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ARTICLE

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Translocations maintain genetic diversity and increase connectivity in sea otters, *Enhydra lutris*

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Abstract

Sea otters, *Enhydra lutris*, were once abundant along the nearshore areas of the North Pacific. The international maritime fur trade that ended in 1911 left 13 small remnant populations with low genetic diversity. Subsequent translocations into previously occupied habitat resulted in several reintroduced populations along the coast of North America. We sampled sea otters between 2008 and 2011 throughout much of their current range and used 19 nuclear microsatellite markers to evaluate genetic diversity, population structure, and connectivity between remnant and reintroduced

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populations. Average genetic diversity within populations was similar: observed heterozygosity 0.55 and 0.53, expected heterozygosity 0.56 and 0.52, unbiased expected heterozygosity 0.57 and 0.52, for reintroduced and remnant populations, respectively. Sea otter population structure was greatest between the Northern and Southern sea otters with further structuring in Northern sea otters into Western, Central, and Southeast populations (including the reintroduced populations). Migrant analyses suggest the successful reintroductions and growth of remnant groups have enhanced connectivity and gene flow between populations throughout many of the sampled Northern populations. We recommend that future management actions for the Southern sea otter focus on future reintroductions to fill the gap between the California and Washington populations ultimately restoring gene flow to the isolated California population.

KEYWORDS
Enhydra lutris, genetic diversity, microsatellites, population structure, reintroductions, sea otters

1 | INTRODUCTION

The maintenance of genetic diversity within wildlife populations and the recovery of lost diversity through facilitated gene flow among isolated groups is an important component of wildlife management (Caro & Laurenson, 1994; Frankham et al., 2017; Miller et al., 2012; Ralls et al., 2018; Vander Wal et al., 2014; Whiteley et al., 2015). Maintaining genetic diversity is vital to avoid inbreeding depression and sustain adaptive potential (Frankham, 2005; Frankham et al., 2017; Lankau & Strauss, 2007). Population size reduction or isolation of previously connected populations may precipitate a loss of genetic variability (Frankham et al., 2017; Lankau & Strauss, 2007, Ralls et al., 2018). One approach to restore extirpated populations is to reintroduce individuals into previously occupied habitats (Bodkin et al., 1999; Jameson et al., 1982; Weeks et al., 2011). Characterizing genetic diversity and differentiation of both remnant and reintroduced populations is important to determine the effects of past translocations and to assess the need for additional translocations to recover populations, and perhaps even the species (Frankham et al., 2017; Vilà et al., 2003; Weeks et al., 2011).

Sea otters, *Enhyrda lutris*, have an extensive history of population extirpations and reductions, primarily from the documented harvests during the international maritime fur trade (Beichman et al., 2019; Bodkin, 2015; Jameson et al., 1982; Kenyon, 1969; Lensink, 1962). Sea otters were once abundant along the North Pacific Rim from northern Japan in the northwestern Pacific to Baja California, Mexico, in the eastern Pacific (Kenyon, 1969), totaling an estimated population of 300,000 animals (Johnson, 1982; see Figure 1 for historical and contemporary range). During the 18th and 19th centuries, the fur trade eliminated an estimated 99% of the sea otter population from most of its original range, leaving only 13 small, isolated, and scattered populations, 11 of which survived to found the remnant populations extant today (Bodkin, 2015; Kenyon, 1969; Lensink, 1962). Although widespread international hunting had diminished sea otter populations to the point of effectively ending commercial hunting by the late 1800s, sea otters did not receive international legal protection until the Treaty for the Preservation and Protection

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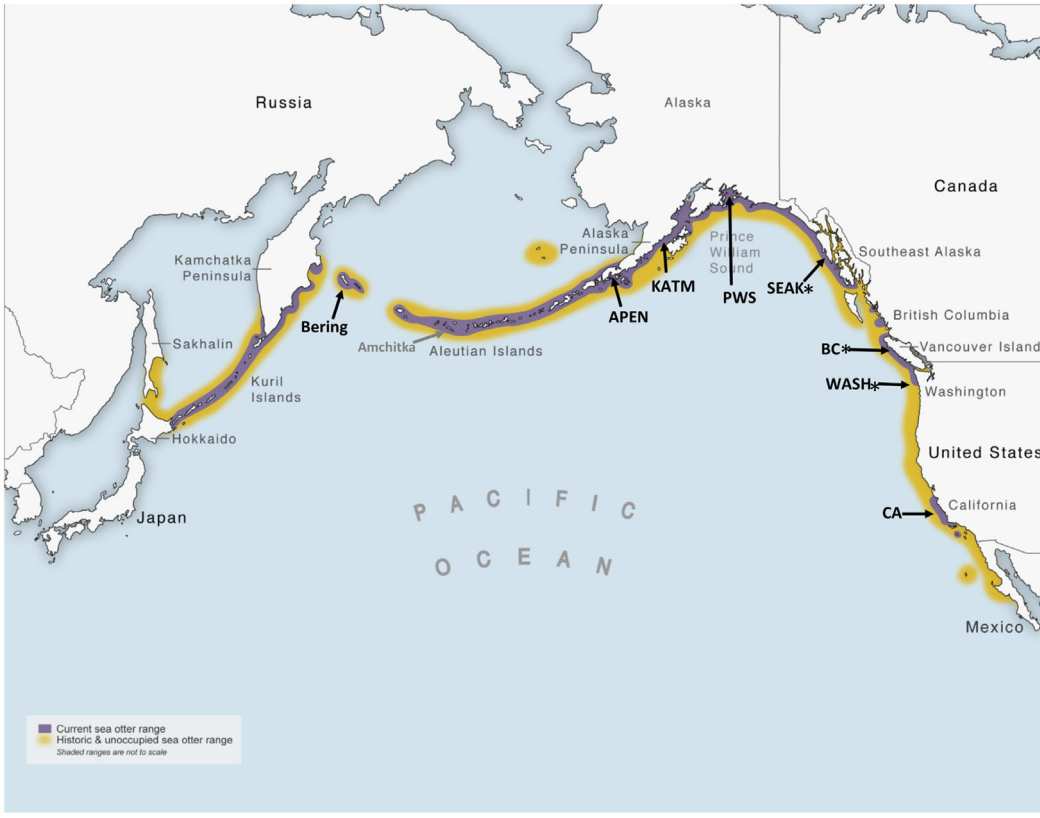

FIGURE 1 Map of historical and present-day sea otter distribution with the eight sampled locations identified in bold and the reintroduced populations (SEAK, BC, and WASH indicated by an asterisk). The purple shading is the current sea otter range, the gold shading is the historic range and the gold shading with no corresponding purple shading the historic range that remains unoccupied. Map originally published in Larson and Bodkin (2015) and reprinted with permission from Elsevier.

of Fur Seals in 1911, in which they were recognized as a fur bearing marine mammal in need of protection along with fur seals, *Callorhinus ursinus* (Kenyon, 1969; Van Blaricom, 2015). Recovery varied with many populations in Alaska returning to preexploitation levels by the middle of the 20th century, while recovery in California remained slow (Bodkin, 2015; Kenyon, 1969). Range expansion in portions of the Aleutian Archipelago was relatively rapid, less than 60 years in some cases, with recovery facilitated by the presence of several remnant populations and the relatively short distances between some of the Aleutian Islands, on the order of 10s of kilometers, which is within the normal movement range of the sea otter (Garshelis & Garshelis, 1984; Kenyon, 1969; Lensink, 1962). However, most of the historic sea otter habitat along the west coast of North America remained vacant, from the remnant population in Prince William Sound, Alaska, to the remnant California population centered around Big Sur (Estes, 1990; Kenyon, 1969).

To address this gap in sea otter populations, state and federal agencies made several translocations from the late 1960s through the early 1970s to vacant habitat between Alaska and California (Jameson et al., 1982). In total, 715 otters were captured at Amchitka Island in the Aleutian chain and in Prince William Sound, Alaska, and released into previously occupied habitats in Southeast Alaska, Vancouver Island in British Columbia, Washington, and Oregon (Bodkin et al., 1999; Estes, 1990; Jameson et al. 1982). The Southeast Alaska and Vancouver Island translocations were founded by animals from both Amchitka and Prince William Sound, while the Washington and Oregon translocations were founded only by Amchitka animals (see Table 1 for descriptions about this study's populations and their origins; see also Bodkin, 2015; Bodkin et al., 1999; Estes, 1990; Jameson et al. 1982 for translocation

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TABLE 1 History of sea otter sampling locations between 2008 and 2011: populations sampled, sample size, remnant versus reintroduced population and, if reintroduced, source population numbers.

Population	Sample size	Remnant	Numbers reintroduced and source populations
Bering	28	Yes	
Alaskan Peninsula (APEN)	34	Yes	
Katmai (KATM)	29	Yes	
Prince William Sound (PWS)	67	Yes	
Southeast Alaska (SEAK)	61	No	369 (Amchitka) 43 (PWS)
Vancouver Island (BC)	41	No	29 (Amchitka) 60 (PWS)
Washington (WASH)	30	No	43 (Amchitka)
California (CA)	44	Yes	

history). All translocations resulted in viable sea otter populations extant today, except for the one to Oregon, which failed for unknown reasons (Bodkin, 2015; Jameson et al., 1982).

From fur trade extirpations to translocations and subsequent population recovery, many extant sea otter populations may have suffered multiple reductions in population size (Aguilar et al., 2008; Larson, Jameson, Bodkin, et al., 2002; Larson, Jameson, Etnier, et al., 2002). Genetic studies focused on sea otters in the past two decades have employed a variety of variable nuclear genetic markers, specifically microsatellites. Microsatellites are nuclear markers that gained popularity in the 1990s because they are highly variable and abundant within the genome (Bentzen et al., 1991; Park & Moran, 1994, Wright & Bentzen, 1994). They are stretches of DNA with repeating units of 1–6 basepairs (bp) that are assumed to be genetically neutral allowing mutations to accumulate making them variable. Multiple loci can be multiplexed, and they are easily amplified making them affordable. Microsatellite loci within sea otters, many of which were also used in this study, have demonstrated low genetic diversity with expected heterozygosities (H_E) between 0.41 and 0.57 (Aguilar et al., 2008; Cronin et al., 1996; Cronin et al., 2002; Gagne et al., 2018; Larson, Jameson, Bodkin, et al., 2002; Larson, Jameson, Etnier, et al., 2002; Larson et al., 2012; Scribner et al., 1997). In an effort to estimate preexploitation sea otter genetic diversity, Larson, Jameson, Etnier, et al. (2002) and Larson et al. (2012) used nuclear microsatellite markers and 600- to >10,000-year-old sea otter bones found in North American First Nations midden collections. These ancient sea otter samples had levels of H_E ranging from 0.62 in ancient California to 0.86 in ancient Alaska. These values are similar to those in many present day non-exploited mammalian populations, e.g., 0.73 for the domestic dog, *Canis familiarus* (Wayne, 1996) and 0.86 for the common field mouse, *Apodemus sylvaticus* (Harr et al., 2000). In comparison, sea otter populations sampled in the 1990s using some of the same nuclear genetic markers used in the ancient populations had H_E between 0.46 and 0.53 (Larson, Jameson, Bodkin, et al., 2002) that when compared to the pre-fur trade values reported in Larson et al. (2012) represented a loss of about 30%–40% of pre-bottleneck heterozygosity (Aguilar et al., 2008; Gagne et al., 2018; Larson, Jameson, Etnier, et al., 2002). The sea otter population with the highest measured diversity resulted from a translocation founded by more than one source population, Southeast Alaska, $H_E = 0.53$ (Larson, Jameson, Etnier, et al., 2002). Recent whole genome sequencing of modern-day sea otters also described extremely low genome wide heterozygosity similar to endangered taxa such as cheetahs, *Acinonyx jubatus*, when compared to the genome wide diversity of the giant otter, *Pteronura brasiliensis* (Beichman et al., 2019). This project utilizing whole genome sequencing within sea otters also provided evidence of recent inbreeding and demographic histories marked by population declines, suggesting past population bottlenecks, associated both with the fur trade and earlier events, that are thought to have resulted in continued low genetic diversity (Beichman et al. 2019).

Sea otter population structure, including identification of population boundaries, stocks, and subspecies has been investigated previously. There are three recognized sea otter subspecies based on skull morphology: Russian (*E. l. lutris*), Northern (*E. l. kenyoni*), and Southern (*E. l. nereis*) (Wilson et al., 1991). The dominant population structure

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illuminated by analyses using nuclear genetic data is the separation of the Southern and Northern sea otters (Aguilar et al., 2008; Larson, Jameson, Bodkin, et al., 2002). Using two nuclear microsatellite loci, one variable region within the mitochondrial DNA, and phenotypic variation, Gorbics and Bodkin (2001) and Cronin et al. (2002) found further structure defining at least three distinct sea otter stocks or genetic management units within the Northern subspecies (*E. l. kenyoni*): a Southwest stock (SW) including the Aleutian Islands, the Alaska Peninsula, the Katmai peninsula, and Kodiak Island (remnant groups); a Southcentral (SC) stock including Prince William Sound, the Kenai Peninsula, and Cordova (remnant groups); and a Southeastern (SE) stock including the Alexander Archipelago (reintroduced group). The designation of these stocks proved useful for the management of the species in Alaska. For example, a 90% decline in large portions of the SW stock in the 1990s resulted in its listing as threatened under the U.S. Endangered Species Act (ESA) in 2005 (Doroff et al., 2003; Estes et al., 1998; Gorbics & Bodkin, 2001; U.S. Federal Register, 2005; U.S. Fish and Wildlife Service, 2013). The California population is also listed as threatened under the ESA (U.S. Fish and Wildlife Service, 2003). The International Union for the Conservation of Nature (IUCN) classifies all sea otters as endangered, in part because of the large areas of unoccupied habitat and the variability in the growth rates among remnant populations (Doroff & Burdin, 2015).

We evaluated population genetics in sea otters sampled throughout North Pacific coastal regions of the United States, Canada, and Russia between 2008 and 2011, and report on recent genetic variation, gene flow, and population structure. This study represents the most comprehensive sampling to date in terms of the number of independent markers used (19 microsatellites) and geographic range of samples, from Bering Island in Russia south to California, sampling all subspecies and many groups throughout their present range. We ask whether reintroduced sea otters have genetic diversity comparable to that of remnant groups and determine range-wide sea otter genetic structure and the amount of gene flow between sampling locations.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Sea otters were sampled at eight locations between 2008–2011 from Santa Barbara, California in the south to the Bering Islands in the northwest for a total sample size of 334 individuals. Locations sampled, abbreviations, and sample sizes from south to north and then east to west were as follows: California coast (CA, $n = 44$); Washington coast (WASH, $n = 30$); Clayoquot Sound and Nuchatlitz Inlet along the west coast of Vancouver Island, British Columbia, Canada (BC, $n = 41$); Elfin Cove and Whale Bay in Southeast Alaska (SEAK, $n = 61$); Prince William Sound (PWS, $n = 67$); Katmai coast, Alaska (KATM, $n = 29$); the Alaskan Peninsula (APEN, $n = 34$), and Bering Islands in Russia (Bering, $n = 28$) (Figure 1 and Table 1). We sampled all the known sea otter groups between CA and SEAK, however, there are groups of sea otters between SEAK and Bering and to the west of Bering that were not sampled.

2.2 | DNA extraction and amplification

Animals were captured using either tangle nets or Wilson traps (Ames et al., 1986). Each animal was sedated with a combination of fentanyl-citrate and midazolam hydrochloride (Monson et al., 2001; Murray, 2015). Tissue plugs obtained to facilitate hind flipper tagging were used for genetic analysis. Tissue plugs were preserved in 100% ethanol or frozen at -20°C or -80°C. DNA from tissue was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California). For all samples, microsatellite genotypes were determined for twenty loci: Mvi 30 and 87 (O'Connell et al., 1996); Mvis 57, 72, and 75 (Fleming et al., 1999); Lut 453, 457, and 832 (Dallas & Piertney, 1998) and A011, A202, A314, A318, A325, B110, B118, B313, B317, D119, D311, and D320 (Arias et al., 2016). Amplification of the 20 microsatellites was conducted at the Veterinary Genetics Laboratory, University

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of California at Davis, and was carried out in three multiplexed PCR reactions (see Table 2 for reaction details including primer concentration). Reactions were 12.5 μl total volume, each containing 3.5 μl of primer mix, 8 μl of master mix, 1 μl of DNA template (50–150 ng/μl quantified using a GeneQuant II spectrophotometer, Pharmacia Biotech), with an additional 15 μl of nonreactive Chillout added to each well to prevent evaporation for a final volume of 27.5 μl. The master mix in PCR reaction contained 1X Taq Buffer with $(NH_4)_2SO_4$ (Thermo Fisher), 1 unit of GoTaq Flexi DNA Polymerase (Promega), 2.5 mM $MgCl_2$, 0.2 mM dNTPs and 2% DMSO. Amplifications were carried out on Applied Biosystems 2720 Thermal Cyclers (Life Technologies) with the following conditions: 95°C for 5 min, 85°C for 10 min, 5 cycles of 94°C for 1 min, 60°C (Mix A and B) or 58°C (Mix C) for 30 s, 72°C for 30 s, 26 cycles of 94°C for 45 s, 60°C (Mix A and B)/58 (Mix C) for 30 s, 72°C for 30 s, with a final extension at 72°C for 15 min. Primer mix and DNA were combined first and set on the thermal cycler for the first step of denaturation after which master mix was then added during the 85°C step, and PCR cycling resumed.

PCR products were run on an Applied Biosystems 3730 DNA Analyzer (Life Technologies) with GeneScan 500 LIZ (Life Technologies) as the internal size standard. All samples were amplified and genotyped twice for confirmation, and each plate of DNA contained positive (DNA from one animal used for PCR assay optimization) and negative (water) controls. Two people separately used STR Analysis Software (Toonen & Hughes, 2001) to score and bin alleles. No genotype mismatches were observed between duplicate runs. MSToolkit (Park, 2001) was used to identify potential duplicate samples. Two pairs of samples had matching genotypes at all 20 loci and the suspected duplicates were removed from the data set.

TABLE 2 Multiplexed primer sets used for PCR and genotyping of 20 microsatellite loci in sea otters, including fluorescent dye labels and primer concentrations in uM (micromolar) within the 3.5 ul of primer mix.

Multiplexes	Dye label	Primer concentration in PCR (μM)
Primer Mix A		
Mvi57	FAM	0.68
B110	FAM	0.11
A318	PET	0.18
D320	VIC	0.15
B118	FAM	0.13
B317	NED	0.07
Primer Mix B		
A314	PET	0.16
A325	FAM	0.15
D311	VIC	0.11
A011	FAM	0.80
D119	PET	0.13
A202	NED	0.20
B313	VIC	0.28
Primer Mix C		
Mvi87	FAM	0.16
Lut453	VIC	0.06
Mvis75	FAM	0.15
Lut457	VIC	0.08
Lut832	FAM	0.15
Mvi30	PET	0.60
Mvis72	VIC	0.15

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2.3 | Genetic structure and diversity analyses

GENEPOP 4.7 (Rousset, 2008) was used to test for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium with default Markov chain parameters as follows: 1,000 dememorizations, 100 batches, and 1,000 iterations. Sequential Bonferroni adjustments were applied to correct for multiple testing (Rice, 1989). GENEPOP was also used to determine microsatellite null alleles and scoring errors. Genetic diversity indices such as expected and observed heterozygosity (H_E and $H_{O,}$ respectively), number of alleles (Na), fixation index (F), diversity information index (I) to standardize diversity, unbiased expected heterozygosity (uH_E) accounting for sample size, and Analyses of Molecular Variance (AMOVAs) were computed using GENALEX 6.5 (Peakall & Smouse, 2006). To test for differences in diversity metrics among sampling locations, we used a bootstrapping method that estimates measures of genetic diversity and accounts for influences of population size by resampling the data at different population sizes (Liu et al., 2015, 2018). We ran analyses with 1,000 replicates and considered sample sizes of 26, 27, and 28. Analyses were run using modified R scripts developed by Liu et al. (2015; http://datadryad.org/resource/doi:10.5061/dryad.4nj00). We followed the guidance of the developers (Liu et al., 2015) of this analysis in selecting sample sizes. The largest bootstrap resampling size cannot be larger than the smallest actual sample size, which was 28. We chose to test multiple sample sizes to ensure that the results were robust to selection of the sampling size. However, we also kept them as large as possible because one would expect fewer samples to be less accurate in estimating genetic diversity of the population.

2.4 | Structure

Genetic structure was explored and characterized using F_{ST} metrics generated from GENALEX, STRUCTURE 2.3.4 (Pritchard et al., 2000) and TESS 2.3 (Chen et al., 2007). STRUCTURE was used to assign individuals to groups based on Hardy–Weinberg and linkage equilibrium using a Bayesian clustering algorithm. The analyses were run with individual labels and with population data (POPDATA) turned on for each individual, no other priors, the admixture model, correlated allele frequencies among populations, and a burn-in period of 10,000 with 100,000 simulations and 10 replicates for each K. The number of clusters explored, or K, ranged between 1 and 8 for the total data set and between 1 and 7 for the subset of Northern sampling locations without the CA group. These two scenarios were explored to determine population structure in the whole data set and in the Northern sampling locations only. Best fit of K from STRUCTURE results was determined using CLUMPAK software based on ΔK by Evanno with Q values indicating the percentage of an individuals' ancestry assigning to each cluster (Evanno et al., 2005; Kopelman et al., 2015). We did not assign individuals to clusters; rather sampled populations were clustered together or not based on the K value with the amount of admixture within populations an indication of individuals with genetic identity similar to other clusters.

The program TESS 2.3.1, a Bayesian clustering program that incorporates geographic locations of samples, was used to conduct a spatially explicit population structure analysis (Durand et al., 2009). The TESS software uses a conditional auto-regressive residual term to account for spatially auto-correlated random effects in order to determine genetic admixture across the sampled area. It models broad-scale genetic clustering of individuals across a multidirectional surface while accounting for the influence of isolation by distance and/or geographic clustering of closely related individuals (Durand et al., 2009). The program was first implemented using the no admixture model for $K = 2$ up to $K = 10$ with ten runs per K (number of clusters) with a burn-in of 10,000 followed by 100,000 iterations. We weighted the Voronoi neighborhood using pairwise great circle geographic distances and used the CAR model (Durand et al., 2009). These runs calculated the deviance information criterion (DIC) needed to identify the upper bound of K but results were inconclusive with no clear plateau. Following developer recommendations, we then ran the admixture models using the same parameters (Durand et al., 2009). Again, no clear best K was identified with the model selection procedure. Thus, we mapped multiple Ks and visualized the data using the package

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POPSutilities implemented in R (R Core Team, 2016), which overlays the population assignment probabilities (*Q* coefficients) on the sampling location (Jay et al., 2012).

Isolation by distance (IBD) was measured between sampling locations and individuals. Isolation by distance occurs when limited dispersal of individuals results in an increase of genetic dissimilarity with geographic distance (Guillot et al., 2009; Rousset, 1997). We conducted IBD analyses using the regression method implemented in GENEPOP 4.7 based on the expected linear relationship between genetic and geographic distances (Rousset, 1997, 2000). Mantel tests with 10,000 permutations were used to assess the significance of the correlation between matrices of genetic and geographic distances. The slope of the IBD regression line provides a robust estimator of $1/D\sigma^2$, the inverse of neighborhood size, where D is the effective density of individuals, and σ^2 the mean squared parent-offspring dispersal distance (Rousset, 1997, 2000). The IBD analysis was run with GPS coordinates for sampled animals using the pairwise genetic differentiation estimator *ar* calculated between individuals (Rousset, 2000). The logarithm of Euclidean geographic distances between individuals and a minimum distance between individuals of 10 km was used to exclude pairs of geographically close individuals from the regression.

To further investigate the spatial genetic differentiation, we used Mapping Averaged Pairwise Information (MAPI) (Piry et al., 2016) implemented in the R package *mapi*. This program smooths pairwise genetic measures attributed to a network of ellipses linking the sampling points to produce geographical layers representing a global "image" of spatial variation of the genetic relationships between sampled individuals. Coordinates of sampled individuals were projected to WGS 84/North Pole LAEA Bering Sea cartesian coordinates (EPSG:3571). An error circle radius was set globally to 1 km for each individual. The MAPI hexagonal grid resolution was computed using a beta parameter of 0.25 (random sampling) due to patchy sampling and distribution of otters, which resulted in a cell halfwidth of 76 km × 76 km. The genetic metric used in this MAPI analysis was the *ar* estimator from Rousset (2000) as described above. In order to avoid intralocation ellipses and to enhance interpopulation signals, the dMin parameter (i.e., minimal distance between two samples) was set to 100 km. Other MAPI parameters left as default. Significant areas were estimated using 10,000 permutations of sample locations.

2.5 | Gene flow/connectivity

We used three methods to evaluate gene flow or recent migrants between sampling locations. Each program uses different algorithms to determine individual assignments to populations, either population of origin or another, based on genetic similarities. Each program estimated recent or first-generation migrants. Multiple analyses were conducted to determine consensus or common migrants identified in each method lending greater credence to those migrants specifically. We determined the putative number of migrants between sampling locations using STRUCTURE 2.3.4 (Pritchard *et al*., 2000), GeneClass2 (Piry et al., 2004) and BayesAss 3.0 (Wilson & Rannala, 2003). STRUCTURE uses a Bayesian iterative algorithm to assign individuals into groups that share similar patterns of variation. We ran the analysis with known population information turned on (POPDATA), a burn-in period of 10,000 with 100,000 simulations and 10 replicates with a migrant significance level of 0.05, which is the default setting for migrant analysis in STRUCTURE. GeneClass2 (Piry et al., 2004) estimates the number of migrants between sampling locations using a maximum likelihood ratio method and the individual assignment probability on the basis of multilocus genotype data (Paetkau et al., 1995). We ran 1,000 simulated individuals using the Paetkau (2004) algorithm, the maximum likelihood ratio and Nei's standard distance to evaluate the probability of membership in each reference population. Finally, we ran BayesAss 10 independent times with 50 million iterations of Monte Carlo multiple comparisons (MCMC), a burn-in of 1 million, and a sampling interval of 10,000. Although results were consistent across runs, evaluation of the trace file using Tracer v1.9 (Rambaut et al., 2018) revealed a jump in the log probability and a relatively low (~23) effective sample size (ESS) score. As a result, an additional ten runs were conducted with a burn-in of 10 million, sampling interval of 20,000 and 100 million MCMC.

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3 | RESULTS

Ninety-seven percent ($n = 324$) of samples were genotyped at all loci. Mvi87 was found to be sex linked to the X chromosome as no males were heterozygous and females were either homozygous or heterozygous, thus this marker was eliminated in further population genetic analyses. After Mvi87 was removed, GENEPOP indicated there was linkage disequilibrium in less than 1% (5/1,368 comparisons or 0.36%) of all locus comparisons in all populations, which failed to show significant differences after Bonferroni corrections. Diversity metrics, including Na, H_E, H_O, uH_E, I, F, and HWE, are in Table S1. All loci in all sampling locations were in HWE after Bonferroni corrections. GENEPOP indicated that two loci, B313 and Mvis75, had null alleles in greater than half of the sampling locations and genotyping errors ranged from 0% in most loci and populations up to 12% in locus B313 in BC (Table S2). To determine if null alleles were interfering with interpretation of results, we ran diversity metrics in GeneAlEx and STRUCTURE on data sets with all remaining loci (19) and with B313 and Mvis75 removed (17). Diversity metrics results (Na, I, H_O, H_E, uH_E) from both data sets were not found to differ significantly (Mann Whitney U test = 0.494, $p = .624$) and STRUCTURE CLUMPAK best Evanno $\Delta K = 2$ for both data sets. Thus the final data set for remaining analyses included 19 loci.

3.1 | Genetic diversity

Diversity in all metrics (Na, I, H_O, H_E, and uH_E) was not found to differ significantly between remnant and reintroduced groups (Table 3). The only significant difference in the bootstrapping comparisons was that CA, a remnant population, had a lower number of alleles compared to other populations ($p = .038$). The highest diversity, as measured by uH_E, was found in the reintroduced sampling locations SEAK and WASH (Table 3), whereas the lowest diversity metrics were found in Bering (as measured by H_O, H_E, and uH_E), followed by CA (Table 3). The AMOVA analysis indicated that 83% of the variation found was within individuals, 16% was among sampling locations, and only 1% was among individuals. Thus, the vast majority of the genetic variation found here was within each individual sea otter with only a relatively small portion between populations.

TABLE 3 Genetic diversity estimates and comparisons between remnant and reintroduced groups including sample size (N), number of alleles (Na), number of alleles resampled via bootstrapping (NaRs), diversity index (I), observed heterozygosity (H_O), expected heterozygosity (H_E), and unbiased expected heterozygosity (uH_E).

	N	Na	NaRs	I	H_O	H_E	uH_E
Remnants							
BERING	28	3.6	3.44	0.84	0.48	0.46	0.47
APEN	34	5.0	4.66	1.01	0.56	0.55	0.55
KATM	29	4.7	4.33	1.00	0.55	0.55	0.55
PWS	67	4.5	3.99	1.00	0.55	0.54	0.54
CA	44	3.4	3.2	0.90	0.49	0.49	0.49
Averages	40	4.3	3.99	0.95	0.53	0.52	0.52
Reintroduced							
SEAK	61	5.3	4.66	1.15	0.58	0.59	0.60
BC	41	4.9	4.44	1.07	0.52	0.54	0.55
WASH	30	3.9	3.77	1.01	0.55	0.55	0.56
Averages	44	4.7	4.22	1.08	0.55	0.56	0.57

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3.2 | Population structure

Genetic differences between sampling locations as measured by F_{ST} were lowest between those related by translocations (SEAK and BC, $F_{ST} = 0.01$, and SEAK and WASH, $F_{ST} = 0.02$) and between adjacent sampling locations (BC and WASH, $F_{ST} = 0.03$). Values were highest between CA and all other sampling locations (from $F_{ST} = 0.18$ with SEAK to $F_{ST} = 0.26$ with Bering; Table 4). All comparisons were significant ($p < .0001$) and the values seen here fall within the acceptable range for microsatellite F_{ST} values with those 0.00–0.05 representing little genetic differentiation (e.g., those related by translocation, SEAK and BC, and/or adjacent, BC and WASH), those values 0.05–0.15 representing moderate genetic differentiation (e.g, Bering and the rest of the Alaska populations), and those values 0.15–0.25 representing substantial genetic differentiation (e.g., comparisons between CA and all others; Hartl & Clark 1997).

Alpha convergence was determined in preliminary runs with very good convergence using the STRUCTURE parameters and this data set (Figure S1). STRUCTURE best supported two genetic clusters ($\Delta K = 2$) (Figure 2a); a Southern group containing only CA, and a Northern group containing all other sampling locations. It is worth noting that when CA is compared to the other populations as in 2a there is very little admixture, or some individuals that seem they may belong to another population as indicated by a color different than their cluster. This is striking when compared to Figures 2b through 2d where there is clear admixture among groups. There was also some support, although less than the $\Delta K = 2$, for both three (Figure 2b) and four genetic clusters (Figure 2c) for all populations. Three clusters (Figure 2b) indicated CA and PWS as distinct with all other Northern populations in the same cluster. Four clusters (Figure 2c) show individuals largely clustering with their geographic sampling location: (1) CA; (2) WASH, BC, and SEAK (related by translocation); (3) PWS; and (4) KATM, APEN, and Bering (west Alaska and Aleutian chain). There is admixture apparent in all these figures except for Figure 2a with KATM, SEAK, and BC having colors from PWS cluster likely due to geneflow between KATM and PWS and common founders in SEAK and BC from Amchitka in the west Aleutians closest to the sampled APEN and Bering populations. To further investigate substructure, STRUCTURE was rerun only on the Northern group and using the same settings and parameters (Figure 2d). Within the northern groups two genetic clusters were best supported with a cluster representing PWS and a cluster representing the remaining sampling locations with APEN, KATM, SEAK, and BC having admixture between the two clusters as indicated by the orange lines within the blue (Figure 2d). This is thought to be due to both the geneflow between PWS and KATM and APEN as well as PWS founders in the reintroduced populations, SEAK and BC. See Figure S3 for CLUMPAK output barplots for all K values for all populations, ΔK by Evanno graphs and LnPD graphs for all groups and Northern groups, respectively.

Spatial clustering using TESS analysis identified three to four main clusters, with highest support for four clusters. The three clusters were: (1) All Northern groups except for PWS, e.g., Bering, APEN, KATM, SEAK, BC, and

TABLE 4 F_{ST} pairwise comparisons. All *p*-values for pairwise comparisons <.0001.

FST	Bering	APEN	KATM	PWS	SEAK	BC	WASH	CA
Bering	—							
APEN	0.05	—						
KATM	0.07	**0.03**	—					
PWS	0.09	**0.06**	**0.04**	—				
SEAK	0.05	0.04	0.04	***0.05***	—			
BC	0.04	0.04	0.05	0.06	***0.01***	—		
WASH	0.07	0.06	0.07	0.08	0.02	***0.03***	—	
CA	0.26	0.19	0.20	0.20	0.18	0.20	0.20	—

Note: Bold = adjacent sampling locations and italic = sampling locations related by translocations.

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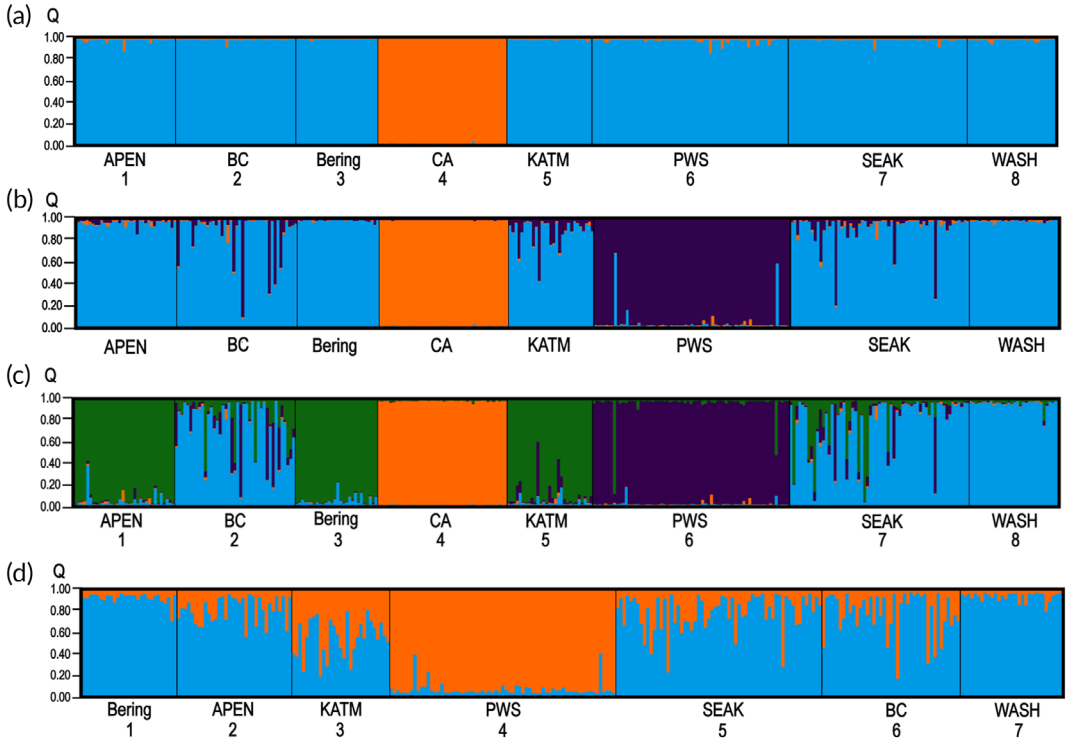

FIGURE 2 Results from STRUCTURE analysis run using POPDATA. (a) All populations returned a best K = 2 based on the Evanno method-CA and all others. (b) K = 3 for all populations with all northern populations clustering together except for PWS and CA also distinct. (c) K = 4 for all populations with CA and PWS as distinct, Bering, APEN, and KATM clustering together, and SEAK, BC, and WASH clustering together. (d) Northern populations only (CA data excluded) and best K = 2 by Evanno-PWS as distinct and all others.

WASH; (2) PWS; and (3) CA. The four clusters were: (1) Bering, APEN, and KATM; (2) PWS; (3) SEAK, BC, and WASH; and (4) CA (Figure 3). The TESS four cluster result (Figure 3) had the highest overall confidence because the majority of individuals had very strong assignment (Q > 0.90) to their clusters except for in KATM (1 of 29 samples) and SEAK (22 of 61 samples), where individuals also had assignment to PWS, similar to the admixture found in the STRUCTURE results (Figure 3). Increasing the number of clusters (K) to five or more resulted in overall low individual assignment probabilities (smaller than 0.25) to the additional clusters and was not further considered.

There was a clear and significant IBD pattern in the sea otter sampling locations. Our first IBD analysis across the entire geographic range (Figure 4a) showed a significant correlation between genetics and geographic distance ($ar = -0.44 + 0.045$ ln(distance), Mantel p-value = .00). However, differentiation between CA samples (red points) and all others was high compared to the cloud of all other pairwise differentiation values, and this potentially obscured IBD patterns among other groups (Figure 4a). For this reason, we ran a second IBD analysis excluding the CA samples, which then indicated a significant and similar IBD pattern (Figure 4b, $ar = -0.11 + 0.015$ ln(distance) and Mantel p-value = .00). In this second IBD analysis, no particular pattern emerged for Bering (red points), the most isolated by distance group sampled compared to the other Northern sampling locations, suggesting that the IBD pattern is similar for all sampled locations except for CA. Results from the MAPI analysis (Figure 5) were concordant with the IBD analyses. There are a range of colors from dark red (low genetic distance) to dark blue (high genetic distance). A significant discontinuous area (designated by hatched hexagons) separates CA and all other sampling locations, while three significant continuous areas (enclosed in dotted lines) encompass Western and Central Alaska (Bering, APEN, KATM, PWS), the reintroduced populations (SEAK, BC, WASH,) and CA, respectively. The top-left single blue hexagon near Bering is a side-effect of

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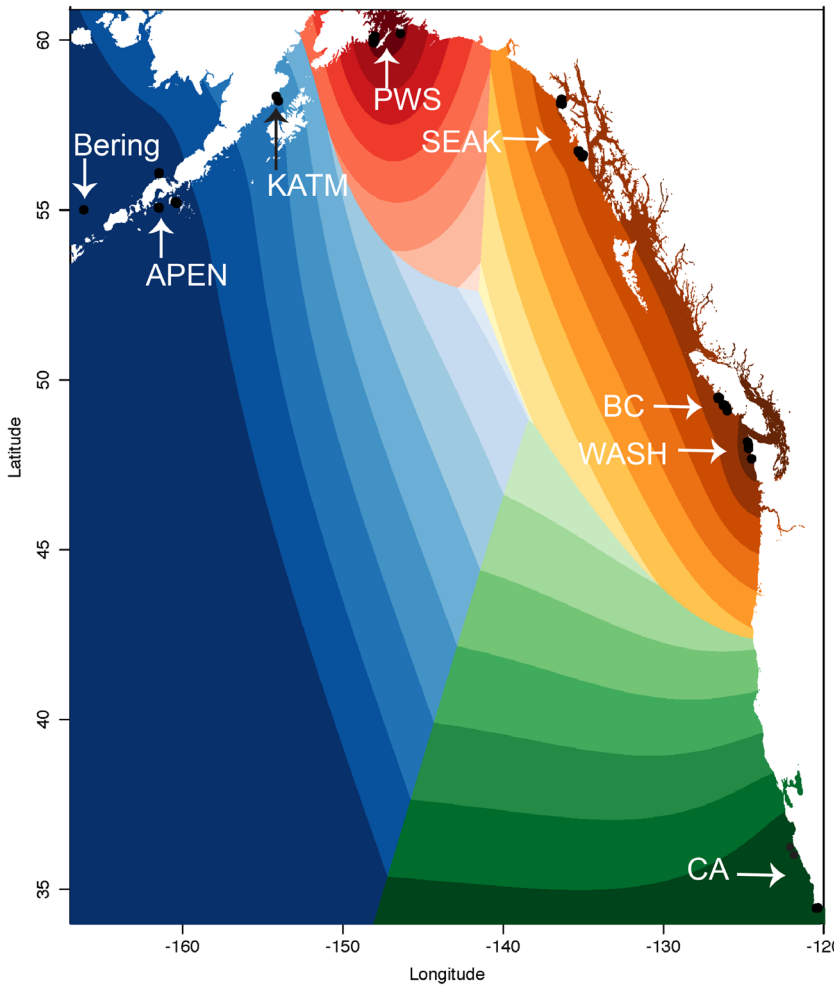

FIGURE 3 TESS results mapping K = 4. 1. Bering, APEN and KATM; 2. PWS; 3. SEAK, BC, and WASH; 4. CA. The assigned genetic cluster is indicated by the color, with the shade corresponding to the proportional assignment to that cluster (the lightest shade represents Q Coefficients 0.5 to 0.6 and the darkest shade Q > 0.9). To illustrate the result, we have extended colors out into the ocean, where sea otters actually would be absent. Similarly, the color will extend longer distance when there is a gap in sampling or sea otters. Such as between Washington and California where sea otters are absent.

long-range ellipses. MAPI showed a spatially homogeneous IBD pattern for all the Northern sampling locations up to Bering Island but with much higher differentiation between CA and all other sampling locations, resulting in two clusters.

3.3 | Gene flow/connectivity

All methods showed recent migration (first generation migrants) between sampled sea otter populations. STRUCTURE migrant analysis resulted in seven significant miss-assignments (i.e., suspected migrants), GeneClass2 resulted in 15 significant migrants per generation, and BayesAss found 24 migrants out of 334 individuals (Tables 5 and S3 (c) for detailed output). The majority of migrants were between adjacent groups, e.g., between BC and WASH, between APEN and KATM and between PWS and SEAK (Table 5) as well as those related by translocations such as

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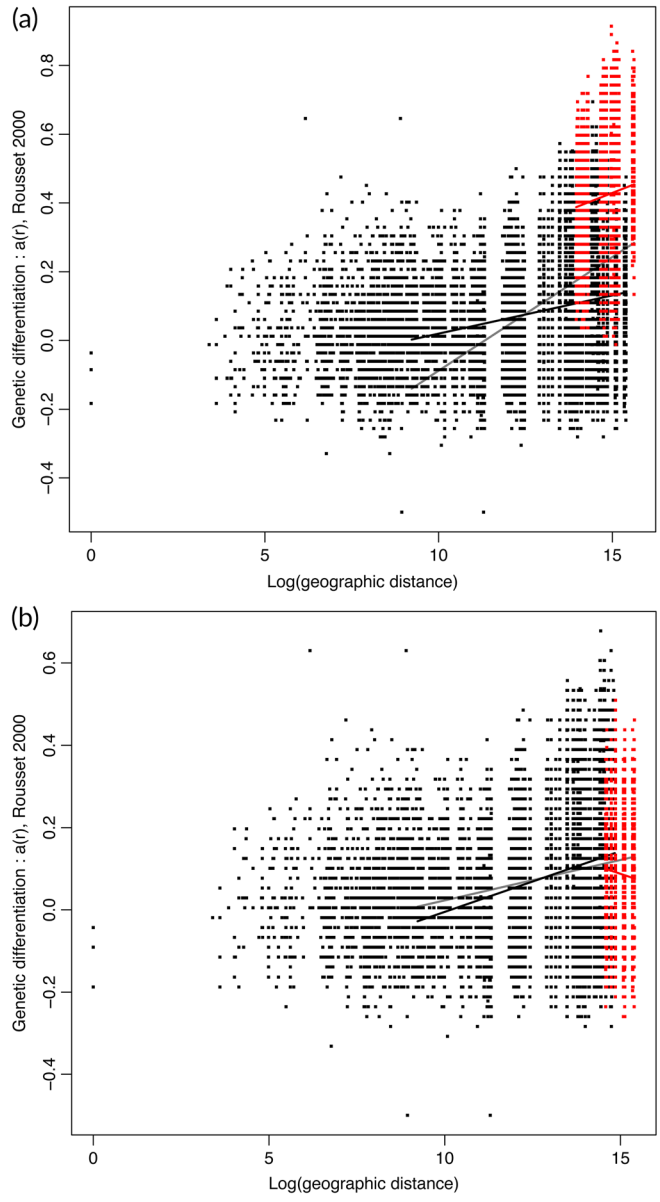

FIGURE 4 Isolation by distance analysis for sampled sea otter populations, shown as the regression of pairwise genetic differentiation between individuals against the logarithm of distance between the populations. (a) IBD all. Red points represent pairwise comparisons between CA samples and other localities. Regression lines: The gray line is the regression on all points (black and red points, $ar = -0.75 + 0.066$*log (distance); The black line is the regression for the black points only, i.e., excluding CA ($ar = -0.20 + 0.022$*log (distance); The red line is the regression for the red points only, i.e., the differentiation between CA and all others ($ar = -0.17 + 0.040$*log (distance). (b) IBD without CA. Red points represent pairwise comparisons between Bering samples and other localities. Regression lines: The gray line is the regression on all points (black and red points, ($ar = -0.17 + 0.020$*log(distance); The black line is the regression for the black points only, i.e., excluding Bering ($ar = -0.30 + 0.030$*log(distance); The red line is the regression for the red points only, i.e., the differentiation between Bering and all others ($ar = 0.52–0.029$*log(distance).

between WASH and SEAK and between PWS and BC (Table 5). All of the BayesAss migrants (between APEN and KATM, SEAK and WASH, PWS and WASH, and BC and WASH), including the direction of the migration, were also detected in at least one of the other programs lending greater credence for those migrants.

4 | DISCUSSION

4.1 | Genetic diversity

Genetic diversity was comparable across reintroduced and remnant sea otter locations. The sampling locations with the greatest diversity, SEAK and WASH, as measured by uH_E (0.60 and 0.56, respectively) are approaching 80%

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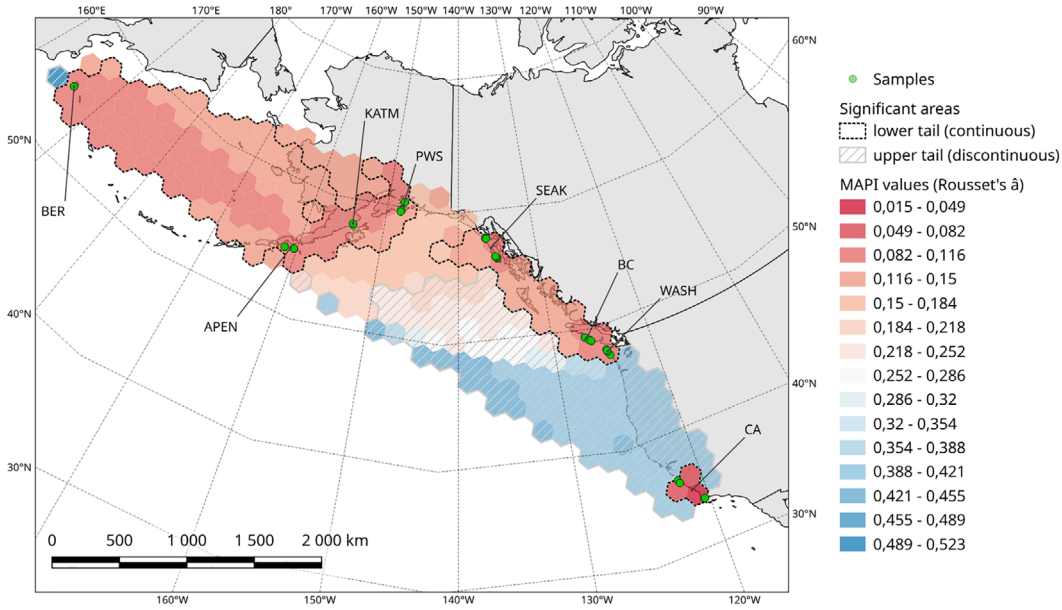

FIGURE 5 MAPI analysis output showing mean genetic differentiation among samples using individual locations. There is a range of colors from dark red (low genetic distance) to dark blue (high genetic distance). A significant discontinuous area (designated by hatched hexagons) separates CA and all other sampling locations, while three significant continuous areas (enclosed in dotted lines) encompass Western and Central Alaska (Bering, APEN, KATM, PWS), the reintroduced populations (SEAK, BC, WASH) and CA, respectively. The top-left single blue hexagon near Bering is an side-effect of long-range ellipses. Cells should not be interpreted one-by-one but altogether form an image of the spatial relationships.

(78% and 72% respectively) of estimated pre-bottleneck genetic diversity of pre-fur trade sea otters (Larson et al., 2012). Those sampling locations are now close to the diversity estimates from microsatellites found in other nonthreatened species such the gray wolf, *Canis lupus*, $H_E = 0.62$ and the domestic dog, *Canis familiarus*, $H_E = 0.73$ (Frankham et al., 2017; Wayne, 1996). The remnant sampling locations APEN, KATM, and PWS also had relatively high genetic diversity estimates compared to Bering or CA. The only significant difference in genetic diversity observed was a lower number of alleles in CA. In general, our findings highlight similar genetic diversity measures in sea otters across their range with the exceptions of the sampled locations nearest the ends of the range, e.g., Bering and CA.

Our diversity results show that even sampling locations founded with animals from a single source, e.g., WASH, resulted in new populations with genetic diversity equal to that in the population from which the founders were taken. It is important to note that we only measured the diversity within one of the source populations, PWS. We did not sample Amchitka Island, from which animals were taken to partially found SEAK and BC and all of WASH. However, Larson, Jameson, Bodkin, et al. (2002) measured the diversity in Amchitka using a subset of the microsatellite markers used here and also found that diversity in the reintroduced sampling locations SEAK ($H_O = 0.49$ and $H_E = 0.46$) and WASH ($H_O = 0.44$ and $H_E = 0.43$) was similar to Amchitka ($H_O = 0.41$ and $H_E = 0.43$).

Bering and CA had the lowest genetic diversity and are at near the ends of the sea otter's range, with CA remaining the most genetically distinct and geographically isolated population. Both are thought to have suffered from significant population bottlenecks during the maritime fur trade and perhaps earlier bottlenecks (Aguilar et al. 2008; Larson, Jameson, Bodkin, et al., 2002; Larson, Jameson, Etnier, et al., 2002; Larson et al. 2012). Genetic diversity within CA has been measured previously using microsatellites and has not changed significantly over a 15-year timespan, the maximal age for wild sea otters (Gagne et al., 2018). Bering has not been studied as

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TABLE 5 Number of first-generation migrants estimated between sampling locations from STRUCTURE, GeneClass2, and BayesAss analyses.

Program	Direction of migration		Number of migrants
	From	To	
STRUCTURE			
	PWS	*BC*	*2*
	APEN	BC	1
	SEAK	**PWS**	**1**
	WASH	**BC**	**2**
	BC	**WASH**	**1**
			7 total
GeneClass2			
	APEN	**KATM**	**3**
	BC	APEN	1
	BC	*PWS*	*1*
	BC	**WASH**	**1**
	KATM	SEAK	1
	PWS	**KATM**	**1**
	PWS	**SEAK**	**1**
	SEAK	***BC***	***2***
	SEAK	*WASH*	*1*
	WASH	**BC**	**2**
	WASH	*SEAK*	*1*
			15 total
BayesAss			
	SEAK	***BC***	**9**
	APEN	**KATM**	6
	SEAK	*WASH*	6
	PWS	*SEAK*	2
	BC	***WASH***	**1**
			24 total

Note: Bold = adjacent populations and italic = related by translocation.

extensively as CA thus we do not know how its diversity has changed over time. Bering, like CA, did not have evidence of gene flow between sampled locations as suggested by STRUCTURE admixture in the barplots and the migrant analyses but clustered with the other sampled Northern populations in all STRUCTURE K scenarios. Although we do not have direct genetic evidence, we believe Bering is likely experiencing gene flow from unsampled nearby populations, e.g., the Kuril or Kamchatka sea otter populations further west. More sampling in these areas near Bering is needed to determine the degree to which it is isolated. The lower diversity in these two populations sampled nearest to the ends of the sea otter's range, CA and Bering, agrees with the "centre–periphery hypothesis," which states that genetic variation decreases from the center to the edge of a species' geographic range (Pironon et al., 2017). However, patterns of diversity in sea otters are likely complicated by the history of extirpation, isolation, recovery, and translocations. The CA population likely has lower genetic diversity, not because it is located towards the historical southern limits of the species, but also because it underwent an extreme bottleneck (i.e., ~50 individuals remained after fur trade exploitation) and has remained isolated from other sea otter populations (Bodkin, 2015). At least in

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part due to their low genetic diversity, the CA and Bering populations may be the most vulnerable to any further reduction of diversity through predation, oil spills, or other means and may have reduced adaptive potential to respond to novel diseases or environmental changes associated with climate change.

4.2 | Population structure

The population structuring found here likely reflects historic bottlenecks, management actions such as translocations, and geography. This is evidenced by the clear distinction found with all methods (F_{ST}, STRUCTURE, TESS, and MAPI) between the Southern (CA) and all the Northern populations and corroborates sea otter subspecies designations among these groups using skull morphometrics (Wilson et al., 1991). Prior to the successful reintroductions the closest neighbor to the CA population was over 3,000 km to the north in PWS (Kenyon, 1969). Our results indicate that PWS was also isolated in its recent history as indicated by the STRUCTURE (Figure 2b–d) and TESS results. Even though Best K by Evanno found K = 2 in all populations (Figure 2a), delta K tends to find the highest hierarchical level of structure. We found evidence to support higher levels of K specifically K = 4 for all populations and K = 3 for Northern groups where the LnPd starts to stabilize (Figure S1). The K = 4 in STRUCTURE (Figure 2c) corroborates the TESS results of four clusters, with CA and three clusters in Northern populations. This is in agreement with the Northern subspecies population separation identified by Cronin et al. (2002) into three genetic stocks: the SW stock that includes the Aleutian chain (APEN, KATM sampled here), and Kodiak Island; the SC stock that includes PWS (sampled here), the Kenai Peninsula, and Cordova; and the SE stock that includes SEAK (sampled here). There was also significant admixture in the STRUCTURE barplots of many Northern populations, particularly in KATM, SEAK, and BC suggesting connectivity between KATM and PWS and common founders within SEAK and BC from both western Alaska (Amchitka) and PWS (Figure 2b–d).

The results of the IBD analyses suggest that sea otter populations experience significant genetic differentiation with geographic distance, with the CA population being the most distinct from all other populations. The analysis run without CA otters shows that the IBD pattern holds for Northern sea otters (Figure 4b), and previous analyses of genetic data within CA otters demonstrated that IBD also occurs at within-region scales (Gagne et al., 2018). The occurrence of IBD in sea otters likely reflects their relatively small home ranges (10–15 km) and generally high site fidelity (Gagne et al., 2018; Gorbics & Bodkin, 2001; Tarjan & Tinker, 2016). However, sea otters do sometimes make long-distance movements, and some males in both PWS (Garshelis & Garshelis, 1984) and CA (Jameson, 1982) frequently (at least yearly) make trips of 80–145 km up to 450 km between breeding territories and male areas (Jameson 1989: Ralls et al., 1996), and may be familiar with much of the geographic range of their population. Although males generally travel longer distances than females, radio telemetry studies have also documented females moving long distances as well, up to 100 km (Garshelis & Garshelis 1982; Laidre et al., 2009; Ralls et al., 1996;). In addition, females are thought to have moved regularly between islands to recolonize the Aleutian Islands post fur trade extirpation (Reidman & Estes, 1990). The functional relationship between genetic isolation and geographic separation appears mostly consistent, although the CA population exhibits greater isolation than expected based on a simple Euclidian measure of geographic isolation (compare Figures 4a and b). This may be because the effective geographic distance between CA and the Northern populations is greater than estimated by a Euclidian measure. Sea otters are unlikely to disperse linearly along a nonlinear coastline but rather tend to follow the meandering coastal margins within waters that are shallow enough for forging, typically less than 70 m depth (Breed et al., 2017; Riedman & Estes, 1990). The highly convoluted coastline topology of Alaska and British Columbia translates into a true coastline distance from CA to PWS (the closest remnant population) that is far greater than the linear distances between any of the Northern populations. If one accounts for this "effective" distance for dispersing sea otters, the genetic isolation of the CA population is more consistent with the general IBD function fit to other populations.

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Our IBD results and those of Gagne et al. (2018) agree with studies indicating that sea otter populations are demographically independent at smaller spatial scales than currently defined management units such as subspecies or stocks (Estes et al., 2010; Tinker et al., 2006, 2008, 2019). However, if a localized threat to sea otters was identified, managers could identify a smaller stock affected by the localized threat. This can be done if a population segment meets the criteria to be recognized as a "distinct population segment" (DPS) under the U.S. Endangered Species Act (e.g., the SW stock of the Northern sea otter was recognized as a DPS and was listed as threatened; 70 FR 46366) (Endangered Species Act of 1973, as amended in 16 U.S. Code § 1531 et. seq.; U.S. Federal Register, 1996). Similarly, demographically independent "stocks" can be specified under the Marine Mammal Protection Act; such designation is meant to prevent substantial localized reduction or extinction of a species and has been implemented to manage Northern sea otters (Moore & Merrick, 2011).

4.3 | Gene flow/connectivity

The migrant results identified gene flow between many sampling locations, except for those at the ends of the sampled range, Bering and CA. All BayesAss migrants were found in at least one other analysis, including the migration direction, corroborating those results. The reintroduced groups have filled in large gaps of previously occupied habitat and have enhanced the connectivity between populations, especially those that are separated by relatively small geographic distances such as WASH and BC. The gene flow detected between PWS and KATM, and PWS and SEAK, could explain the increased genetic diversity in PWS that we found compared to earlier studies. It is likely that there has been genetic exchange between sea otters in PWS and those to the west and north as population growth along the Katmai/Kamishak/Kachemak coasts has resulted in a continuous sea otter population across the Gulf of Alaska (PWS to APEN). In addition, there is evidence of gene exchange southward from PWS to SEAK, which is not surprising as sea otter populations are thought to have been continuous from SEAK to PWS since the mid-2000s (Larson & Bodkin 2015). Even with the recent gene flow between PWS and adjacent populations, PWS remains genetically distinct from the other Northern sampling locations, which is likely the result of its long period of isolation following extirpation. Although the suggested gene flow between both WASH and BC and SEAK could be confounded by shared source populations (Amchitka and PWS), the use of rapidly evolving microsatellite markers in this study makes it possible to detect genetic structure and gene flow over short periods of time, thus our results likely represent contemporary gene flow (Haasl & Payseur, 2011). Additionally, in recent years the BC population has continued to expand to the north and the south, making the distribution of sea otters nearly contiguous between SEAK, BC, and WASH (Nichol et al., 2020). Finally, the geneflow detected here between BC and WASH is not surprising given the short distance between the two known population boundaries, 60 km, a distance easily traveled by sea otters. Additional evidence of movement between these populations is provided by a female sea otter tagged in BC that stranded off of the central Washington Coast (J.B., unpublished data; Deanna Lynch, personal communication, 2018).

The successful reintroductions of sea otters to Washington, Vancouver Island, and Southeast Alaska were responsible for approximately 32,000 animals in 2012 (the last survey for the Southeast population), which represented approximately 25% of the estimated 125,000 sea otters existing at that time (Bodkin, 2015). In addition, as evidenced by the migrant analyses, these reintroductions have been instrumental in increasing population connectivity. Indeed, reintroductions have been the most successful management tool employed to recover extirpated sea otter populations in over 80 years of conservation efforts. However, because of the failure of the Oregon translocation there remains a large (approximately 1,200 km) section of unoccupied habitat from Northern California to Southern Washington (Larson & Bodkin, 2015). Sea otters are thought to be at carrying capacity throughout the center portion of their range in California (Laidre et al., 2001; Thometz et al., 2014; Tinker et al., 2008), and thus population growth and range expansion at the Northern and Southern peripheries of the range in California will be critical for further recovery. Unfortunately, the recent increase in mortality due to great white sharks, *Carcharodon*

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carcharias, has been greatest at the range peripheries (Tinker et al., 2016) and is thought to be one of the primary causes preventing the California population from expanding its range.

4.4 | Conservation implications

Our results have several important conservation implications: (1) persistent low genetic diversity in some populations (e.g., CA and Bering) likely increases their vulnerability to threats, and ultimately to reduced population resilience and viability; (2) sea otter demography varies at small spatial scales relative to current management units; and (3) the successful reintroductions resulted in comparably genetically diverse populations, even though they were founded by relatively small populations 40 years prior to sampling, and have resulted in increased genetic connectivity between groups proving they are an effective tool to enhance the recovery of sea otters and their associated ecosystem functions.

Accelerating the recolonization of coastal and estuarine habitats by sea otters in Oregon and Northern California could help fill in the remaining large gap in distribution between California and Washington, thereby increasing the potential for gene flow between the Northern and Southern populations and restoration of genetic diversity to the California population. Recolonization could also have important ecosystem-level benefits for food web structure and function in both coastal and estuarine habitats (Estes et al., 2010; Hessing-Lewis et al., 2018; Hughes et al., 2013, 2019; Markel & Shurin, 2015).

Future management actions for the Southern sea otter could focus on filling in this gap of sea otter occupation. However, a traditional translocation of wild-captured otters may not be the most effective approach. Sea otters were translocated from the central California coast to San Nicolas Island in the 1980s to establish a new population, separate from the mainland population as a safeguard (Rathbun et al., 2000). Although this translocation eventually resulted in a sustained population (Tinker & Hatfield, 2016), initially up to 90% of the translocated individuals dispersed from the island, traveling more than 100 km to return to the mainland, or disappearing. Rather than using wild-captured otters, reintroducing surrogate-reared rescued pups, which have been proven to survive better in the wild than human raised pups and tend to remain in release locations when released with other similarly raised pups (as is the case in Elkhorn Slough; Mayer et al., 2019), may greatly reduce the likelihood of the reintroduced individuals dispersing from the release site, although more research needs to be done on releasing these pups outside of currently occupied areas (Hughes et al., 2013, 2019; Mayer et al., 2019). Further reintroductions that include surrogate raised pups into unoccupied estuarine habitats between CA and WA, such as San Francisco Bay or Drakes Estero north of the currently occupied CA range and the Oregon Coast, may reduce the likelihood of high mortality from shark predation thought to limit the CA range expansion (Tinker et al., 2016), thus aiding the recovery of the CA sea otter and restoring connectivity and genetic diversity across the greatest remaining span of unoccupied sea otter habitat.

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AUTHOR CONTRIBUTIONS

Shawn Larson: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing-original draft; writing-review & editing. **Erick Gange:** Formal analysis; investigation; methodology; resources; validation; visualization; writing-original draft; writing-review & editing. **James Bodkin:** Conceptualization; investigation; visualization; writing-original draft; writing-review & editing. **Michael Murray:** Data curation; methodology; writing-original draft; writing-review & editing. **Katherine Ralls:** Formal analysis; investigation; methodology; validation; visualization; writing-original draft; writing-review & editing. **Lizabeth Bowen:** Formal analysis; methodology; validation; visualization; writing-original draft; writing-review & editing. **Raphael Leblois:** Investigation; methodology; software; validation; visualization; writing-original draft; writing-review & editing. **Sylvain Piry:** Methodology; software; validation; visualization; writing-original draft; writing-review & editing. **Maria Penedo:** Data curation; formal analysis; validation; writing-review & editing. **M. Tim Tinker:** Data curation; formal analysis; methodology; validation; visualization; writing-original draft; writing-review & editing. **Holly Ernest:** Validation; visualization; writing-original draft; writing-review & editing.

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