they were pooled into a single pre-gene flow sample. To test for differences between the pre-gene flow sample and each subsequent cohort, we then used a two-sided paired t-test with locus as the paired measure for H_E and A_R and a two-sided two sample t-test for r. We tested the differences between cohorts for normality using a Shapiro-Wilk test. When these differences were not normally distributed, we either log-transformed the data or used a paired Wilcoxon sign rank test instead of a t-test. We used a Type I error rate of $\alpha = 0.05$ for all significance tests.

Table 1 Sample sizes and years of birth for genotyped bears with definite origins in the Selkirk, Yaak, or Cabinet populations

	Selkirk	Yaak	Cabinet
Definite origin bears	138	60	45
Years of birth	1981–2021	1986–2020	1973–2020

There was only one bear in our dataset with known YOB and definite Cabinet origins prior to 1997, the first year that augmentation-mediated gene flow was detected in this population. Because a single pre-augmentation individual would not be sufficient to compare pre- and post- augmentation genetic diversity, we instead tested for significant differences in H_O, H_E, A_R, and r between each post-augmentation cohort in the Cabinet population to evaluate if these metrics are changing over time. We used the same methods as described above for the Selkirk and Yaak populations. A summary of all statistical tests used is given in Table S1.

Effective population size

We estimated effective population size using the temporal and LD methods. The temporal method assumes that changes in allele frequencies over time are due solely to genetic drift and involves sampling a population at two or more intervals and estimating N_e across that time period (Nei and Tajima 1981). The LD method is a single sample approach based on the principle that gamete disequilibrium depends on N_e in a given generation and the effective number of breeders (N_b) that produced a given cohort (Hill 1981).

We estimated N_e and N_b over three generations (1990–2020) for the Selkirk and Yaak populations and over two generations (2000–2020) for the Cabinet population. These time intervals were chosen to both maximize sample sizes and maximize the time between samples to obtain the strongest possible drift signal (Nei and Tajima 1981). For the temporal method, we took two population samples with all bears known to be alive in the first and last years of the chosen time period and used the unbiased estimator described in Jorde and Ryman (2007). We used Plan I sampling, which requires the input of a census population size for the first year of sampling. In the SE and Yaak, we used previously estimated population growth rates (Kasworm et al. 2023a, b) to back-calculate to 1990 from published population estimates. In the Cabinets, we used a census size of 6 in 2000 due to population growth rates in the CYE primarily reflecting the Yaak portion of the recovery area.

For the LD method, we used three-year cohorts based on YOB and estimated N_b for each cohort, then averaged across all cohorts to get an estimate of N_b over the same time period as the temporal method (Kamath et al. 2015). We removed any bears with known origins outside the focal population.

We assumed three years as the absolute minimum age of reproduction in grizzly bears, so by using three-year cohorts based on YOB we were able to avoid including parents and offspring in the same cohort which would have violated the LD assumption of discrete generations. We used NeEstimator V2.1 (Do et al. 2014) for the temporal and LD methods, assumed a random mating system, and used a critical allele frequency of 0.01.

We estimated the effective to census population size ratio, or N_e/N_c for all three populations. N_c values were obtained from the most recent available population estimates in our study area. These population estimates included bears of all age classes. There are some disadvantages to comparing temporal N_e (which applies to the entire time period over which sampling occurs) with a population estimate from a portion of the study period (Waples 2005), but estimation of N_e/N_c at a finer scale was not feasible due to the scarcity of published demographic estimates in our populations over time.

Genetic drift

To estimate what current H_E would have been in the absence of gene flow we used the equation for genetic drift given by:

$$H_t = H_0(1 - \frac{1}{2N_e})^t$$

Where H_t is the heterozygosity at generation t, H_0 is the initial heterozygosity, and N_e is the initial effective population size (Wright 1931). We used H_E estimates for the generation immediately preceding the occurrence of gene flow and we used the estimated N_e for the generation prior to gene flow in each population from the LD methods described above. We performed the drift calculation at each locus, then used a one-sided paired t-test with loci as the paired measure to evaluate if our actual H_E estimates were significantly higher than what would be predicted under the drift equation.

Results

Dataset summary, Hardy-Weinberg, and linkage disequilibrium

Our final dataset consisted of 452 bears that were genotyped at 21 loci, 11 bears that were genotyped at 15 loci, and one bear with complete genotypes at 18 loci and partial genotypes at three loci. The number of alleles per locus ranged from 5 to 11 and averaged 7.5. Our HWE and LD tests did not support removing any loci from the dataset, so all 21 loci were used in subsequent analyses (Supplemental Material).

Genetic diversity over time

We did not observe a significant difference in H_O, H_E, A_R, or r between pre-gene flow cohorts (1981–1995 and 1996–2005) in the SE, so we pooled these cohorts for further analysis (Supplementary Material Table S1). Observed heterozygosity of native bears in the SE ranged from 0.59 to 0.61 throughout our study period (Supplementary Material Table S2). We observed a significant increase in H_O at one locus (G10C) and a significant decrease at one locus (G10X) from the pre-gene flow period to 2006–2015, and a significant decrease at 2 loci (MU50 and G10X) from the pre-gene flow period to the 2016–2021 cohort. Expected heterozygosity ranged from 0.56 to 0.60, and we did not observe a significant change in H_E from the pre gene flow sample to either of the cohorts after gene flow. Estimates of A_R ranged from 4.24 to 4.61 and A_R was significantly elevated in both the 2006–2015 and 2016–2021 cohorts when compared to the pre-gene flow condition. Pairwise relatedness was between 0.002 and 0.110 and was significantly lower in the 2006 and 2016 cohorts than in the sample prior to gene flow (Fig. 2).

Similar to the Selkirk population, we did not observe a significant difference in H_O, H_E, A_R, or r between pre-gene flow cohorts (1986–1989 and 1990–1999) in the Yaak population, so we pooled these cohorts for further analysis. Observed heterozygosity of native bears in the Yaak ranged from 0.61 to 0.66 throughout our study period and did not significantly change at any locus from the pre-gene flow period to the 2000 cohort. However, H_O increased at two loci (MU23 and REN145P07) and decreased at two loci (G10C and REN144A06) between the pre-gene flow sample and the 2010 cohort (Supplementary Material Table S3). Expected heterozygosity and allelic richness of native bears in the Yaak throughout our study period ranged from 0.54 to 0.60 and 3.76–4.15, respectively, and we did not observe a significant change in H_E or A_R from the pre gene flow sample to either of the cohorts after gene flow. Pairwise relatedness ranged from 0.069 to 0.213 and was significantly lower in the 2000 and 2010 cohorts than in the sample prior to gene flow.

Observed heterozygosity for the pre-gene flow individual in the Cabinets was 0.71; however, we could not estimate H_E or A_R with a sample size of one (Supplementary Material Table S4). Observed and expected heterozygosity of post-augmentation bears in the Cabinets ranged from 0.59 to 0.73 and 0.54–0.56, respectively. There was no significant change in H_O at any locus or H_E between the post gene flow cohorts. Allelic richness ranged from 2.76 to 3.42 and significantly increased from the 1997 cohort to the 2007 cohort, and from the 2007 cohort to the 2017 cohort. Pairwise relatedness

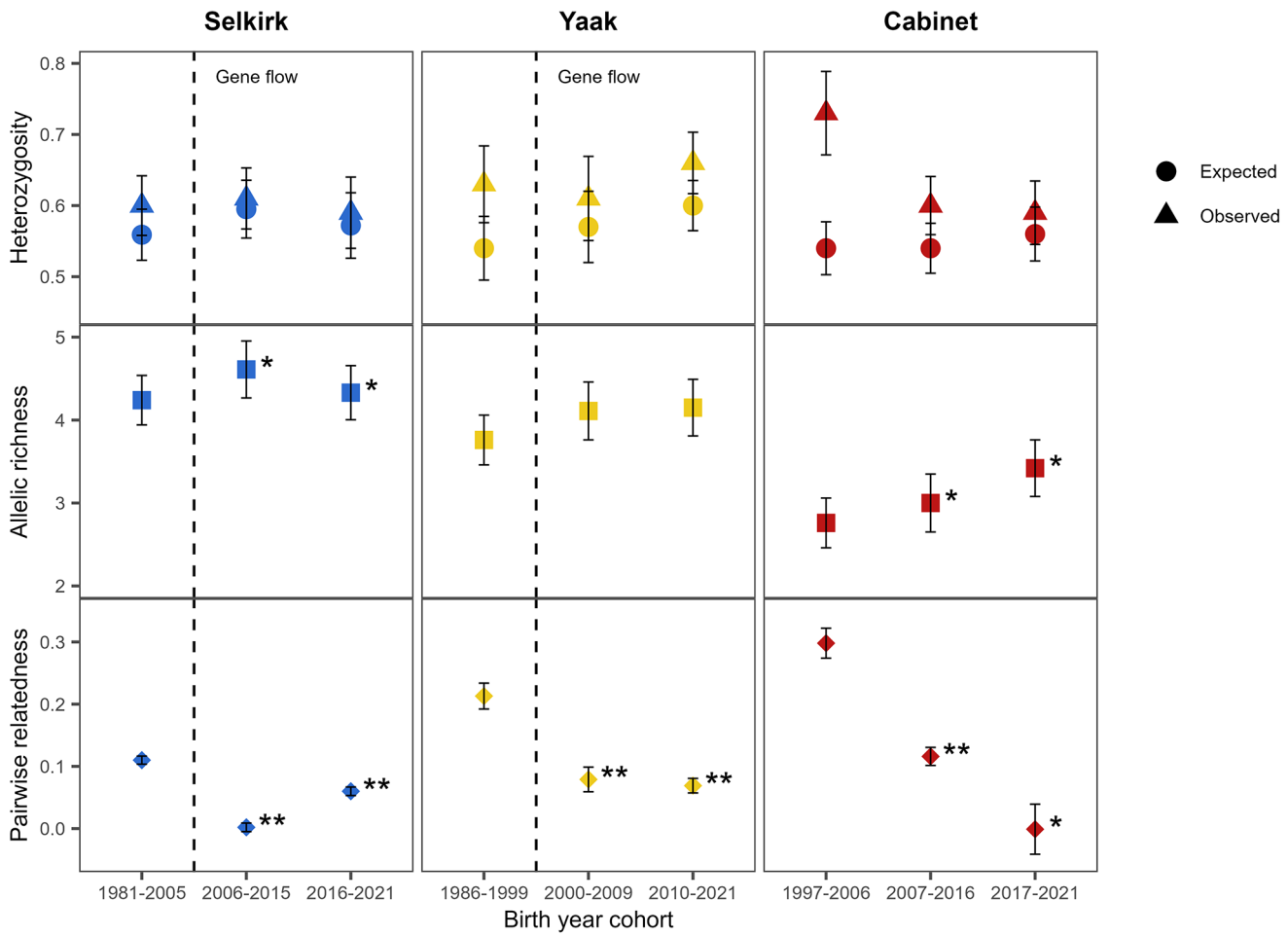

Fig. 2 Observed and expected heterozygosity, allelic richness, and average pairwise relatedness for the Selkirk, Yaak, and Cabinet populations. Asterisks (* = $p<0.05$, **=$p<0.01$) denote a significant difference from the pre-gene flow period (Selkirk and Yaak) or the preceding generation (Cabinet). Error bars represent standard error. Allelic richness is only comparable over time within each population and should not be compared between populations due to different rarefaction sample sizes

Table 2 Estimates of temporal and linkage disequilibrium effective population size, effective to census population size ratios, and the time period over which each estimate applies

Population	Time period	Temporal N_e (95% CI)	LD N_e	N_e/N_c
Selkirk	1990–2020	15.8 (10.1–36.6)	15.2	0.15–0.16
Yaak	1990–2020	17.5 (11.1–42.1)	15.4	0.44–0.50
Cabinet	2000–2020	8.9 (7.6–10.7)	5.6	0.19–0.31

was between −0.001 and 0.298 and significantly decreased in each subsequent generation.

Effective population size

In the SE, N_e from 1990 to 2020 was 15.8 (95% CI: 10.1–36.6) using the temporal method and 15.2 using the LD method (Table 2). In the Yaak, N_e from 1990 to 2020 was 17.5 (95% CI: 11.1–42.1) using the temporal method and 15.4 using the LD method. In the Cabinets, N_e from 2000 to 2020 was 8.9 (95% CI: 7.6–10.7) using the temporal method and 5.6 using the LD method. 95% confidence intervals for all LD cohorts are available in the Supplementary Material (Tables S5-S7), and cohorts yielding an infinite estimate of N_b in the LD method were not included in calculations of the mean over the sample period. Using contemporary N_c estimates of 100 for the Selkirk population (Proctor et al. 2022), 35 for the Yaak population (Proctor et al. 2018, 2024; Kendall et al. 2016), and 29 for the Cabinet population (Kendall et al. 2016), N_e/N_c ratios were 0.15–0.16, 0.44–0.50, and 0.19–0.31, respectively.

Projections of genetic drift

Effective population size for the Selkirk cohort prior to gene flow (1996–2005) was 11.7 based on the LD estimator and H_E for the same cohort was 0.56. Using the drift equation, H_E would have declined to 0.54 and 0.51 after one and two generations without gene flow, respectively (Fig. 3). These projected estimates were significantly lower than the

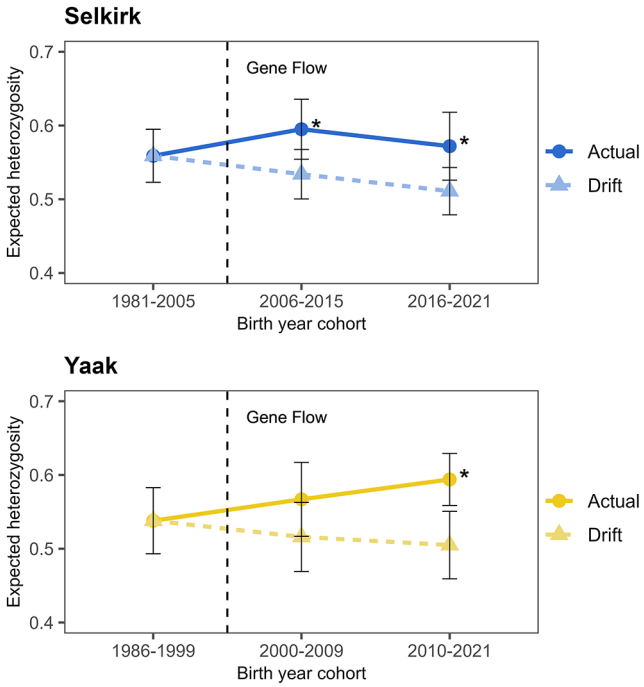

Fig. 3 Expected heterozygosity (H_E) from the Selkirk and Yaak populations compared to H_E predicted under a drift-only scenario. Error bars represent standard error. Asterisks denote H_E that is significantly higher than predicted under drift ($p < 0.05$). Vertical dashed line divides sample into pre (left) and post (right) gene flow cohorts

H_E values we observed in the SE in both generations post gene flow ($p = 0.032$ and $p = 0.045$). N_e for the Yaak cohort prior to gene flow (1990–1999) was 22.5 based on the LD estimator, and H_E for this cohort was 0.53. Using the drift equation, H_E would have declined to 0.52 and 0.51 after one and two generations without gene flow, respectively. The projected drift estimate of H_E after one generation was not significantly lower than our actual H_E estimates, but the projected estimate after two generations was significantly lower ($p = 0.028$). Extremely small sample sizes made LD N_e estimation of the 1997–2006 Cabinet cohort difficult, and we decided that uncertainty was too high to realistically estimate loss of diversity due to drift in this population.

Discussion

Genetic monitoring is particularly important for small, fragmented populations such as grizzly bears in the SE and CYE. However, obtaining the detailed genetic information necessary to evaluate the effects of gene flow and historic isolation, particularly in such a long-lived species, is challenging. Long-term monitoring in the SE and CYE provided a unique opportunity to examine genetic changes over four generations of grizzly bears. Our research shows that genetic diversity has increased or remained constant in these populations, with no evidence of declines. However, our estimates of N_e are small. Monitoring N_e in small populations is critical to understand how quickly genetic variation may be lost due to drift, and as the first N_e estimates for the Selkirk, Cabinet, and Yaak populations, our results will be a valuable baseline for future monitoring.

Genetic diversity and relatedness

We predicted that the onset of gene flow into each population would increase H_O, H_E, and A_R and decrease r of bears born into these populations. Previous studies on genetic rescue have shown increased H_E and A_R with the addition of just one breeding migrant in a small cougar population (Gustafson et al. 2017), increased H_E after population augmentation in sage grouse (Zimmerman et al. 2019), and decreased r after outcrossing in salmon (Pregler et al. 2023). In our study area, A_R significantly increased after gene flow in the SE and remained significantly elevated two generations after the advent of gene flow. Allelic richness also increased over the two most recent generations in the Cabinets, which is likely due to the ongoing augmentation program. This, combined with significant decreases in r in all three populations, provides evidence of the beneficial impact gene flow is having on these populations. We also observed an upward trend in A_R in the Yaak after gene flow, although this change was not statistically significant. While the Selkirk and Cabinet populations have a history of isolation to both sexes, the Yaak has historically only been isolated to female movements, not those of males (Proctor et al. 2012). It is possible that this limited male migration resulted in some undetected gene flow earlier than 2000, which may have reduced the visible impact of gene flow after the early 2000s in our study.

We also detected no significant increase in H_O (at more than 2 of 21 loci) or H_E in any of our study populations after gene flow or augmentation. Expected heterozygosity is one of the most common indicators of genetic diversity and is valuable for evaluating a population's adaptive potential (Allendorf 1986). However, A_R will respond more quickly to a genetic bottleneck or genetic rescue than H_E because it is based on the number of alleles present at a locus rather than relative allele frequencies (Allendorf 1986). The different responses we observed between H_E and A_R demonstrate the importance of monitoring multiple indices of genetic variation when evaluating the impacts of gene flow or genetic rescue.

Although we did not observe an increase in H_E or H_O in any of our populations, we also did not find any decreases in heterozygosity between the pre- and post- gene flow samples. In the absence of gene flow, genetic diversity will decline quickly in populations with small N_e due to drift.

The maintenance of genetic diversity in these small populations should not be overlooked as a positive outcome of management efforts. The results of our genetic drift projection also lend support to this conclusion by showing that H_E values in the SE and Yaak are significantly higher than they would likely be in the absence of gene flow, although there is some uncertainty associated with the projected H_E estimates. This, combined with the observed increases in A_R, indicates that gene flow as a result of natural movements and augmentation are likely occurring at sufficient levels to mitigate the effects of genetic drift. However, population genetic responses to changes in gene flow often occur slowly, particularly in species with long generation intervals such as grizzly bears. Continued monitoring of genetic trends, potentially with genomic markers such as single nucleotide polymorphisms, will be important to ensure that genetic diversity continues to increase or be maintained by gene flow.

For all our pre and post gene flow comparisons, we estimated genetic diversity only for bears with known origins and YOB; thus, our estimates differ slightly from estimates obtained through population-wide sampling such as those found in Proctor et al. (2012) and Kendall et al. (2016). Population-wide samples may include migrants that are present in the population at the time of sampling but if these migrants do not reproduce, they will not impact genetic diversity long-term. Our genetic diversity methods were designed to measure the impacts of gene flow on each population by only including offspring of migrants and excluding migrants themselves.

Cabinet population

Genetic diversity estimates prior to augmentation-mediated gene flow were not possible in the Cabinet population due to a sample size of one and estimates of N_b for the individual cohorts necessary for the drift equation had high levels of uncertainty. Thus, we were unable to test the hypothesis that augmentation significantly increased genetic diversity in the Cabinets or estimate what current H_E would have been in a drift-only scenario. Despite the difficulties associated with small sample sizes in the Cabinets, we felt that it was valuable to conduct analyses of the Cabinet population separately from the Yaak population even though both populations occupy the same recovery area. Migration into the Cabinet population is limited and the only documented gene flow into the Cabinets has come from the augmentation program. In addition, a majority of litters born in the Cabinets after 2000 were fathered by one male bear (770), suggesting that inbreeding may be a concern in this population (Kendall et al. 2016; Kasworm et al. 2023a). Combining the Yaak and Cabinet populations for analysis may obscure patterns unique to each population and may bias estimates toward the Yaak population (Kasworm et al. 2021a).

One of the aims of this research was to evaluate the genetic effects of the Cabinet augmentation program. Based on the small N_e estimates from 2000 to 2020 in the Cabinets we would expect this population to lose genetic diversity quickly over just a few generations in the absence of gene flow. We found no decline in H_E and H_O and an increase in A_R, which is consistent with gene flow through augmentation. Observed heterozygosity was much higher than expected in the 1997–2006 Cabinet cohort, which could indicate a recent bottleneck in the Cabinet population (Nei et al. 1975). This pattern in heterozygosity may also indicate an isolate-breaking effect, where the mixing of two previously isolated populations causes H_O to temporarily increase over H_E. While we did not perform a formal bottleneck test as part of our research, both of these hypotheses would suggest a Cabinet population that had a low number of breeders and was isolated prior to the introduction of augmentation bears from the NCDE. The Cabinet population was extremely small prior to augmentation (< 15 bears) and almost all Cabinet bears today are augmentation bears or their descendants (Kendall et al. 2016; Kasworm et al. 2023a). While the genetic impacts of the augmentation program are undoubtedly relevant, the augmentation program likely saved the Cabinet population from the more immediate threat of demographic extinction (Kasworm et al. 2023a). As the Cabinet population continues to grow and the likelihood of demographic extinction diminishes, restoring natural gene flow between the Cabinets and other grizzly bear populations will be important to maintain long-term genetic diversity.

Effective population size

Estimates of N_e using temporal and LD methods were similar in all three of our populations. LD-based estimates were derived from averaging N_b estimates across cohorts, but N_e and N_b are not always the same and their relationship is complex and variable across taxa. However, in grizzly bears the ratio of N_b/N_e is close to 1 and single-sample estimates of N_b can generally be compared to multi-sample estimates of N_e if they are estimated over the same time period (Waples et al. 2013). LD estimates of N_b were variable across cohorts and this method yielded an infinite estimate for one cohort in the Selkirk population, two cohorts in the Yaak population, and three cohorts in the Cabinet population (Supplementary Material Tables S5–S7). Sample sizes for our cohorts ranged from $n = 3$–24 and the infinite N_b estimates resulted from cohorts with $n = 3$–7, suggesting that the poorest performance was linked to the smallest sample sizes. Kamath et al. (2015) demonstrated that LD-based methods are less

biased by small sample size than other methods; however, a sample size of $n > 30$ for each cohort has been previously recommended (Waples 2006; Waples and Do 2010). Meeting this target was not possible due to the small size of our study populations and variable sampling effort over time. However, the agreement between the temporal and LD methods in each of our populations lends support to our LD estimates and provides more robust estimates of N_e overall.

Most methods of estimating N_e or N_b are based on an idealized Wright-Fisher population and assume a population is closed to migration. Migration does occur in our system at relatively low levels and may bias estimates of N_e (Wang and Whitlock 2003). However, the LD and temporal methods can be robust to violations of population closure as long as migration rates are less than approximately 5–10% (Waples and England 2011). We did not directly estimate migration rates, but we were able to identify migrants in each population using sampling records, radio collar records, and GENECLASS. When these known migrants were removed from our temporal samples, the resulting estimates of temporal N_e were very similar to those including migrants, differing by a maximum of 0.7 (Supplementary Material Table S8). Although this method is imperfect, it provides some evidence that migration rates may be low enough to not significantly bias our temporal N_e estimates. The similarity of our migrant-inclusive temporal N_e estimates and our LD-based estimates, which exclude migrants, also suggests a minimal migration bias. However, explicitly quantifying migration rates and incorporating them into global N_e estimates would be beneficial for future work.

The effective to census population size ratio, or N_e/N_c is useful to evaluate the relative contribution of demographic and genetic factors to population persistence (Palstra and Fraser 2012). Our N_e/N_c ratios of 0.15–0.50 are comparable to ratios obtained through simulations (0.24–0.32; Harris and Allendorf 1989), higher than ratios in Alaskan brown bear populations obtained from a stepwise mutation model (0.04–0.19; Paetkau et al. 1998), and lower than recent ratios in the GYE obtained from the estimator by parentage method (0.42–0.66; Kamath et al. 2015). While comparison of N_e/N_c ratios between different populations can indicate biological differences between populations, it is important to note that differences in N_e/N_c ratios may also reflect differences or statistical uncertainty based in methodology (Palstra and Fraser 2012). The small N_c values of our populations may also contribute to higher N_e/N_c ratios (Palstra and Ruzzante 2008). Although our N_e/N_c ratios are within the bounds of those previously estimated for brown bears, our N_e estimates do not meet the 50/500 rule. First proposed by Franklin (1980), this rule suggests a minimum inbreeding effective population size of 50 to avoid immediate deleterious effects from inbreeding and a minimum variance effective population size of 500 to retain evolutionary potential long-term. There is some debate regarding the general applicability of this rule, and some suggest the 50/500 rule is not sufficient and should be increased to 100/1000 (Frankham et al. 2014). Applying the 50/500 rule to populations that are part of a metapopulation can further complicate the interpretation of this rule (Ryman et al. 2019). In well-connected metapopulations, the target of $N_e > 500$ should apply to the metapopulation rather than each individual subpopulation (Laikre et al. 2013, 2016). Although we did not estimate inbreeding N_e, our temporal estimates of variance N_e were 8.9, 17.5, and 15.8 for the Cabinets, Yaak, and SE, respectively. These effective population sizes are likely too small to maintain genetic diversity according to the 50/500 rule without migration and augmentation. Our estimates of N_e also fail to meet the target of $N_e > 500$ set by the Kunming-Montreal Global Biodiversity Framework (Convention on Biological Diversity 2022). However, providing a target N_e in any population is difficult, and this process must take into account realistic constraints on N_c and connectivity opportunities across the landscape (Jamieson and Allendorf 2012). As inter-population connectivity increases, estimating metapopulation N_e for the international Selkirk and Purcell Mountain ecosystems would be a worthwhile goal for future research.

Our research adds to the growing body of work that emphasizes a need for connectivity between the SE, CYE, and Canadian grizzly bear populations. Unlike other U.S. recovery zones and many Canadian populations, the SE and CYE lack large national parks or wilderness areas that can serve as core habitat. As a result, the SE and CYE have higher anthropogenic influence which contributes to relatively low bear densities compared with other North American populations (Kendall et al. 2016). Grizzly bears dispersing across our study area often must spend prolonged periods of time navigating human development associated with highways between populations, resulting in elevated mortality associated with both vehicle strikes and conflict with humans (Proctor et al. 2012, 2015). This mortality risk is particularly acute for female bears, who naturally disperse more gradually than males (McLellan and Hovey 2001). The combination of both direct and indirect highway-associated mortality requires a multifaceted approach to restoring connectivity between populations. A small number of wildlife crossing structures such as overpasses and underpasses have been constructed along highways in our study area and may provide some reduction in vehicle strike mortality on highways. However, extensive human presence across the landscape, particularly along highway corridors, is a stronger predictor of fragmentation among grizzly bear populations in the U.S. than highways themselves (Proctor et al. 2012, 2018). To address this, managers in the SE and CYE have employed a variety of human-bear conflict reduction strategies such as outreach and education efforts, electric fencing cost-shares, subsidized bear-resistant garbage container programs, and backcountry access management to increase non-lethal

management options for bears in areas with increased human development (Proctor et al. 2018, 2022). Managers and non-profit partners have also used conservation easements and land purchases to maintain and restore key grizzly bear connectivity areas (Proctor et al. 2018). These efforts to boost connectivity are likely a major reason for the maintenance of H_E and the increase in A_R we observed in our results.

The importance of gene flow into the Selkirk and Cabinet-Yaak grizzly bear populations has previously been emphasized by Proctor et al. (2005, 2012) and Kendall et al. (2016), among others. Long-term genetic monitoring enabled us to evaluate the impact of gene flow and estimate N_e for the first time in these populations. Our results show elevated allelic richness and decreased relatedness post-gene flow, while heterozygosity is being maintained. Although N_e is low in all three populations, we did not see evidence that genetic diversity is eroding due to genetic drift. Our results suggest that management actions undertaken to increase connectivity are having a positive impact on population genetics and their continuation would be beneficial given the small demographic and effective population sizes in the SE and CYE.

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Author contributions M.T., L.W., W.K., and J.T. designed the study. M.T. contributed to sample collection, performed analyses, created figures, and wrote the manuscript. M.F. helped with statistical methods. M.P. contributed numerous bear genotypes from his work in British Columbia. L.W., W.K., J.T., and M.F. reviewed all manuscript drafts. All authors read and reviewed the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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