










# Genomic analyses reveal range-wide devastation of sea otter populations

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## Abstract

The genetic consequences of species-wide declines are rarely quantified because the timing and extent of the decline varies across the species' range. The sea otter (*Enhydra lutris*) is a unique model in this regard. Their dramatic decline from thousands to fewer than 100 individuals per population occurred range-wide and nearly simultaneously due to the 18th–19th century fur trade. Consequently, each sea otter population represents an independent natural experiment of recovery after extreme population decline. We designed sequence capture probes for 50 Mb of sea otter exonic and neutral genomic regions. We sequenced 107 sea otters from five populations that span the species range to high coverage (18–76×) and three historical Californian samples from ~1500 and ~200 years ago to low coverage (1.5–3.5×). We observe distinct population structure and find that sea otters in California are the last survivors of a divergent lineage isolated for thousands of years and therefore warrant special conservation concern. We detect signals of extreme population decline

James Bodkin is Retired.

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in every surviving sea otter population and use this demographic history to design forward-in-time simulations of coding sequence. Our simulations indicate that this decline could lower the fitness of recovering populations for generations. However, the simulations also demonstrate how historically low effective population sizes prior to the fur trade may have mitigated the effects of population decline on genetic health. Our comprehensive approach shows how demographic inference from genomic data, coupled with simulations, allows assessment of extinction risk and different models of recovery.

#### KEYWORDS

conservation genomics, demographic simulations, genetic load, population bottleneck, sea otters

## 1 | INTRODUCTION

The historical range of the sea otter (*Enhydra lutris*) spanned the Northern Pacific Rim from the Kuril Islands of Russia to Baja California (Figure 1a; Figure S1A). Across this range, sea otter populations were hunted to extinction or the brink of extinction by the 18th–19th century fur trade (Kenyon, 1969; Riedman & Estes, 1990). The few populations that survived in the Kuril Islands, Kamchatka Peninsula, Commander and Aleutian Islands, central Alaska, and California contained fewer than 100 survivors apiece, a severe population bottleneck from previous population sizes estimated at 10,000–20,000 individuals per population (Riedman & Estes, 1990). The loss of sea otters triggered a trophic cascade that led to the disappearance of kelp forests through overgrazing by sea urchins (Estes & Palmisano, 1974). Sea otters were formally protected in 1911 by the International Fur Seal Treaty, and populations have increased and been reintroduced into southeast Alaska, British Columbia, Washington State and southern California where they had been fully extirpated (Jameson et al., 1982). Sea otters in Baja California, Mexico, were declared probably extinct due to the fur trade (Kenyon, 1969), but there have continued to be sporadic sightings in the region (Gallo-Reynoso & Rathbun, 1997; Schramm et al., 2014). The recent history of sea otter populations provides a unique model for species decline, because rarely are bottlenecks so uniform and near-concurrent across an entire species distribution. Each surviving sea otter population represents a natural replicate of an extreme bottleneck event, offering a powerful system to investigate the genomic consequences of near-extinction.

Previous genetic studies of the sea otter considered microsatellite loci and mitochondrial control region sequences which showed population structure and low average levels of genetic variation (Bodkin et al., 1999; Gagne et al., 2018; Larson, Jameson, Bodkin, et al., 2002; Larson, Jameson, Etnier, et al., 2002; Larson et al., 2012, 2021). Here, we use a more comprehensive genomic approach to investigate the genomic impacts of the fur trade bottleneck on remnant sea otter populations. Using sequence capture, we assess genic and nongenic regions of the genome in 107 sea otters (Figure 1a;

Figure S1A–C; Table S1) sampled from five remnant populations across the species range (Figure 1a), as well as three historical samples from California dating from ~1500–200 years ago (Figure 2a; Table S2). We use these sequence data to analyse sea otter population structure, genetic continuity, and demographic history. These results then serve as the basis for forward-in-time simulations to explore the impact of the fur trade on genetic load and recovery. We find that although the historical population structure has been preserved through the persistence of survivors in each population, the fur trade bottleneck may have had long-lasting effects on population fitness and plans for restoration.

## 2 | MATERIALS AND METHODS

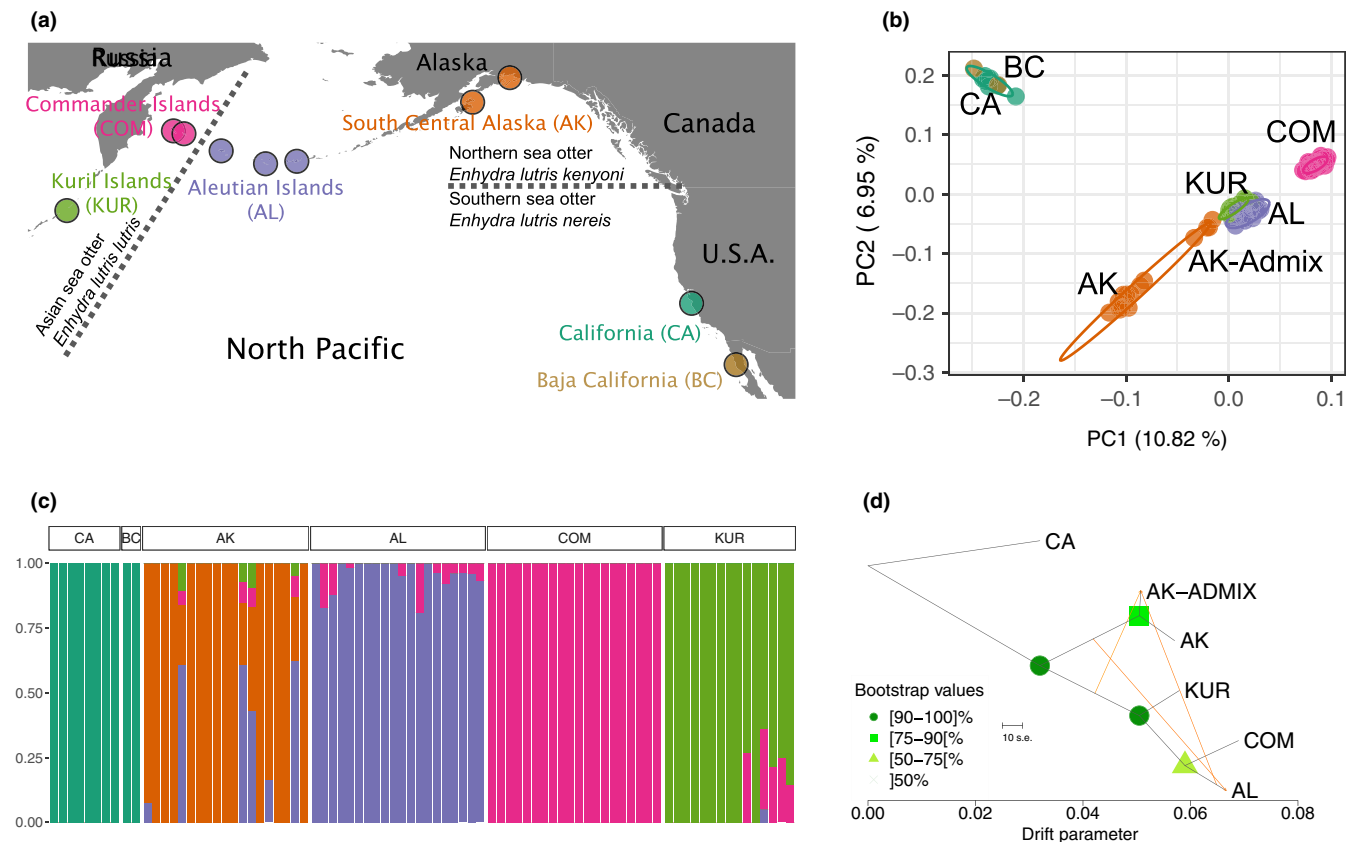
The Supplemental Methods provides additional details on all analyses described below.

### 2.1 | Sample information

Sea otters were sampled from the Kuril Islands (KUR,  $n = 13$ ), Commander Islands (COM,  $n = 45$ ), Aleutian Islands (AL, consisting of Attu, Amchitka and Adak Islands,  $n = 21$ ), south central Alaska (AK,  $n = 19$ ), California (CA,  $n = 7$ ) and Baja California (BC,  $n = 2$ ). All samples derive from sea otter research expeditions from 1974 to 1994 (Supplemental Methods; Figure 1a; Figure S1A–C; Table S1), except for the two recent (2008–2011) samples recovered from stranded individuals from Baja California (Schramm et al., 2014) (Table S1; Figure S1D). Three historical Californian samples, excavated from coastal shell middens and dating from ~1500–200 years ago, were also analysed (Supplemental Methods; Figure 2a; Table S2).

### 2.2 | Sequence capture probe design

We designed a custom sequence capture based on the annotation of the southern sea otter genome from Beichman et al. (2019) (*Enhydra*

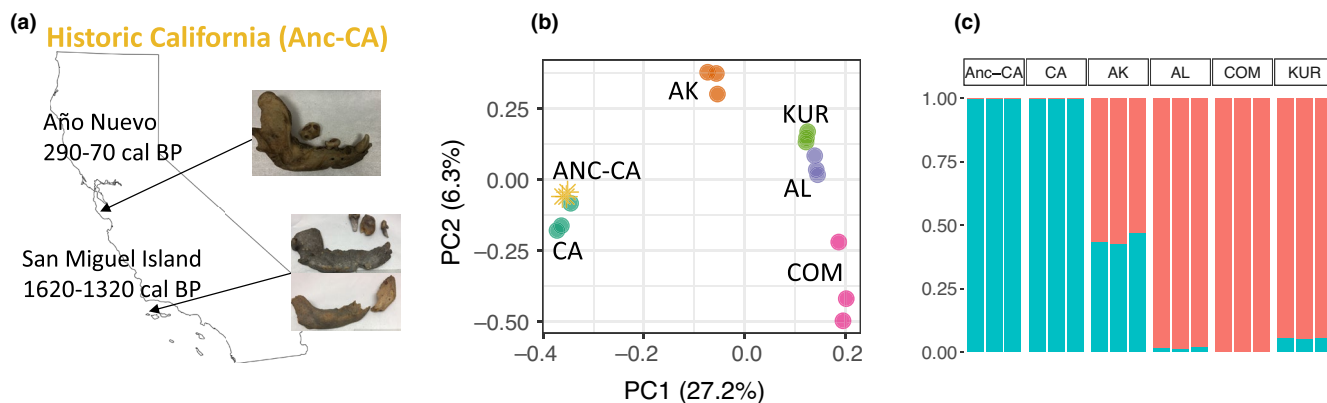


**FIGURE 1** Sea otter population structure mirrors their historic distribution. (a) Locations of sea otter samples used in this study. Sea otters were sampled from the Kuril Islands (KUR,  $n = 13$ ), Commander Islands (COM,  $n = 45$ ), Aleutian Islands (AL, consisting of Attu, Amchitka and Adak Islands,  $n = 21$ ), south central Alaska (AK,  $n = 19$ ), California (CA,  $n = 7$ ), and Baja California (BC,  $n = 2$ ). Dashed lines indicate the previously designated subspecies boundaries between southern, northern and Asian sea otters. See Figure S1 for finer-scale sampling location maps, Supplemental Methods for sample information, and Tables S1 and S3 for sample and coverage details. (b) Principal components analysis (PCA) of modern sea otter samples. Multivariate  $t$ -distribution 95% data ellipses are shown. See Figure S3A–C for PCA analyses of the northern and Asian populations without California which provide greater resolution of their population structure. (c) FASTSTRUCTURE analysis of the samples with the number of clusters ( $K$ ) = 5. See Figure S3F for additional values of  $K$ . (d) Bootstrapped TREEMIX analysis showing drift between populations, with migration events shown as orange lines. California was set as the root. Admixed south central Alaskan individuals are separated in the analysis (AK-Admix) from the nonadmixed (AK). Baja California was excluded as it appears to be an expansion of the California population, rather than a distinct population. TREEMIX residuals are shown in Figure S3G. See also Figures S1 and S3 and Tables S1, S3, S4 and S5

*lutris nereis*, accession GCA\_006410715.1). Fifty megabases of sequence was included in the capture design, including all annotated exonic sequence passing custom filters, 1-kb regions upstream of genes and 10 Mb of neutral regions far from genes and therefore unlikely to be under natural selection. We chose these target regions for three reasons. First, they provided enough information to carry out high-resolution analysis of population structure. Second, they enabled us to generate the neutral site frequency spectrum (SFS) from regions far from genes for demographic inference. Third, we could generate the synonymous and missense SFSs from exonic regions, allowing us to calibrate our simulations of coding sequence under our inferred demographic models. The custom sequence capture probe pool was built based on the coordinates of these regions by KAPA Biosystems.

### 2.3 | Extraction, library preparation, sequence capture and sequencing

Blood and tissue samples were extracted using Qiagen's DNEasy Blood and Tissue kit. Libraries were prepared using the KAPA HyperPlus library preparation kit, and sequence capture was carried out using the KAPA SeqCap EZ Hyperflow workflow kit, with 12–13 uniquely indexed samples per capture pool. Capture success was evaluated using the Agilent Bioanalyzer and Qubit. Captures were sequenced on the Novaseq 5000 (150PE) at Medgenome. An average of 6 Gb of sequence was generated per sample, with 93% of bases  $\geq 30$  base quality, and per-individual coverage of 18–76 $\times$  (Table S3). Four samples from California were excluded from subsequent analysis due to low sequencing yields.



**FIGURE 2** Historical DNA suggests genetic stability in California. (a) Sampling locations and ages of three historical sea otter samples from California shell middens. (b) PCANGSD principal components analysis of three historical California samples (“Anc-CA,” gold stars) and 15 modern sea otter samples based on genotype likelihoods. Each modern population—California (CA), Alaska (AK), Aleutian Islands (AL) and Kuril Islands (KUR), and the Commander Islands (COM)—was downsampled to three individuals to equal the sample size of the three historical samples. (c) PCANGSD admixture analysis, based on genotype likelihoods, including the three historical California samples (“Anc-CA”). See also Figure S2 and Table S2

Samples from Baja California were extracted at the Autonomous University of Baja California, Ensenada, Mexico. Library preparation and sequence capture was carried out using the workflow described above and the samples were sequenced on the NextSeq 500 at the National Laboratory of Genomics for Biodiversity in Mexico (LANGEBIO-CINVESTAV, Irapuato, Mexico).

The three historical samples were prepared in UCLA's ancient DNA laboratory, a clean room that is separate from all modern DNA laboratories. DNA was extracted using the Gamba et al. (2014) protocol. The historical samples with the highest concentration were chosen for library preparation at Arbor Biosciences using NEB Next Fast DNA library preparation. Sequence capture was carried out and the historical samples were sequenced to 1.5–3.5× coverage of capture regions on a HiSeq3000 at the UCLA Technology Center for Genomics & Bioinformatics. The historical samples were radiocarbon dated at the W.M. Keck Carbon Cycle Accelerator Mass Spectrometer at the University of California, Irvine, and dates were calibrated as described in the Supplemental Methods.

## 2.4 | Read mapping, genotype calling, and filtering (modern samples)

Following the strategy of Beichman et al. (2019), reads were mapped to the domestic ferret reference genome (*Mustela putorius furo*, accession GCA\_000215625.1) in order to use a reference genome that is an outgroup from all sea otter populations with a good annotation and pre-existing resources, particularly the Ensembl Variant Effect Predictor database. Reads were mapped using the PALEOMIX pipeline (Schubert et al., 2014) with BWA-MEM (Li & Durbin, 2009) as the alignment algorithm. Genotypes were called using GATK's HaplotypeCaller (Van der Auwera et al., 2013) and genotypes were hard-filtered as described in the Supplemental Methods. Fifteen individuals with

greater than the mean +1 SD worth of missing genotypes were excluded from downstream analyses.

After genotype filtering, the average coverage per individual of sites was 36× (based on sites with at least 10× coverage). Each individual had an average of 61.4 Mb called genotypes (Table S3). Approximately 60,000 total segregating sites were discovered.

To ensure that our choice of a divergent outgroup reference genome would not bias estimates of heterozygosity or demographic history, we also mapped all individuals to the southern sea otter reference genome (accession GCA\_006410715.1) generated in Beichman et al. (2019). Mapping rates were highly similar between the two reference genomes (Table S3). We repeated all analyses described below with reads mapped to each reference genome.

## 2.5 | Read mapping, genotype likelihoods and filtering (historical samples)

The historical samples were mapped to both the ferret and sea otter reference genomes using the PALEOMIX pipeline (Schubert et al., 2014) with the BWA backtrack (Li & Durbin, 2009) alignment algorithm. MAPDAMAGE (Jónsson et al., 2013) was used to detect DNA-damage profiles and to rescale the quality score of bases that are likely to be misincorporations (Figure S2A). Three modern samples from each remnant population were selected to be analysed alongside the historical California samples. Genotype likelihoods, genotype posterior probabilities, and per-site depths were determined using ANGSD (Korneliussen et al., 2014). The reads from historical samples showed a mapping preference toward the sea otter reference genome (Table S2), probably due to lower coverage and read quality. However, mapping to an ingroup genome from one sea otter population can cause reference biases. Therefore, analyses are presented mapped to both the domestic ferret genome and to the southern sea otter genome.

## 2.6 | Population structure analyses (modern samples)

Relatedness between individuals was assessed using PLINK's identity-by-descent method of moments approach (*snpgdsIBDMoM*) (Purcell et al., 2007) (Table S4). *SNPRELATE* (Zheng et al., 2012) was used to carry out linkage disequilibrium (LD) pruning of single nucleotide polymorphisms (SNPs) and principal components analysis (PCA) (Figure 1b; Figure S3). Weir and Cockerham's (1984)  $F_{ST}$  was calculated for each population-pair using *SNPRELATE* (Zheng et al., 2012) (Table S5; Figure S3). Population structure was assessed using *FASTSTRUCTURE* (Raj et al., 2014) (Figure 1c; Figure S3). *TREEMIX* (Pickrell & Pritchard, 2012) was used to assess historical population divergence and gene flow using covariance of allele frequencies (Figure 1d; Figure S3). We constructed a neighbour-joining (NJ) phylogenetic tree to explore the relationship between the Baja California sea otters and the California population using the *APE* package in R (Paradis & Schliep, 2019) (Figure S3).

## 2.7 | Population structure analyses (historical samples)

*PCANGSD* (Meisner & Albrechtsen, 2018) was used to carry out PCA and structure analyses for the three historical samples and a subsample of three modern individuals from each population. Since the historical data mapped better to the sea otter reference genome than to the ferret reference genome, the analysis was done for each reference genome, with similar qualitative results (Figure 2; Figure S2).

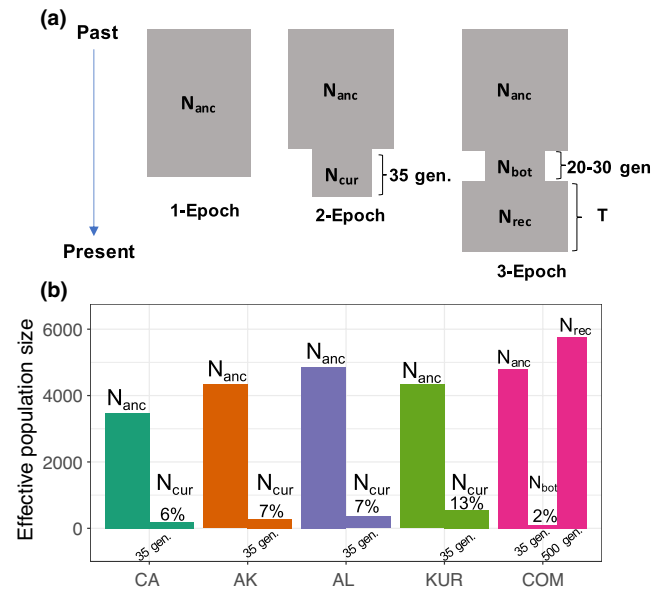
## 2.8 | Generating site frequency spectra

*ENSEMBL*'s Variant Effect Predictor (VEP) (McLaren et al., 2016) was used to classify SNPs as synonymous, missense or stop-gained based on the domestic ferret genome annotation database. For the neutral SFS, putatively neutral regions >10 kb away from ferret exon sequences that did not intersect with CpG islands or repeat regions were chosen. For each population, the neutral, synonymous and missense folded SFSs were generated using a hypergeometric projection to smooth over missing data (Table S6).

$S$  (total number of segregating sites),  $\pi$  (average pairwise heterozygosity), Watterson's  $\theta$  ( $\theta_W$ ) (Watterson, 1975) and Tajima's  $D$  (Tajima, 1989) were calculated from the projected folded SFSs for each population based on Wakeley (2009).

## 2.9 | Demographic inference

The neutral SFSs were used for demographic inference in *∂a∂i* (Gutenkunst et al., 2009) and *FASTSIMCOAL2* (Excoffier et al., 2013) for



**FIGURE 3** Signals of extreme decline detected in every remnant population using the site frequency spectrum. (a) The three single-population demographic models that were compared: a 1-Epoch model in which no size change occurs, a 2-Epoch model in which a single contraction occurs, and a 3-Epoch bottleneck model in which a recovery period is modelled. For the 2-Epoch model, a wide variety of contraction durations was explored using a grid search approach (Figure S4B). However, in order to compare the magnitude of decline across populations, results shown have the duration of the contraction fixed to ~35 generations, representing the start of the fur trade ~245 years prior to sampling. (b) For California (CA), Alaska (AK), Aleutian Islands (AL) and Kuril Islands (KUR), a 2-Epoch contraction model with parameters inferred using a grid search in *∂a∂i* fits the SFS best.  $N_{anc}$  represents the inferred ancestral size and  $N_{cur}$  is the inferred contraction size if the contraction duration is fixed to ~35 generations to be concordant with the timing of the fur trade. The ratio of the inferred postcontraction effective population size to ancestral effective population size is shown as a percentage for each population. For the Commander Islands (COM), a 3-Epoch model with a bottleneck size ( $N_{bot}$ ) and recovery size ( $N_{rec}$ ) was a significantly better fit to the SFS, but this signal did not appear when mapped to the southern sea otter genome and therefore may be artefactual (Table S7; Supplemental Methods). See also Figures S4 and S5 and Tables S6 and S7

the following single-population models (described forward in time; model diagrams in Figure 3a):

- 1-Epoch model: no size changes, infers the ancestral size ( $N_{anc}$ ) of the population.
- 2-Epoch model: a single size change from  $N_{anc}$  to the current size ( $N_{cur}$ ) occurring  $T$  generations ago.
- 3-Epoch model: two sizes changes from  $N_{anc}$  to a bottleneck ( $N_{bot}$ ) followed by recovery ( $N_{rec}$ ) that lasts  $T$  generations. The duration of the bottleneck is set to 20–30 generations.

For each model, 50 replicates of the inference were carried out to confirm that both parameters and log-likelihoods converged and

parameters with the maximum log-likelihood were chosen. Models were compared using likelihood ratio tests (LRTs) (Table S7).

Since the duration and magnitude of a contraction are difficult to disentangle, we also used a grid search approach in  $\partial a \partial i$  to explore the range of parameter combinations that fit the data (Figure S4B). To compare the magnitudes of decline across populations, we fixed the duration of contraction at 245 years since the advent of the fur trade in 1741 (Kenyon, 1969) and the time of sampling ~1986 (~35 generations assuming a 7-year generation time; Gagne et al., 2018; Ralls et al., 1983).

We used the joint SFS between California and central Alaska sea otters to infer the shared demographic history and divergence time of the populations using *FASTSIMCOAL2* and  $\partial a \partial i$ , as above (model diagrams in Figure S4D).

Finally, we compared our models inferred here using the SFS to those we previously inferred using *MSMC* (Schiffels & Durbin, 2014) from a single southern sea otter genome from California and a northern sea otter genome from the translocated southeast Alaska population (Beichman et al., 2019) (Figure S5).

## 2.10 | Wright-Fisher simulations with selection

We designed models based on the inferred demographic parameters for the California population to determine the impact of population contractions on deleterious variation (model diagram in Figure 4a). We then simulated a period of partial recovery after the contraction to reflect the current recovery status of the population (diagram in Figure 4b).

To explore the role of serial bottlenecks, we designed a model loosely based on the history of serial bottlenecks in the Alaskan and Aleutian Island sea otter populations due to the Exxon Valdez oil spill and predation by orcas, respectively (Ballachey et al., 1994; Estes et al., 1998) (diagram in Figure 4c).

Finally, to model the potential benefits of gene flow between populations, we designed a joint simulation between the California and south central Alaska populations in which reciprocal translocations occur at a rate of either one individual per generation, 25 individuals per generation, or a single burst of 25 individuals being exchanged for two generations and then ceasing (diagram in Figure 5). Detailed model descriptions are given in the Supplemental Methods.

Under these different demographic models, we simulated neutral and coding sequence using forward-in-time Wright-Fisher simulations in *SLIM 2* (Haller & Messer, 2017). We assumed that the dominance and selection coefficients of the mutations were inversely related, such that the most strongly deleterious mutations are also the most recessive (Henn et al., 2016; Huber et al., 2018) (Figure S6). The selection coefficients of new missense mutations were drawn from the distribution of selection coefficients inferred by Kim et al. (2017). The burden of deleterious variation, known as genetic load ( $L$ ), was calculated every two generations of the model as described in the Supplemental Methods.

Since the precise distributions of dominance and selection coefficients in sea otters are unknown, we explored a variety of relationships between dominance and selection which strongly affect the genetic load dynamics. We carried out simulations in which deleterious mutations are only partially recessive (Deng & Lynch, 1997), or all mutations are fully additive or fully recessive. We also carried out simulations under a distribution of selection coefficients that contains a higher proportion of strongly deleterious and lethal mutations suggested by Kardos et al. (2021). See Note S1 for additional discussion.

## 2.11 | Non-Wright-Fisher simulations with selection

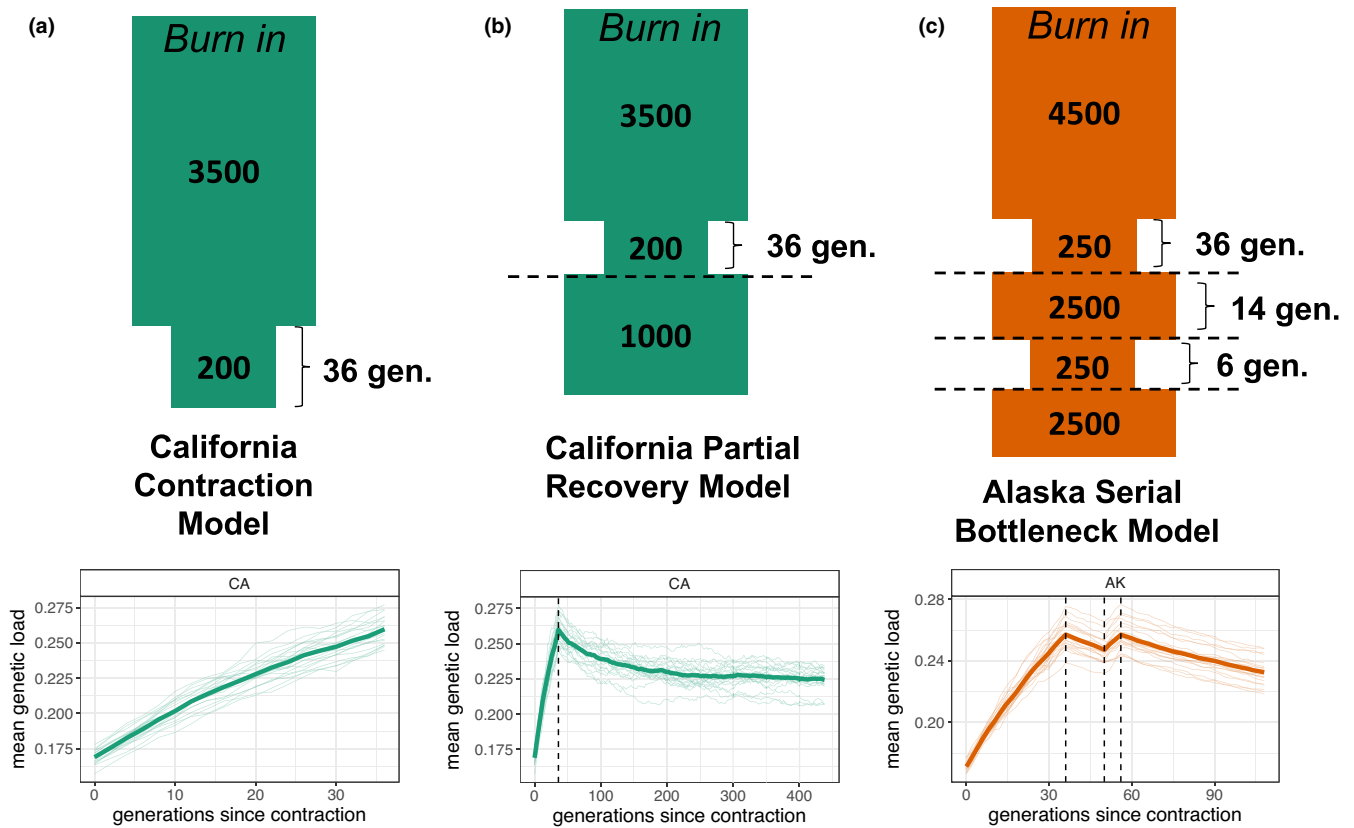
To explore the extent to which the California sea otter population may be threatened by extinction due an accumulation of deleterious mutations, we conducted simulations with the non-Wright-Fisher (nonWF) models in *SLIM 3* (Haller & Messer, 2019), following the approach of Kyriazis et al. (2021) with modifications described in the Supplemental Methods. These models aim to facilitate more ecologically realistic population genetic simulations by relaxing many of the assumptions of the Wright-Fisher model. The simulated population size is determined by the absolute fitness of individuals in the population and a user-defined carrying capacity. As a result, the simulated population can go extinct when fitness declines. In addition, this model provides a natural framework for incorporating environmental stochasticity and natural catastrophes, which may also impact the persistence of sea otter populations.

Finally, to explore the potential impact of an increased historical population size on levels of inbreeding depression and extinction risk in the California sea otter population, we ran the above simulations with the ancestral carrying capacity increased three-fold, with all other parameters kept the same (diagram in Figure 6; Table S8). See Note S1 for additional discussion.

## 3 | RESULTS

### 3.1 | Genetic structure of sea otter populations

Individuals from each sampling location clustered by genotypes in both PCA (Figure 1b) and *FASTSTRUCTURE* (Figure 1c) analyses, and values of  $F_{ST}$  increased with distance between populations (Figure S3D; Table S5), agreeing with previous studies (Larson et al., 2012, 2021; Larson, Jameson, Bodkin, et al., 2002). Sea otters in California are the most divergent population and represent the only surviving remnant of the southern sea otter subspecies (*Enhydra lutris nereis*) (Figure 1b,d). All other sea otter populations surveyed, which span the northern (*Enhydra lutris kenyoni*) and Asian (*Enhydra lutris lutris*) subspecies range, clustered according to geography once the California individuals were excluded (Figure S3A). Inference of the population split time between

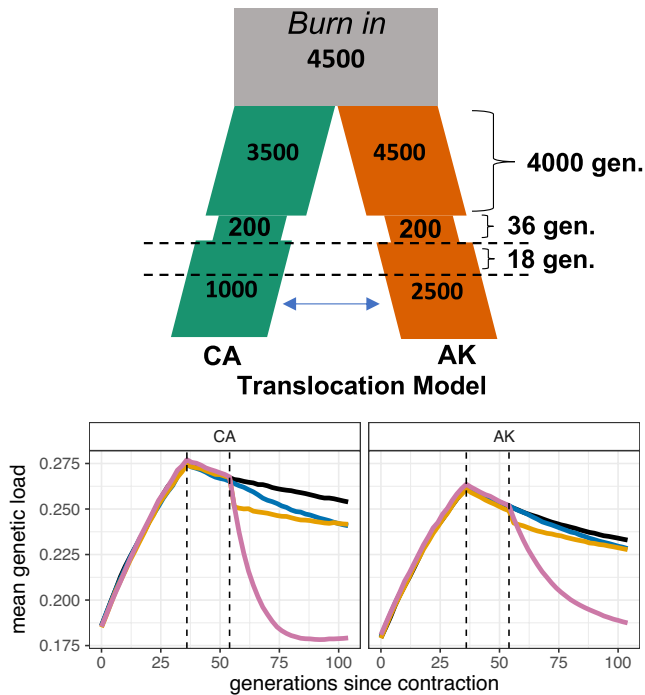


**FIGURE 4** Recessive genetic load is predicted to increase during decline and persist after recovery. (a) Forward-in-time Wright–Fisher simulations of genetic load (reduction in fitness due to deleterious variation) under the demographic model of recent population decline we inferred for the California sea otter population. The demographic model is shown above, with sizes in diploid individuals and times in generations. The simulation results below the model diagram show an increase in genetic load during the inferred population contraction (contraction occurs at generation 0). The thick line represents the mean of 20 simulation replicates, with individual replicates as faint lines. The contraction duration was increased from 35 to 36 generations to accommodate sampling of load every even-numbered generation in the simulation framework. (b) Simulations of genetic load under a model of partial recovery for the California population in which the contraction modelled in (a) is followed by a partial recovery to an effective size of 1,000 individuals. The simulation results below the model are as described in (a). The partial recovery occurs at the dashed line. (c) A model of serial declines in the south central Alaska population, showing the possible impacts of post-fur trade events such as the Exxon Valdez oil spill or orca predation. The simulation results below the model are as described in (a). The first population contraction occurred at generation 0, followed by a brief recovery (starting at the first dashed line), then another rapid contraction and partial recovery (second and third dashed lines). Results based on alternative distributions of dominance and selection coefficients are shown in Figure S6C–E

California and Alaska using the joint SFS dates the divergence to ~4000 generations ago (~28 thousand years ago [ka]), with only low amounts of genetic exchange between the populations (Table S7; Figure S4D,E). The best-fitting model was one which also included a recent contraction in both populations (inferred in the single-population models below).

To assess the history of connectivity among the northern and Asian sea otter populations, we inferred historical migration events between these populations using TREEMIX (Figure 1d) and individual ancestry profiles in FASTSTRUCTURE (Figure 1c). We found that northern and Asian sea otter populations have a complex history of connectivity, with evidence for fine-scale spatial structure and a history of admixture events. Twelve individuals showed evidence of admixture, notably four from the Kenai Peninsula and Kodiak Island in south central Alaska which have mixed Alaskan, Aleutian, Kuril, and Commander Island ancestry (multicoloured AK columns in

Figure 1c; labelled “AK-Admix” in Figure 1b,d; Figure S1C), and five in the Kuril Islands that had a portion of Commander Islands ancestry. Interestingly, the highly admixed Alaskan individuals sampled from Kodiak Island all had kinship coefficients  $\geq 0.1$  to each other and are unrelated to any other otters sampled in Alaska (Table S4). Historical migration (orange arrows in Figure 1d) from the Aleutian Islands and the common ancestor of the Kuril/Commander/Aleutian populations into the admixed Alaskan group, and in the opposite direction from Alaska into the Aleutian Islands, was inferred using TREEMIX (Pickrell & Pritchard, 2012) based on the covariance of allele frequencies. Within the Aleutian Islands, samples were obtained from Adak, Amchitka, and Attu Islands (Figure S1B). When analysed alone, we found that the three islands clustered separately in the PCA (Figure S3B) with very low values of  $F_{ST}$  (0–0.02) (Table S5). We did not observe any divergence between the two Commander Islands (Figure S3C; Table S5).



**FIGURE 5** Gene flow may mitigate genetic load. An isolation-migration model exploring the theoretical benefits of enabling gene flow between California and south central Alaska, based on the joint population histories of the populations we inferred. The simulated populations split 4000 generations ago, then both experience contractions followed by partial recovery, and then hypothetical translocations at varying levels of intensity begin 18 generations after the populations have partially recovered from their respective declines. The forward-in-time Wright-Fisher simulations of genetic load under this model show a decrease in recessive genetic load caused by high levels of gene flow between populations. The partial recovery occurs at the first dashed line, and gene flow begins at the second dashed line. Levels of gene flow range from no gene flow (black), one individual per generation (blue), a burst of 25 individuals exchanged for two generations (yellow) or sustained high levels of gene flow of 25 individuals per generation (pink). Each line represents the mean of 20 simulation replicates. Results based on alternative distributions of dominance and selection coefficients are shown in Figure S9

### 3.2 | Historical DNA suggests genetic stability in California

To examine the temporal continuity of the southern sea otter population, we sequenced DNA from three historical sea otter tooth roots sampled from prehistoric and historical shell middens on the California coast. The oldest samples were from San Miguel Island, dating from 1610–1320 calibrated calendar years before present (cal BP), and the third was a historical sample from Año Nuevo, dating from 290–70 cal BP (Figure 2a; Table S2). The low sequencing coverage (1.5–3.5 $\times$ ) of these samples was not high enough to explore levels of heterozygosity, but was sufficient for population structure analyses. We randomly chose three individuals from every modern population, including California, to analyse alongside the historical samples. The three historical samples cluster with modern

Californian sea otter samples in the PCANGSD (Figure 2b) and admixture (Figure 2c) analyses based on genotype likelihoods.

We found the population structure results were qualitatively similar regardless of the reference genome used for mapping (Figure S2B,C; Table S2).

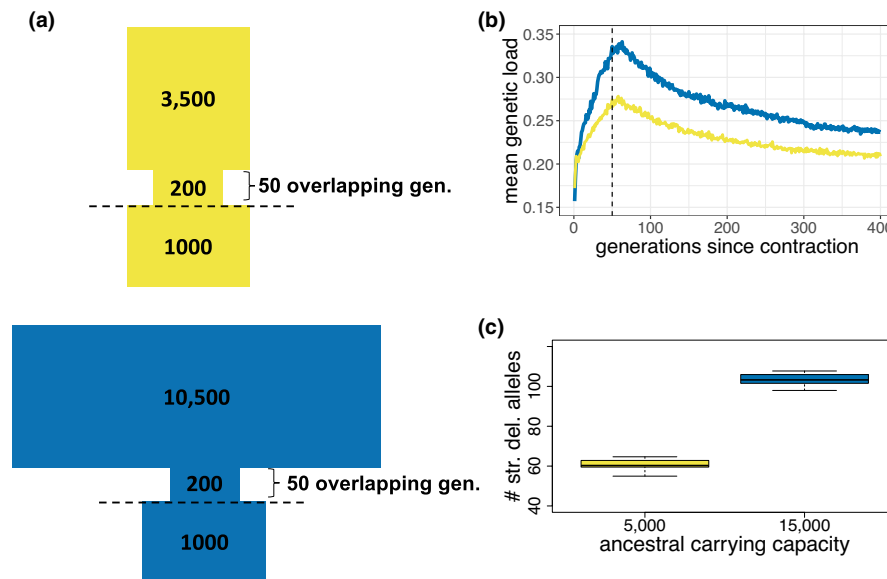
### 3.3 | Expansion of southern sea otters into Baja California

The two modern individuals sampled in Baja California clustered with the California samples in the PCA (Figure 1b) and FASTSTRUCTURE (Figure 1c) analyses. They had an  $F_{ST}$  of  $\sim 0$  compared to the California sea otters (Table S5) and clustered within the California clade in a NJ tree (Figure S3E). Although historical levels of genetic differentiation between California and Baja California sea otter populations are unknown, the local population structure we observe in other remnant sea otter populations (Figure S3B,D) suggests that the two populations would be genetically distinguishable. The lack of differentiation that we observe therefore suggests that the Baja California samples may be migrants or the offspring of migrants from the California population, rather than survivors of a historically differentiated Baja California population. Indeed, we observed moderate levels of kinship (0.08–0.15) between them and individuals in the California sample (Table S4), indicating that they may even be relatives of extant California individuals.

### 3.4 | Signals of extreme decline detected in every remnant population

Each sea otter population has extremely low neutral genetic diversity, ranging from 1.1 to 1.6  $\times 10^{-4}$  heterozygotes/base pair, irrespective of which reference genome (domestic ferret or southern sea otter) was used for read mapping (Table S6). To explore demographic scenarios that could give rise to low diversity, we conducted demographic inference based on the SFS. We detected low ancestral effective population sizes (3500–5000 individuals per population) and a signal of extreme decline in every sea otter population we surveyed (Figure 3a,b; Table S7). Single-size population models were strongly rejected in favour of two-epoch contraction models (likelihood ratio test  $p < .005$ ; Figure 3a; Table S7). After fixing the duration of the contraction based on historical records (Methods), we inferred that each population suffered an extreme contraction, with only 2%–13% of the ancestral effective population size estimated to have survived the decline (Figure 3b). Qualitatively similar results of a steep decline in every population were obtained using either the domestic ferret or southern sea otter reference genome to map sequence reads (Table S7; Supplemental Methods). Since our inference method averages over the period of decline, sea otter populations in some regions may have reached even lower population sizes than those in our model, as suggested by field observations (Riedman & Estes, 1990).





**FIGURE 6** Long-term low population size may mitigate recent bottleneck effects. (a) More ecologically realistic non-Wright-Fisher models of the impact of historical population size on genetic load. Values represent effective population sizes, though the model uses carrying capacity ( $K$ ) (see Table S8 for corresponding  $K$ ). The yellow (upper) model represents a population with ancestral effective population size based on our demographic inference, and the blue (lower) model is a theoretical population that has a 3 $\times$  higher ancestral effective size. Each population goes through an extreme decline as in the model, then partially recovers. The contraction duration was increased from 35 to 50 generations to account for overlapping generations in the non-Wright-Fisher framework. (b) Non-Wright-Fisher simulations of genetic load under the models in (a). The yellow line represents the yellow model in (a) based on our inferred demographic parameters, and the blue line represents the blue model in (a) with the 3 $\times$  larger ancestral size. Recovery occurs at the dashed line. (c) The average number of strongly deleterious alleles per individual for each ancestral carrying capacity described in (a). Results based on alternative distributions of dominance and selection coefficients are shown in Figure S10

We used forward-in-time simulations to demonstrate that this extreme bottleneck would only moderately lower neutral heterozygosity (Figure S6A), and therefore long-term small population size, rather than a rapid contraction, is probably responsible for the extremely low genetic diversity we observe. However, a decline of this severity would shift the SFS toward intermediate frequency variants (Figure S4C), with implications for genetic load (discussed below).

Our inference was not able to detect the very recent recovery of sea otter populations using the SFS, except in the case of the Commander Islands. Specifically, a three-epoch model with a recovery period was the best fit to the Commander Islands SFS (Figure 3b; likelihood ratio test  $p = 5 \times 10^{-7}$ ; Table S7). However, the inferred recovery duration is too long (500 generations) to be consistent with the time since the fur trade. Furthermore, the evidence of a recovery in the Commander Islands is diminished because the signal is not found when demographic inference used reads mapped to the southern sea otter reference genome (Table S7; Supplemental Methods).

Finally, we compared our SFS-based demographic models to those previously obtained from single whole genome sequences (Beichman et al., 2019) and found that the fit of the whole-genome models to the empirical SFS was improved by including the recent decline that we detect with the SFS. The combination of these inference methods increases confidence in our findings of an extreme decline in recent time occurring in every sea otter population (Figure

S5). For California, we found that a model that included a shallow pre-fur trade decline in population size in addition to the recent extreme bottleneck was more consistent with the whole-genome inference than the simpler two-epoch model inferred using the SFS (Figure S5).

### 3.5 | Severe population contraction is predicted to increase genetic load

To explore how changes in population size could affect deleterious variation, we used forward-in-time Wright-Fisher simulations of coding sequence to assess the reduction in fitness due to deleterious mutations (i.e., genetic load) before and after the inferred fur trade bottleneck. Our simulations showed that contractions of the magnitude we infer, while brief, could increase the genetic load of the population up to 54% (Figure 4a). This increase in load is due to deleterious recessive mutations that were shielded from selection in the larger ancestral population being exposed as homozygotes in the contracted population, allowing them to reduce fitness (Figure S7A). This shift in the frequency of missense mutations can be seen in the shift of variants from low to intermediate frequency in the simulated postcontraction and empirical missense SFSs compared to the simulated precontraction SFS (Figure S8A). Additional model conditions are presented in Figures S6C, S7B–E and S8B–E and discussed in Note S1.

### 3.6 | Simulations indicate genetic load may persist, but not cause extinction

To assess the possible long-term genetic impact of the fur trade and subsequent bottlenecks, we carried out additional Wright–Fisher simulations of sea otter recovery. We found that even with partial recovery (defined as a return to an effective size of 1000 individuals for the California population), genetic load did not return to pre-fur trade levels even after 400 generations (2800 years) (Figure 4b; Figure S6D). Subsequent declines in population size further slowed the reduction in genetic load (Figure 4c).

To explore whether the increase in genetic load could cause the extinction of sea otter populations, we made the simulations more ecologically realistic by allowing the simulated population size to depend on population fitness such that the simulated population can go extinct when fitness declines or due to environmental stochasticity, following the approach of Kyriazis et al. (2021). Under this model, the California otter population did not decline to extinction due to genetic factors at any point during its 400-generation-long partial recovery from the decline (Table S8). Additional model conditions are presented in Figure S6D,E and discussed in Note S1.

### 3.7 | Gene flow may mitigate the impact of genetic load

To explore the potential consequences of restoring gene flow between otter populations, we used Wright–Fisher simulations to model a scenario in which there are varying levels of gene flow between the bottlenecked Alaska and California populations (Figure 5). We found that genetic load decreases more swiftly than in the no-gene flow scenario if at least one migrant is reciprocally exchanged per generation. Equivalently, it is decreased the same amount if a single burst of 25 individuals are reciprocally exchanged for just two generations and then gene flow ceases (a translocation-like scenario) (Figure 5). However, genetic load is still slow to return to pre-fur trade levels in each population except in the case of extremely high gene flow of 25 individuals reciprocally exchanged per generation, which is also highly impractical in reality. Additional model conditions are presented in Figure S9 and discussed in Note S1.

### 3.8 | Long-term low population size may mitigate effects of bottlenecks

Finally, we explored the potential role of the relatively small historical effective population sizes on the accumulation of genetic load and extinction risk in the California sea otter population. Specifically, we hypothesized that, if sea otters had a larger historical effective population size, they might have experienced more severe genetic effects from this bottleneck, due to the higher numbers of heterozygous recessive strongly deleterious mutations

harboured in large populations (Hedrick, 2002; Hedrick & Garcia-Dorado, 2016; Kyriazis et al., 2021; Robinson et al., 2018). These mutations, when made homozygous by inbreeding following the bottleneck, could lead to a greater increase in genetic load and potentially drive extinction. To explore the consequences of a larger historical population, we ran non-WF simulations with an ancestral population size that was three times larger than our empirically inferred size (Figure 6a). The historically larger population had a greater increase in recessive genetic load due to the recent contraction than the historically smaller population (117% vs. 61% increase, Figure 6b; Table S8). The higher load is a direct consequence of the higher number of recessive strongly deleterious mutations in the larger ancestral population (Figure 6c). Nevertheless, the historically larger population did not go extinct in any simulation replicates. Additional model conditions are presented in Figure S10 and discussed in Note S1.

### 3.9 | Simulation dynamics under differing dominance and selection distributions

Due to uncertainty in the distribution of selection coefficients and the relationship between dominance and selection in sea otters, we carried out additional simulations under a variety of selection and dominance parameters (Note S1). We found that the population contraction would result in an increase in genetic load if mutations were recessive, but not if they were additive or only partially recessive (Figure S6C). Strongly deleterious additive mutations are not shielded from selection in the heterozygous state and are continuously eliminated by selection. This finding is consistent with theoretical studies suggesting that recent demography may only have a limited impact on additive load (Do et al., 2015; Lohmueller, 2014; Simons et al., 2014) and that recessive load caused by strongly deleterious genotypes in the homozygous state may be more of a threat to population persistence. In addition, if there is a substantial proportion of recessive lethal mutations present in the simulated population ( $\geq 5\%$ ) as proposed by Kardos et al. (2021), we found that overall genetic load would be much higher, but the percentage gain in load during the contraction would be moderate ( $\sim 9\%$ ) and rapidly return to baseline after population recovery (Figures S6C–E). Also, translocations and ancestrally small population sizes would show no benefits (Figures S9 and S10). Each of these different model conditions is shown in Figures S6–S10 and discussed fully in Note S1.

## 4 | DISCUSSION

### 4.1 | Sea otters exhibit broad- and fine-scale population structure

Defining management units for sea otters, whether at fine or broader scales, remains a challenge to conservation efforts (Davis et al., 2019). Genetic structure as defined by PCA and admixture

analyses can provide insight into natural genetic groupings, but cannot characterize microscale structuring of sea otter familial groups (Bodkin, 2015; Davis et al., 2019; Gagne et al., 2018; Larson et al., 2021; Tinker et al., 2019). We found that the geographically driven population structure of sea otter populations remained after the fur trade bottlenecks, leading to five broad geographical groupings in our sample: the Kuril Islands, Commander Islands, western Aleutian Islands, south central Alaska, and California. This shows that a key element of the successful restoration of sea otters was their survival and numerical increase from small remnant populations distributed throughout their geographical range rather than long-distance dispersal (Larson, Jameson, Bodkin, et al., 2002; Larson et al., 2012, 2021). This fortuitous survival allowed both rapid range-wide restoration of former abundance and preserved historical genetic structure and perhaps even local specializations that enhanced survival (Fujii et al., 2015).

The importance of these remnant populations is exemplified by the southern sea otter, whose former distribution probably spanned from Washington to Mexico (Wilson et al., 1991), but was fully eliminated, except for 30–50 individuals on the California coast (Riedman & Estes, 1990). Our results suggest that southern sea otters, represented by the only surviving population in California, diverged from the four northern and Asian populations before the latter populations differentiated from each other. The ~28 ka we infer for this north–south split means it may have occurred during the Last Glacial Maximum (~20–27 ka). Sea otters cannot tolerate extensive ice cover that restricts access to foraging habitat (Schneider & Faro, 1975) and much of the Alaskan and British Columbian coast was glaciated during that period (Lesnek et al., 2018; Mann & Hamilton, 1995; Mann & Peteet, 1994). This may have isolated northern and southern populations, with only low levels of gene flow occurring subsequently along the North American Pacific coast after the ice retreated. Since the intermediate populations between Alaska and California are now extinct, we cannot easily differentiate between a demographic model of long-term isolation of northern and southern sea otter populations, or isolation by distance with low dispersal between populations, both of which are consistent with our results. Ancient mitochondrial DNA and morphological characteristics of extinct Oregon sea otters analysed in Valentine et al. (2008) suggest they were more similar to California sea otters, but recent ancient mitogenome haplotype analyses in Wellman et al. (2020) show clustering between northern sea otters and ancient Oregon sea otters, possibly indicating that the southern sea otters in California and Mexico may have been genetically distinct from Oregon populations, and/or that Oregon was a location in which northern and southern sea otters mixed (Larson et al., 2012; Lee Lyman, 1988). The genetic legacy of the southern sea otter lineage would have entirely been lost if not for the preservation of the last relict population in California.

Our analysis of population structure in northern and Asian sea otter populations demonstrates a complex and interconnected history shaped by gene flow, recolonization, and fine-scale population

structure. Migration appears to have occurred in both directions along the island chains of the north Pacific, probably aided by the high abundance of sea otters in these areas prior to the fur trade. The four interrelated individuals sampled from Kodiak Island, Alaska, that had high levels of Aleutian Island ancestry are particularly intriguing. Conceivably, Kodiak Island was a sink for migration from the Aleutian Islands when otters were abundant. Previously, mitochondrial and microsatellite data identified Kodiak Island otters as a separate stock from those in Prince William Sound and the Kenai Peninsula, with greater similarity to the Aleutian Islands (Gorbics & Bodkin, 2001). Our findings add evidence that the Kodiak Island sea otters are a distinct genetic grouping with mixed south-central Alaskan and Aleutian Island ancestry.

Among the northern populations, the differentiation we observed between the Aleutian Islands indicates that the populations among these islands are highly interconnected, but there may nevertheless exist fine-scale structure at the level of only hundreds of kilometres between the islands. The lack of similar differentiation between the Commander Islands could indicate enduring high levels of gene flow between Bering and Medny Island or is also consistent with the theory that the Bering Island colony was fully extirpated during the fur trade and recolonized by otters from Medny Island (Bodkin et al., 2000).

#### 4.2 | Historical DNA suggests genetic stability in California

Only 30–50 southern sea otters are thought to have survived the fur trade near Big Sur, California, a minuscule fraction of their historical range (Riedman & Estes, 1990). We had hypothesized that the fur trade bottleneck may have caused considerable genetic differentiation in extant sea otters which are descended from this relict population compared to historical sea otters sampled from the same region due to founder effects, drift or local extinction, and recolonization from genetically differentiated subpopulations (Hastings & Harrison, 1994). Local extinction and recolonization is frequently observed in mammal populations using ancient DNA (Brace et al., 2012; Cooper et al., 2015; Foote et al., 2012; Hofreiter & Barnes, 2010; Hofreiter & Stewart, 2009; Nichols et al., 2007; Pilot et al., 2010), and population replacement has found to be widespread in human populations (Allentoft et al., 2015; Lazaridis et al., 2016; Olalde et al., 2018). However, we found that modern sea otters from the central coast of California cluster closely with a historical sample from the central coast (290–70 cal BP) and two historical samples from southern California (1620–1320 cal BP). Our comparison is global in scale, and therefore cannot detect more subtle signals of differentiation caused by the population bottleneck, but the clustering of historical and extant California sea otter samples from the northern and southern regions of the state does not support extinction after an extreme population bottleneck followed by recolonization from a genetically divergent population.

### 4.3 | Southern sea otters are expanding into Baja California

The sea otter population in Baja California, Mexico, was declared “probably extinct” in the late 1960s (Kenyon, 1969) based on aerial surveys, but since the 1970s fishermen and scientists have reported sporadic sightings of sea otters (Gallo-Reynoso & Rathbun, 1997; Rodriguez-Jaramillo & Gendron, 1996) and the population is currently classified as endangered (Norma Oficial Mexicana, 2010). A previous study that analysed 318 bp of the mitochondrial control region of the two samples used in this study (Schramm et al., 2014) did not have the resolution to determine if these otters were recent migrants from California (Hatfield, 2005; Rathbun et al., 2000) or were survivors of the historical population. We found that the two individuals we sampled are probably migrants or the descendants of migrants from California, representing a southward expansion of the population, rather than survival of the original Baja California sea otter population. Though additional sampling will be necessary, this finding has immediate conservation and management implications for the southern sea otter subspecies. First, binational efforts to protect sea otters in California and Mexico should be increased to ensure that the California population remains the source of individuals for Baja California with the objective that in the long term, a permanent self-sustaining Baja California population would be re-established. Second, the protection of the few individuals in the Mexican Pacific and their habitat is key to increase population numbers, which would facilitate the recolonization of their old geographical range and provide redundancy for regional extinctions should they occur (Eisaguirre et al., 2020). Finally, protecting the southern subspecies could help restore kelp forest ecosystems (Wilmers et al., 2012), which is critical for coastal fisheries in both the USA and Mexico.

### 4.4 | Low diversity indicates long-term small population size

The extremely low diversity we detect in all sea otter populations probably preceded the fur trade (Beichman et al., 2019). We estimate small ancestral effective sizes ( $N_{anc}$ ) from the SFS for every sea otter population prior to the fur trade contraction (3500–5000 individuals per population). The low ancestral effective population sizes we infer are ~20% of the historical census sizes of these populations, which range from 10,000 to 20,000 individuals (Riedman & Estes, 1990). This low effective/census ratio is expected (Frankham, 1995; Nunney, 1993, 1996), and may be due to several factors. Resource availability may set biological limits on the amount of successful reproduction for each population (Davis et al., 2019; Tinker et al., 2019), which may explain similar ancestral sizes across all populations. Overlapping generations and high variance in reproductive success between males and females due to a highly polygynous mating system may have limited successful breeding to a smaller fraction of the census population (Nunney, 1993, 1996; Riedman & Estes,

1990). The linear geography of sea otter populations along coastlines or around islands may increase local structure which could further decouple census and effective population sizes (Gagne et al., 2018). Finally, hunting by aboriginal peoples may have kept otter population numbers low or caused local depletions prior to the fur trade (Aguilar et al., 2008; Braje & Rick, 2011; Erlandson et al., 2005; Hildebrandt & Jones, 1992; Larson et al., 2012; Simenstad et al., 1978). Our estimates of effective size provide insight into long-term levels of genetic diversity of these populations, showing that sea otters have maintained low genetic diversity throughout the history of the species.

### 4.5 | Signals of extreme decline detected in every remnant population

We detected a strong signal of independent population collapse in every sea otter population surveyed using SFS-based demographic inference methods, consistent with worldwide near-extinction caused by the fur trade. This decline may have had lasting impacts on genetic diversity. As predicted theoretically by Ralls et al. (1983), this extreme contraction would only moderately lower heterozygosity, and is therefore not responsible for the very low diversity we report above. However, the contraction may have increased genetic load in the surviving populations (discussed below).

For the California population, we found that a model that combines SFS and whole genome-based demographic inference methods supported an extreme recent bottleneck as well as an additional earlier contraction. Several studies have raised the possibility of multiple declines in the California sea otter population, possibly caused by hunting by Native Californians (Aguilar et al., 2008; Beichman et al., 2019; Erlandson et al., 2005; Larson et al., 2012), and we demonstrate that a model incorporating multiple declines could integrate both whole-genome and SFS-based inferences.

### 4.6 | Impact of genetic load in sea otter populations

Our simulations show that the extreme fur trade bottleneck, although brief, may have increased the genetic load of surviving sea otter populations due to the exposure of deleterious recessive alleles as homozygotes (Balick et al., 2015; Do et al., 2015; Hedrick & Garcia-Dorado, 2016; Kimura et al., 1963; Lynch et al., 1995; Ohta, 1973). Many sea otter populations quickly recovered after their protection in 1911 under the International Fur Seal Treaty (Kenyon, 1969; Riedman & Estes, 1990). The California population, however, has yet to recover beyond one third of its estimated pre-fur trade levels, with a current census size ~3000 individuals (Hatfield et al., 2019), down from an estimated ancestral size of 10,000–20,000 individuals (Riedman & Estes, 1990). Populations in Alaska and the Aleutian Islands have also suffered serial declines due to cataclysms such as the Exxon Valdez oil spill in Prince William Sound in 1989

or due to predation by orca in the Aleutian Islands (Ballachey et al., 1994; Estes et al., 1998). We therefore simulated a variety of recovery models including partial recovery and serial declines to determine their effects on genetic load. We found that under a scenario of partial recovery, recessive genetic load did not return to pre-fur trade levels even after 400 generations (~2800 years). Serial declines further slowed this process, highlighting the critical importance of protecting sea otter populations from further bottlenecks as robust populations are needed to erode the increase in genetic load caused by the fur trade.

Since selection can act much more strongly on partially recessive and additive mutations, the dynamics of genetic load are strongly affected by the distribution of dominance and selection coefficients (Note S1) (García-Dorado, 2012; Hedrick & Garcia-Dorado, 2016). Additional research on the distribution of dominance and selection coefficients in mammals is therefore needed to gain a more precise understanding of the extent to which the fur trade bottleneck led to an increased genetic load in sea otters.

Despite the persistence of genetic load in our simulations, we found that this accumulation of genetic load is unlikely to cause extinction in more ecologically realistic non-WF simulations. This result is encouraging, as it indicates that the decline due to the fur trade was not severe enough to cause species extinction due to genetic factors alone. Critically, the environmental threats that sea otters currently confront, particularly predation by sharks and orca, lack of habitat or prey availability, and environmental catastrophes such as oil spills are likely to have a far greater impact on population recovery (Ballachey et al., 1994; Estes et al., 1998; Tinker et al., 2016; U.S. Fish & Wildlife Service, 2003). In particular, our models do not currently incorporate detailed information about local population density or habitat availability, which have been shown to be important for predicting sea otter carrying capacity and range expansion (Tinker et al., 2008, 2021). Incorporating these spatial dynamics into future simulations might provide even greater resolution on the impact of genetic load on sea otter recovery.

Our Wright–Fisher simulations show that translocations between populations could decrease genetic load through heterosis of population-specific recessive deleterious variants. Such heterosis would have the effect of returning sea otter populations to pre-fur trade levels of fitness more quickly (Frankham, 2016; Hedrick & Garcia-Dorado, 2016; Whiteley et al., 2015). Translocated populations of mixed south central Alaskan and Aleutian ancestry in southeast Alaska and British Columbia, which were not analysed in this study, may have already benefitted in this way from the mixing of populations through translocation (Bodkin et al., 1999; Jameson et al., 1982; Larson et al., 2021). While our simulations suggest a potential benefit of translocation for the southern sea otter, admixture of southern and northern sea otters could also result in the loss of local adaptations and the distinct evolutionary legacy of this long-diverged population (Bell et al., 2019; Edmands, 2007; Waller, 2015). Genetic exchange between southern and northern sea otters may occur naturally in the future if northward migrants from California

overlap in range with southward migrants from the translocated northern sea otter population in Washington.

#### 4.7 | Long-term low ancestral population size may mitigate bottleneck effects

Our simulations indicate that the genetic impact of the fur trade could have been substantially worse if the prebottleneck ancestral populations were larger. Strongly deleterious variants, which tend to be highly recessive, are expected to be present at lower levels in smaller populations, because they are more frequently exposed to selection as homozygotes (García-Dorado, 2012; Hedrick, 2002; Hedrick & Garcia-Dorado, 2016). Recent work by Robinson et al. (2018) and Kyriazis et al. (2021) has suggested that long-term low ancestral population sizes may lead to decreased extinction risk after a population bottleneck due to these populations harbouring fewer recessive strongly deleterious variants. We therefore hypothesized that the relatively small (~3500–5000) historical effective population sizes we inferred for all surviving sea otter populations may have contributed to their apparent low risk of extinction due to genetic factors. We found that a simulated historical population three times larger than what we actually inferred had much greater gains in genetic load during the subsequent population decline (117% vs. 61% increase), indicating that intermediate-sized populations may gain less genetic load through a population bottleneck if strongly deleterious mutations are recessive (Grossen et al., 2020; Kyriazis et al., 2021; Robinson et al., 2018). However, under models in which deleterious mutations are only partially recessive or additive, there was no such benefit to having had a long-term small ancestral population (Note S1). These results highlight the importance of future work to determine the frequency of recessive deleterious variants in genomes, as they determine the degree to which historical demography impacts inbreeding depression. Interestingly, regardless of the mode of dominance, the larger ancestral population model did not have an elevated rate of extinction, suggesting that the recovery of the population is substantial enough to stave off extinction even when genetic load is significantly increased. Furthermore, there may be an important tradeoff in long-term small populations between the elimination of strongly deleterious variation vs. the maintenance of adaptive potential. Large, genetically diverse populations may have greater ability to rapidly adapt to changes in their environment (Hoffmann et al., 2017), though the prediction of adaptive potential based on neutral diversity is not always straightforward (Teixeira & Huber, 2021).

#### 4.8 | Conclusions

In conclusion, we characterized genome-wide genetic diversity in sea otter populations that survived the fur trade. We found that each northern sea otter remnant population is genetically distinct but interconnected via a complex history of gene flow.

The southern sea otter population in California is the last remnant of a long-diverged lineage and is beginning to recolonize Baja California, Mexico. Each of these remnant populations provided a means for rapid recovery of the sea otter and continuity of historical population structure. We detected a strong genetic signal of the fur trade bottleneck in every population, allowing us to estimate its severity and possible impacts on genetic load. We combined these empirical results with genetic simulations to demonstrate an approach for assessing the impacts of extreme population decline and exploring varied recovery scenarios. Several species, such as the Northern elephant seal (Hoelzel et al., 1993), island fox (Robinson et al., 2016, 2018) and North American bison (Hedrick, 2009) have managed to survive extreme population bottlenecks (reviewed in Wiedenfeld et al., 2021). Our simulations indicate that dramatic declines may not doom a species to eventual extinction due to genetic factors alone, provided the recovery is rapid, but the legacy of increased genetic load caused by a bottleneck may be challenging to erode without gene flow between populations. Our genome- and species-wide analysis indicates the need for varied conservation strategies for each sea otter population that reflect region-specific genomic variation, demographic history, and local environmental challenges.

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#### CONFLICT OF INTEREST

The authors have no conflicts of interest.

#### AUTHOR CONTRIBUTIONS










A.C.B., K.P.K., K.E.L. and R.K.W. conceived the study. A.C.B. carried out all analyses and generated all figures except as specified below. P.K. performed demographic analyses in *FASTSIMCOAL2* and designed Wright–Fisher simulations. C.C.K. designed and

completed non-WF simulations. P.K. and C.C.K. contributed figures and supplementary tables. A.A.D. carried out laboratory work and sample management and assisted with study design. S.N.M. performed laboratory work and analyses on the Baja California samples and contributed supplementary figures and discussion with advice from A.M-E. K.P.K. provided samples, guidance, and interpretation of results. G.H. and Y.S. provided samples from Baja California, carried out DNA extractions and advised the project. D.J.K. and M.H. provided historical samples and aided in interpretation of the historical results. D.J.K. developed the radiocarbon chronology for the historical samples. J.B. provided sea otter samples and helped to integrate past sea otter research and conservation strategies. A.C.B. wrote the manuscript with K.E.L. and R.K.W. with input from the other authors. K.E.L. and R.K.W. jointly supervised the study.

#### DATA AVAILABILITY STATEMENT

All sequencing data are available on the Sequence Read Archive (SRA), BioProject accession PRJNA629776. Data files are available on Dryad (<https://doi.org/10.5068/D1ZD4D>). All custom scripts are available on Github (<https://github.com/LohmuellerLab/SeaOtterSequenceCaptureProject>). Benefits Shared: The sea otter samples from Baja California, Mexico, were collected, sequenced and analysed by Mexican researchers, all of whom are co-authors (Gisela Heckel, Yolanda Schramm, Sergio Nigenda-Morales, Andrés Moreno-Estrada), enabling reciprocal exchange of expertise between Mexican and US researchers. The findings will inform sea otter conservation efforts in the USA and Mexico. All collaborators are co-authors, including two undergraduate researchers (Pooneh Kalhori and Amber DeVries). We have disseminated findings via conference talks in the USA and Mexico, and to the public via posts on the Monterey Bay Aquarium blog. Annabel Beichman and co-author Mark Hylkema (Cultural Resources Program Manager & Tribal Liaison/Archaeologist, California State Parks) will coordinate further to disseminate the findings to the public via a virtual webinar. All data and code to generate these analyses are shared publicly (described above).

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