Early Holocene glacial retreat isolated populations of river otters (*Lontra canadensis*) along the Alaskan coast

M.S. Seymour, K.E. Ott, D.A. Guertin, H.N. Golden, D.B. McDonald, and M. Ben-David

Abstract: Pleistocene climatic oscillations have resulted in high rates of speciation. Lesser known are speciation events related to recent glacial retreats. During the early Holocene many Alaskan coastal glaciers receded, exposing much of the Kodiak Island Archipelago (KOD), the Kenai Peninsula, and Prince William Sound (PWS). Using fecal DNA analyses on samples collected in KOD, PWS, Kenai Fjords National Park (KEFJ), Katmai National Park and Preserve (KATM), and Vancouver Island, British Columbia (BC), we found isolation by distance to be an important mechanism for the divergence of populations of river otters (*Lontra canadensis* (Schreber, 1777)) along the Pacific coast. Nonetheless, our results also demonstrated that KOD river otters appear to be more isolated genetically from their mainland conspecifics (approximately 50 km away), as river otters inhabiting PWS are from those in BC (over 2500 km away). In addition, KATM and KOD otters likely differentiated from one ancestral stock that inhabited the southwestern shores of Alaska during the Pleistocene and was isolated from more easterly populations by distance. The low genetic diversity among KOD river otters, compared with similar subpopulations in PWS, is likely the result of a founder effect and limited gene flow among the different islands within the Archipelago. Our observation that glacial retreat, rising sea levels, and formation of the Gulf of Alaska Coastal Current in the early Holocene likely led to divergence of populations of river otters, a highly mobile semiaquatic mammal, highlights the potential for future speciation events related to current climate change and ocean currents in coastal animal populations.

Key words: analysis of molecular variance (AMOVA), bottleneck, isolation by distance, *Lontra canadensis*, probability of identity, structure.

Résumé : Si les oscillations climatiques au Pléistocène se sont traduites par des taux élevés de spéciation, les évènements de spéciation associés aux reculs glaciaires récents sont moins bien connus. Durant l'Holocène précoce, de nombreux glaciers côtiers de l'Alaska ont reculé, exposant du coup une bonne partie de l'archipel Kodiak (KOD), de la péninsule Kenai et du golfe du Prince William (PWS). Des analyses d'ADN de matière fécale provenant d'échantillons prélevés dans le KOD, le PWS, le parc national des Kenai Fjords (KEFJ), le parc national et la réserver Katmai (KATM) et l'île de Vancouver, en Colombie-Britannique (BC) ont révélé que l'isolement par la distance est un mécanisme important pour expliquer la divergence de populations de loutres de rivière (*Lontra canadensis* (Schreber, 1777)) le long de la côte pacifique. Cela dit, nos résultats ont également démontré que les loutres de rivière de KOD semblent plus isolées sur le plan génétique de leurs congénères continentaux (à environ 50 km de distance) que le sont les loutres de rivière habitant le PWS de celles de la BC (à plus de 2500 km de distance). En outre, les loutres de KATM et de KOD se sont vraisemblablement différenciées d'un stock ancestral unique qui occupait le littoral sud-ouest de l'Alaska au Pléistocène et était isolé par la distance de populations plus à l'est. La faible diversité génétique au sein des loutres de rivière de KOD par rapport à celles de sous-populations semblables dans le PWS est vraisemblablement le résultat d'un effet fondateur et d'un flux génétique limité entre les différentes îles de l'archipel. Notre observation à l'effet que le recul glaciaire, la montée des niveaux de la mer et la formation du courant côtier du golfe de l'Alaska à l'Holocène précoce ont vraisemblablement mené à la divergence de populations de loutres de rivière, un mammifère semi-aquatique très mobile, souligne le potentiel d'évènements de spéciation futurs associés aux changements climatiques et aux courants océaniques actuels au sein de populations animales côtières.

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Mots-clés : analyse de la variance moléculaire (AMOVA), goulot d'étranglement, isolement par la distance, *Lontra canadensis*, probabilité d'identité, structure.

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Introduction

Pleistocene climatic oscillations have resulted in high rates of speciation, including current species divergences stemming from periods of glacial advances (Pennington et al. 2004; Knowles and Richards 2005). During glacial advances, landscape fragmentation and genetic drift resulted in the divergence of many extant species and subspecies. For example, genetic divergence of North American black bears (*Ursus americanus* Pallas, 1780; Wooding and Ward 1997), American and Pacific martens (*Martes americana* (Turton, 1806) and *Martes caurina* (Merriam, 1890), respectively; Small et al. 2003; Stone et al. 2002), and long-tailed voles (*Microtus longicaudus* (Merriam, 1888); Conroy and Cook 2000) suggests that populations of these species have been separated into eastern and western refugia until the melting of the Wisconsin glaciers approximately 12 000 years ago. Similarly, Iberian Emerald Lizards (*Lacerta schreiberi* Bedriaga, 1878) have been isolated in glacial refugia on the Iberian Peninsula through several climatic oscillations (Paulo et al. 2001), and montane grasshoppers (*Melanoplus oregonensis* (Thomas, 1876)) were repeatedly isolated in regional glacial refugia in the Rocky Mountains (Knowles and Richards 2005). In addition, some of the differentiation of populations of giant otters (*Pteronura brasiliensis* (Gmelin, 1788)) can be attributed to isolation in Pleistocene rainforest refugia in the Amazon basin (Pickles et al. 2011, 2012). Lesser known are divergence events related to recent glacial retreats.

Early Holocene (10 000 – 9 000 years ago) glacial retreats caused increases in sea levels. Such rising sea levels resulted in genetic isolation of coastal island populations of terrestrial animals including Tasmanian devils (*Sarcophilus laniarius* (Owen, 1838); Jones et al. 2004), Pacific martens (Small et al. 2003), Neotropical water rats (*Nectomys squamipes* (Brants, 1827); Almeida et al. 2005), Rock Iguanas (*Cyclura cychlura* (Cuvier, 1829); Malone et al. 2003), and Galápagos Lava Lizards (*Microlophus albemarlensis* (Baur, 1890); Calsbeek and Smith 2003; Jordan and Snell 2008). The evolutionary consequences of isolation for Tasmanian devils are unclear because populations on mainland Australia became extinct between 5000 and 500 years ago (Jones et al. 2004). Rising sea levels and formation of ocean currents, however, clearly resulted in isolation and limited gene flow leading to significant genetic divergence in Pacific martens, Galápagos Lava Lizards, and the semiaquatic Neotropical water rats, and Rock Iguanas (Calsbeek and Smith 2003; Malone et al. 2003; Small et al. 2003; Almeida et al. 2005; Jordan and Snell 2008).

Throughout the Pleistocene (1 800 000 – 10 000 years ago), most of coastal Alaska was heavily glaciated, although small nunataks (ice-free areas) likely persisted during the last glacial maximum in some areas such as Montague and Hinchinbrook islands in Prince William Sound and on southwest Kodiak Island (Klein 1965; Mann and Hamilton 1995; Lance and Cook 1998). During the last glacial retreat, about 10 000 years ago, many of these coastal glaciers (including the Kodiak ice cap) receded, exposing much of the Kodiak Island Archipelago, the Kenai Peninsula, and Prince William Sound. More importantly, rising sea levels resulted in the northerly retreat of the Alaska coastline and the formation of the Gulf of Alaska Coastal Current (GOACC) and Shelikof Strait, which separates the Kodiak Island Archipelago from the Alaska Peninsula roughly 50 km away (Mann and Hamilton 1995). Paetkau et al. (1998) hypothesized that large tidal fluctuations and strong currents associated with the GOACC and Shelikof Strait were responsible for the isolation and divergence of Kodiak brown bears (*Ursus arctos* L., 1758) from their conspecifics on the Alaska Peninsula.

Populations of North American river otters (*Lontra canadensis* (Schreber, 1777)) occur along the Pacific coastline from northern California to the Alaska Peninsula (Melquist et al. 2003). These semiaquatic mammals are highly mobile, especially females, which are known to disperse 60–90 km (Blundell et al. 2002). Previous studies have identified isolation by distance as the main mechanism for genetic divergence in river otters in both freshwater and marine ecosystems (Blundell et al. 2002; Latch et al. 2008). Nonetheless, anthropogenic disturbance has been implicated in some small-scale subpopulation genetic structuring of river otters (DePue 2007; Guertin et al. 2012) and Eurasian otters (*Lutra lutra* (L., 1758); Hung et al. 2004; Kalz et al. 2006). Because of the sparse human habitation along the coasts of the Kodiak Island Archipelago and the Alaska Peninsula, the relatively short distance between these two shorelines, and the advanced swimming adaptations of river otters (Ben-David et al. 2000; Crait et al. 2012), it is likely that Kodiak river otters, unlike brown bears, would be genetically connected to their conspecifics on the Alaska Peninsula.

Such connectivity, however, may be thwarted if river otters are exposed to high predation risk from sea lions (*Eumetopias jubatus* (Schreber, 1776), killer whales (*Orcinus orca* (L., 1758)), and other top marine predators. Recently, Estes et al. (2006) proposed that the sequential predation on Steller sea lions, harbor seals (*Phoca vitulina* (L., 1758)), and sea otters (*Enhydra lutris* (L., 1758)) by killer whales in Alaskan waters resulted from overharvest of great whales in the late 1800s. Although evidence of predation by sea lions and killer whales on river otters is limited (ADFG 2010), Herreman et al. (2009) demonstrated that harbor seals in Glacier Bay National Park, Alaska, exhibited a substantial change in diet and high emigration rates in response to an increased predation risk from top marine predators. Thus, where marine predators are abundant we expect lower connectivity among subpopulations of river otters irrespective of distance or ocean currents.

In 2004, river otters were identified as a "vital sign" species by the Inventory and Monitoring Program of the Southwest Alaska Network of National Parks (Ben-David and Golden 2009). This designation was based on their role as

keystone species for the land-margin ecosystem (Ben-David et al. 2005) and their sensitivity to contaminants (Bowyer et al. 2003), habitat degradation, harvest (Melquist et al. 2003), and human disturbance (DePue 2007; Gaydos et al. 2007; Guertin et al. 2012). Following this designation, we initiated a large-scale inventory effort using noninvasive genetic sampling in Prince William Sound (PWS), Kenai Fjords National Park (KEFJ), Katmai National Park and Preserve (KATM), Lake Clark National Park and Preserve (LACL), and the Kodiak Island Archipelago (KOD). Using data obtained from this effort and a companion study on Vancouver Island, British Columbia (BC; Guertin et al. 2010; 2012), we investigated the hypotheses: (*i*) genetic distances among river otter populations along the Pacific coast follow the expectations of the isolation-by-distance model, (*ii*) Kodiak river otters are genetically connected to their conspecifics on the Alaskan Peninsula, and (*iii*) migration rates are consistent among the coastal river otter populations we sampled, regardless of the presence of marine predators.

Materials and methods

Latrine site surveys and sample collection

River otters communicate by repeatedly scent marking at specific terrestrial locations (latrines) with urine, feces, and anal gland secretions (Bowyer et al. 2003; Ben-David et al. 2005). Between 2004 and 2007, we surveyed approximately 2100 km of shoreline (354 km in KEFJ in 2004 and 2005; 1145 km in PWS in 2004 and 2006; 190 km in KATM in 2005; 50 km in LACL in 2006; 375 km in KOD in 2007; and 92 km in BC in 2005 and 2006; Fig. 1) and sampled 727 active river otter latrine sites (153 sites in KEFJ; 386 in PWS; 58 in KATM; 4 in LACL; 183 in KOD; and 86 in BC). Each site was visited between 1 and 16 times during the sampling period (once in KEFJ 2004, KATM, LALC, and KOD; twice in KEFJ 2005 and PWS 2004; nine times in PWS 2006; and 16 times in BC). From each site all fresh feces (characterized by their distinctive glossy appearance and strong odor) were individually collected and preserved in 100% ethanol (EtOH). In total, we collected 3419 otter feces (673 in KEFJ; 1529 in PWS; 63 in KATM; 261 in KOD; 893 in BC; no fresh samples were found in LACL). Samples were stored at 4 °C before shipping to the University of Wyoming, Laramie, Wyoming, USA, for genotyping.

DNA extraction and microsatellite genotyping

All samples were initially washed with 100% EtOH through sterilized fine-mesh stainless steel, autoclavable sieves to remove foreign materials, prey, and parasites following protocols reported in Hansen et al. (2008). We isolated genomic DNA from feces using the QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, California, USA). To monitor for contamination, we included a negative control (no DNA) with each batch of eight sample extractions.

We performed DNA amplifications by polymerase chain reaction (PCR) using a PTC-0200 DNA Engine Peltier Thermal Cycler (MJ Research, Inc., Waltham, Massachusetts, USA). We used eight microsatellite primers for PCR amplifications: four developed for river otters (RIO-01, RIO-05, RIO-17, RIO-19; Beheler et al. 2004, 2005) and four developed for Eurasian otters (LUT-701, LUT-733, LUT-801 and LUT-829; Dallas and Piertney 1998). For samples collected on KOD, we also used the primer RIO-20 (Beheler et al. 2005). Positive (blood samples from river otters with known genotypes) and negative (PCR blank) controls were included with each PCR reaction to ensure the reliability of PCRs and to monitor for contamination (Hansen et al. 2008). Successful PCR reactions were resolved on an ABI 3130xl Automated Sequencer (Applied Biosystems (ABI), Foster City, California, USA) at the University of Wyoming Nucleic Acid Exploration Facility. We scored products manually using the software PEAK SCANNER version 1.0 (ABI).

To minimize genotyping error, we followed the comparative multiple tubes approach (Frantz et al. 2003; Hansen et al. 2008), and discarded all samples that could not be assigned a consensus genotype at a minimum of seven loci. Loci that amplified the same heterozygous genotype twice were recorded, and homozygote genotypes were accepted on a provisional basis after three PCRs. If an allele amplified only once to yield one heterozygote genotype in seven reactions, with the other six runs resulting in the same homozygous genotype, we designated the allele as constituting a half-genotype (Frantz et al. 2003). We computed genotyping error rates (i.e., false alleles and allelic dropout) based on the final data set of samples with complete multilocus genotypes according to Broquet and Petit (2004) and the total probability of obtaining an erroneous multilocus consensus genotype following methods described in Prugh et al. (2005).

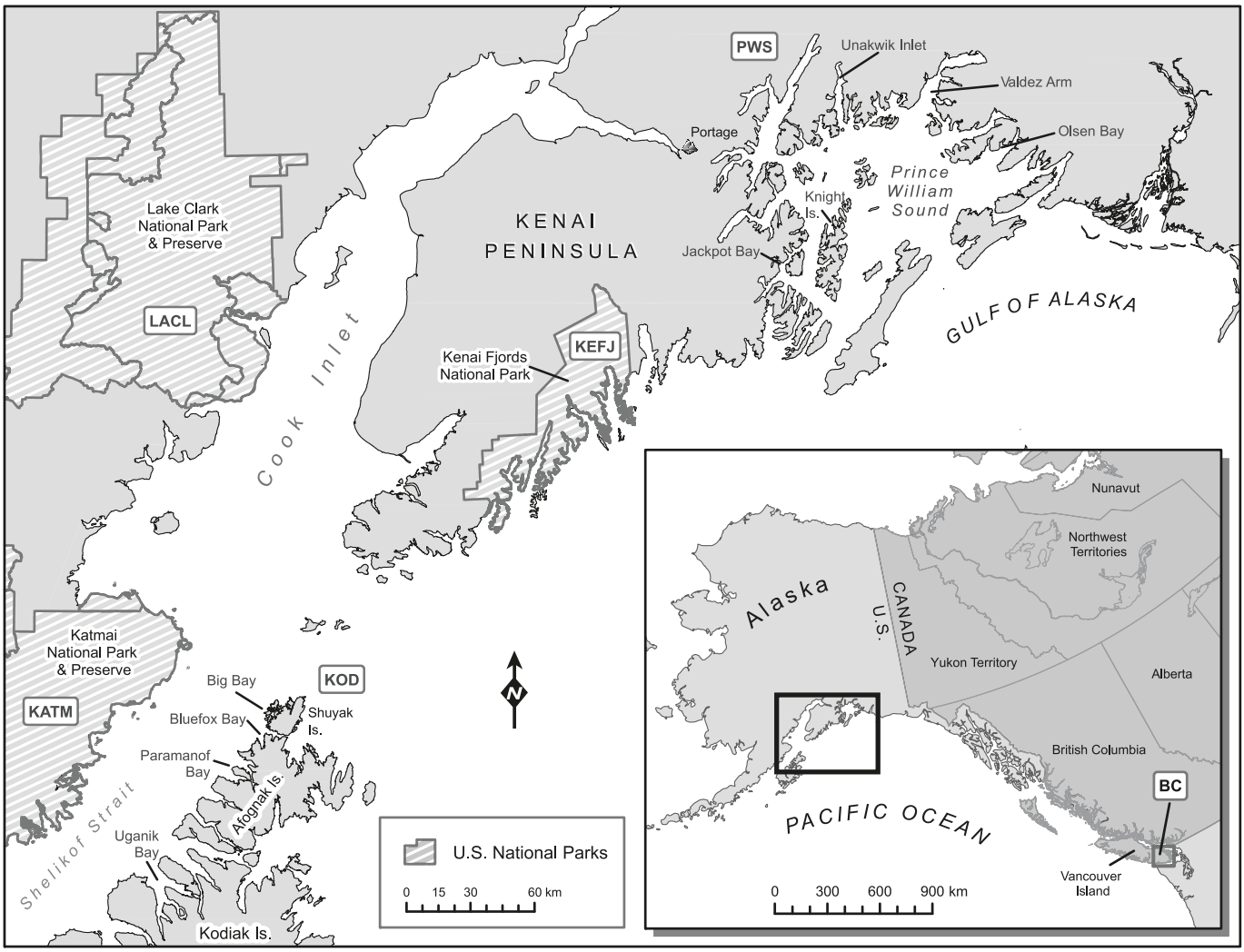
Because inclusion of repeated samples from the same individual may bias population level analyses, we excluded genetic recaptures from the data set. To ensure that we used a sufficient number of loci for individual identification, we calculated the probability of identity (P_{ID}; the probability that two individuals drawn at random from a given population share identical genotypes at all typed loci) using GIMLET version 1.3.2 (Valière 2002). We calculated both the upper limit, $P_{ID\text{-}sib}$, which assumes that the population is composed only of siblings, and should be 0.01 or lower (Mills et al. 2000; Waits et al. 2001), and the lower limit, $P_{ID\text{-}unbiased}$, which assumes a randomly mating population of unrelated individuals in Hardy–Weinberg equilibrium (HWE; Paetkau et al. 1998).

Statistical analysis

We determined population-level genetic diversity by calculating the mean number of alleles per locus, the unbiased estimate of mean expected heterozygosity (H_e), and the observed heterozygosity (H_o) for each of our sampled populations. We tested all loci for departure from HWE using two methods. First, we used GENEPOP version 3.1 (Raymond and Rousset 1995) to conduct probability tests of HWE at each locus using an exact test procedure (Guo and Thompson 1992). Second, we calculated the inbreeding coefficient (F_{IS}; Weir and Cockerham 1984) using FSTAT version 2.9.3.2 (Goudet 1995), and tested for significant deviations from zero using a randomization approach and adjusting the P value for multiple comparisons with the standard Bonferroni procedure (Rice 1989).

To assess overall genetic differentiation, we used analysis of molecular variance (AMOVA) as implemented in ARLEQUIN version 3.5 (Excoffier et al. 2005) with default settings. Genetic differentiation between pairs of sampling

Fig. 1. Location of 5 study areas along the Pacific coast where individual river otters (*Lontra canadensis*) (n = 383) were identified via non-invasive genetic sampling of feces. Approximately 2100 km of shoreline in Kenai Fjords National Park (KEFJ), Katmai National Park and Preserve (KATM), Lake Clark National Park and Preserve (LACL), and Prince William Sound (PWS), Alaska, USA, and southern Vancouver Island, British Columbia (BC), Canada, were surveyed between 2004 and 2007.



localities was estimated from F_{ST} values (Wright 1969) with 95% confidence intervals using FSTAT version 2.9.3.2 (Goudet 1995, 2000). Similarly, we calculated Jost's D values for each pair of samples (Crawford 2010; Jost 2008). Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967) for each pair of locations was calculated using the module GENDIST in PHLYIP version 3.67 (Felsenstein 1989). These measures of genetic distances were then used to construct a tree diagram using Saitou and Nei's (1987) neighbour joining method with 1000 bootstrap replications implemented in the modules NEIGHBOR and CONSENSUS in PHYLIP.

We used two different approaches to explore the assignment of individuals identified in the various localities to any of the other populations. First, we used the genotype assignment feature in ARLEQUIN version 3.5. Because the ARLEQUIN analysis assigns populations a priori, we also used STRUCTURE version 2.2 (Pritchard et al. 2000), which does not use any geographic knowledge for individual assignment. We assumed an admixture model with correlated allele frequencies (Falush et al. 2003). To estimate the most likely number of populations (K) represented in our data, we performed 10 independent runs of K = 1–8 with a burn in of 20 000 followed by 100 000 Markov chain Monte Carlo (MCMC) repetitions for K = 1–8. We determined the most probable number of populations based on the log-likelihood value and by calculating ΔK, a measure of the second-order rate of change in the log-likelihood of K (Evanno et al. 2005). This parameter represents the best-fitted K based on the rate of change in the log-likelihood and the standard deviation of the log-likelihood (Evanno et al. 2005). Last, we estimated the effective number of migrants per generation (Nm) between inferred subpopulations as $Nm = [1/(4F_{ST})] - (1/4)$, and explored for directionality in migration with the Bayesian model implemented in BAYESASS version 1.3 (Wilson and Rannala 2003) with default settings.

To assess whether river otters in KOD exhibit signs of a bottlenecked population from a recent founding event, we used the program BOTTLENECK (Cornuet and Luikart 1997). Recent reduction in effective population size usually results in loss of allelic richness preceding the loss of hetero-

zygosity. We evaluated both the infinite allelic model (IAM) and the stepwise mutation model (SMM) because few microsatellite loci follow the SMM in which heterozygosity excess is not observed (Cornuet and Luikart 1997). We used the program default settings with 1000 replications.

To evaluate the relative importance of predation risk and ocean currents on gene flow of river otters, we compared migration rates and their directionality among the four sampled sites in KOD with those among five sites in PWS. The distances between pairs of sites in both areas were comparable (Fig. 1) and marine predators are common offshore (Angliss and Outlaw 2007); however, PWS represents protected waters, whereas all sites sampled on KOD are exposed to the currents of Shelikof Strait (Fig. 1). We estimated the distances among sites by computing the centre of each sampling area and measuring geographic distances with ArcGIS version 9.0 (Environemtal Systems Research Institute (ESRI), Inc., Redlands, California, USA). We used linear regression to explore the relation between migration rates (as derived from BAYESASS version 1.3) and geographic distance.

Results

Of the 3419 fresh river otter feces we collected, 713 samples yielded consensus genotypes at seven or more loci (120 in KEFJ; 374 in PWS; 15 in KATM; 94 in KOD; and 110 in BC). These samples represented 383 unique individuals (103 in KEFJ; 137 in PWS; 12 in KATM; 82 in KOD; and 49 in BC). The probability of obtaining an incorrect multilocus genotype after replication at all eight microsatellite loci ranged from 0.0004 to 0.009 across all populations (for more detail see Guertin et al. 2012 and Ott 2009). Theoretical $P_{\text{ID-unbiased}}$ for the eight microsatellite loci ranged from 3.38×10^{-4} (or a 1 in 2 959 chance of two unrelated individuals in the population sharing the same multilocus genotype) in KOD to 1.96×10^{-7} (1 in 5 102 041) in PWS (Table 1). $P_{\text{ID-sib}}$ ranged from 2.87×10^{-2} in KOD to 1.98×10^{-3} in PWS (or 1 in 35 chance of two siblings sharing the same multilocus genotype in KOD versus 1 in 505 in PWS; Table 1). The addition of RIO-20 to the battery of primers for KOD samples resulted in $P_{\text{ID-unbiased}}$ of 2.58×10^{-5} and $P_{\text{ID-sib}}$ of 1.076×10^{-2}. After identifying unique individuals in KOD based on all nine primers, we excluded RIO-20 from further analyses except when comparing localities within KOD.

Overall, the mean number of alleles varied across localities, ranging from 4.00 in KOD to 6.63 in PWS (Table 2). Observed heterozygosity ranged from 0.433 in KOD to 0.718 in KATM, with BC and KATM populations exhibiting significant deviation from HWE (Table 2). Similarly, F_{IS} ranged from –0.168 in KATM to 0.106 in BC with all localities (except KEFJ) indicating nonrandom allelic distribution (Table 2).

AMOVA results suggested that KOD otters are genetically distinct from all other sampled populations ($p < 0.001$), although the differentiation among the other four localities was not statistically significant ($p > 0.4$). Nonetheless, pairwise F_{ST} and Jost's D values indicated moderate to high genetic differentiation among all five populations (Table 3). This differentiation, however, could not be explained by geographic distance (Fig. 2). Cavalli-Sforza chord distance with BC rooted as an outgroup indicated strong divergence of KEFJ and PWS from KOD and KATM, with well-supported divergence between KEFJ and PWS (Fig. 3). In contrast, divergence of KOD from KATM was weak, with less than half of the projected trees supporting separation of the two populations (Fig. 3).

Table 1. Probability of identity (P_{ID}) for river otters (*Lontra canadensis*) along the Pacific coast (Fig. 1).

Location	n	$P_{\text{ID-unbiased}}$	$P_{\text{ID-sib}}$
KOD	82	3.38×10^{-4}	2.87×10^{-2}
KATM	12	3.00×10^{-6}	1.88×10^{-2}
KEFJ	103	2.26×10^{-7}	2.01×10^{-3}
PWS	137	1.96×10^{-7}	1.98×10^{-3}
BC	49	2.39×10^{-6}	4.98×10^{-3}

Note: Genotypes were obtained from 383 fecal samples representing unique individuals. Samples were collected between 2004 and 2007. The upper limit $P_{\text{ID-sib}}$, which assumes that the population is composed only of siblings and should be 0.01 or lower (Mills et al. 2000; Waits et al. 2001), and the lower limit, $P_{\text{ID-unbiased}}$, which assumes a randomly mating population of unrelated individuals in Hardy–Weinberg equilibrium (Paetkau et al. 1998). For KEFJ, KATM, PWS, and BC, P_{ID} values were calculated based on eight microsatellite loci. For KOD, the primer RIO-20 was added to the other eight microsatellite loci. KOD, Kodiak Island Archipelago; KATM, Katmai National Park and Preserve; KEFJ, Kenai Fjords National Park; PWS, Prince William Sound; and BC, Vancouver Island, British Columbia.

Results from STRUCTURE supported the presence of four distinct populations: KOD, KEFJ, PWS, and BC, with KATM individuals grouping with the PWS cluster ($\Delta K = 214.4$ for $K = 4$; ΔK values ranging from 0.1 to 11.6 for other K values; Fig. 4). Assignment results suggest that some gene flow may be occurring between PWS and KEFJ. One of the KEFJ individuals (or 1.2%) was misassigned to the PWS–KATM cluster and 17 individuals from PWS (or 8.9%) were misassigned to the KEFJ cluster with probability greater than 0.7. Two KEFJ and 27 PWS animals exhibited admixture ancestry. Similarly, genotype assignment following AMOVA unequivocally consigned all KOD, KATM, and BC individuals to their geographic population, while three PWS (2.2%) and four KEFJ (3.9%) animals were equally assigned to both areas. Results from BAYESASS indicated a low degree of migration across all five locations (Table 4), except between KEFJ and PWS, suggesting little genetic exchange among all other sampled study areas.

Despite the lower allelic richness on KOD relative to the other populations (Table 2), this population exhibits mutation–drift equilibrium based on BOTTLENECK results. For the IAM, the probability of heterozygosity excess ranged from 0.102 using the Sign test to 0.148 using the Wilcoxon test. For the SMM, the probability of heterozygosity excess ranged from 0.997 using the Sign test to 0.999 using the Wilcoxon test.

Within localities on KOD, mean number of alleles varied from 2.11 in Big Bay on Shuyak Island to 3.89 in Uganik Bay on Kodiak Island (Table 2). Expected and observed heterozygosities were similar among bays except for Big Bay (Table 2). Significant F_{IS} values indicated inbreeding due to nonrandom mating in Big Bay and Uganik Bay, whereas river otters in Blue Fox Bay on Afognak Island appeared outbred (Table 2). BOTTLENECK results for these different

Table 2. Sample size (n), mean (±SD) number of alleles (A), expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{IS}) for river otters (*Lontra canadensis*) along the Pacific coast, four sampling locations in the Kodiak Island Archipelago, and five locations within Prince William Sound (Fig. 1).

Location	n	A	H_e	H_o	F_{IS}
All					
KOD	82	4.00±1.86	0.420	0.433	0.098[b]
KATM	12	4.50±1.69	0.622	0.718[a]	–0.168[b]
KEFJ	103	6.13±2.23	0.683	0.674	0.013
PWS	137	6.63±2.36	0.659	0.695	–0.054[b]
BC	49	4.38±2.00	0.570	0.520[a]	0.106[b]
KOD					
Big Bay	15	2.11±1.17	0.296	0.256	0.140[b]
Blue Fox Bay	14	2.89±1.62	0.446	0.487	–0.091[b]
Paramanof Bay	30	3.67±1.87	0.414	0.415	–0.002
Uganik Bay	21	3.89±1.45	0.401	0.332[a]	0.175[b]
PWS					
Knight Island	64	4.88±1.64	0.666	0.671	–0.094[b]
Jackpot Bay	17	4.38±2.00	0.605	0.562	–0.062[b]
Unakwik Inlet	24	5.38±2.07	0.674	0.646	–0.044[b]
Valdez Arm	14	4.75±2.25	0.498	0.551[a]	0.121[b]
Olson Bay	18	4.13±1.46	0.552	0.515	–0.032[b]

Note: Genotypes were obtained from 383 fecal samples representing unique individuals. Samples were collected between 2004 and 2007. KOD, Kodiak Island Archipelago; KATM, Katmai National Park and Preserve; KEFJ, Kenai Fjords National Park; PWS, Prince William Sound; and BC, Vancouver Island, British Columbia.

[a]Significantly different than expected.

[b]Significantly different than zero.

Table 3. Pairwise F_{ST} values (upper diagonal with 95% confidence intervals) and values of Jost's D (lower diagonal) for river otters (*Lontra canadensis*) along the Pacific coast (Fig. 1).

Location	KOD	KATM	KEFJ	PWS	BC
KOD	—	0.254 (0.088–0.449)	0.248 (0.126–0.371)	0.190 (0.081–0.287)	0.394 (0.160–0.472)
KATM	0.155	—	0.070 (0.011–0.130)	0.059 (0.020–0.110)	0.150 (0.083–0.153)
KEFJ	0.254	0.050	—	0.076 (0.035–0.126)	0.197 (0.100–0.314)
PWS	0.126	0.097	0.100	—	0.159 (0.047–0.279)
BC	0.241	0.083	0.195	0.116	—

Note: Genotypes were obtained from 383 fecal samples representing unique individuals. Samples were collected between 2004 and 2007. KOD, Kodiak Island Archipelago; KATM, Katmai National Park and Preserve; KEFJ, Kenai Fjords National Park; PWS, Prince William Sound; and BC, Vancouver Island, British Columbia.

subpopulations suggested recent bottleneck events in all locations (Wilcoxon test IAM p ranged from 0.003 to 0.004, SMM p ranged from 0.004 to 0.019).

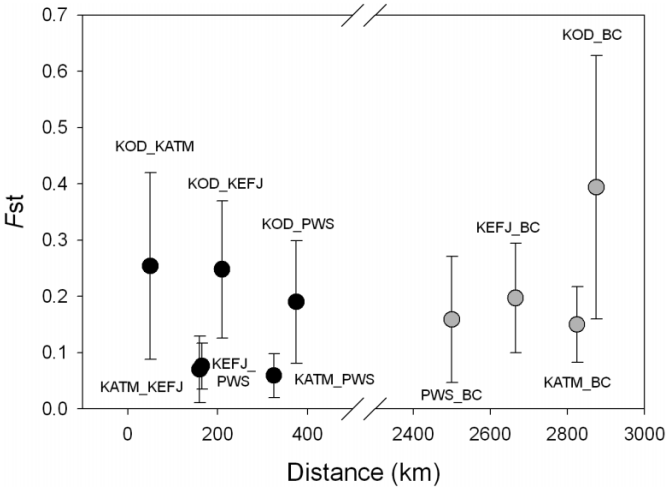
Pairwise F_{ST} values (with 95% confidence intervals (CI)) indicated the occurrence of genetic structuring within KOD (F_{ST} = 0.074, 95% CI: 0.041–0.117; Table 5). Similarly, although estimates of ΔK obtained from STRUCTURE were low (ΔK = 7.7), this analysis indicated the existence of four genetic clusters in KOD (other ΔK values ranging from 0.1 to 4.6). Finally, results from program BAYESASS indicated asymmetrical migration rates between sampling localities on KOD, with higher rates occurring from north to south for the same pairs of locations, except between Blue Fox and Paramanof Bays, both of which occur on Afognak Island (Fig. 4). Migration rates were low between these two sites, but with no obvious directionality.

In contrast, although F_{ST} values indicated some genetic structuring within PWS (F_{ST} = 0.053, 95% CI: 0.036–0.068; Table 5) and results from STRUCTURE supported the existence of four subpopulations within this area (ΔK = 65.2 with other values ranging between 1.1 and 10.3), assignment of individuals to a specific geographic location was equivocal. Assessment of migration rates between pairs of locations within PWS revealed no trend in directionality but a significant isolation by distance (R^2 = 0.38, p = 0.05; Fig. 5).

Discussion

As expected, isolation by distance emerged as an important mechanism for the genetic divergence of river otter populations along the north Pacific coast. River otters from the four locations along the Alaska coast exhibited greater genetic

Fig. 2. Pairwise F_{ST} with 95% confidence intervals for five populations of river otters (*Lontra canadensis*) sampled along the Pacific coast between 2004 and 2007 in relation to geographic distance. Individuals from the Kodiak Island Archipelago (KOD) are genetically distinct from all other populations, regardless of distance. Genotypes were obtained from 383 fecal samples representing unique individuals.

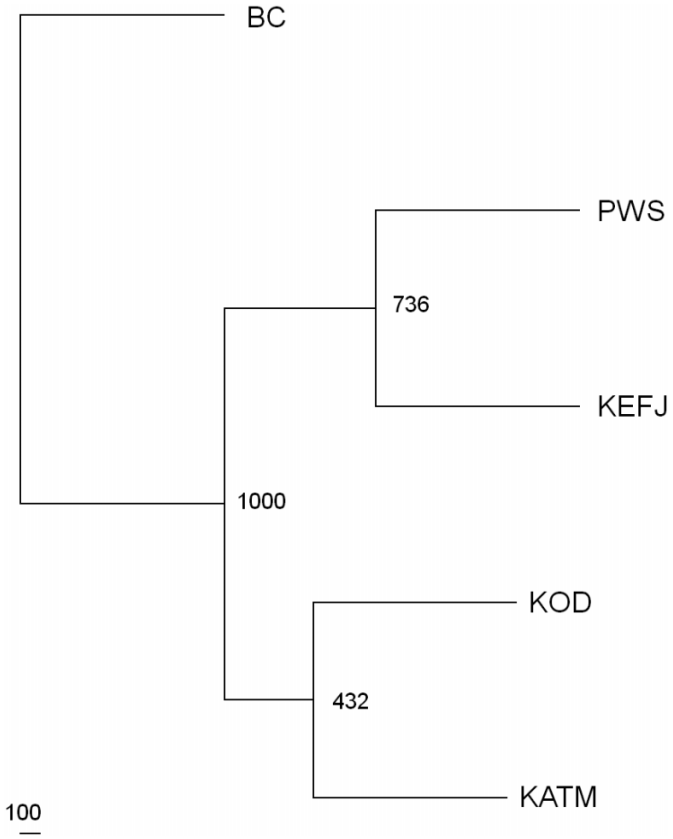


differentiation from the distant population in BC, over 2500 km away than the nearby Alaskan populations, except for KOD. Within Alaska, otters in the adjacent KEFJ and PWS exhibited relatively low genetic differentiation and several individuals identified from feces collected in PWS had genetic characteristics of KEFJ animals. In addition, while Cavalli-Sforza chord distance provided some support for two distinct populations, and the pairwise F_{ST} value was different from zero, AMOVA results indicated that the genetic distance between KEFJ and PWS was relatively low. Similarly, distance influenced migration rates among river otter subpopulations within PWS, suggesting it is a predominant cause for the genetic segregation in this region.

Nonetheless, our results also demonstrated that isolation by distance might not be the only mode of divergence of coastal river otter populations. Despite the relatively short distance between the Kodiak Island Archipelago and the Alaska Peninsula (approximately 50 km away), KOD river otters appear to be more isolated genetically from their mainland conspecifics as otters inhabiting PWS are from BC individuals. The separation of KOD otters from the other Alaskan populations is unlikely to be a recent one, as we did not find the expected heterozygosity excess associated with a recent bottleneck event. Indeed, our genetics data are in agreement with the designation of KOD otters as a separate subspecies (*Lontra canadensis kodiacensis* (Goldman, 1935)); a designation first proposed by Goldman (1935) based on morphometrics and reinforced during taxonomic studies by van Zyll de Jong (1972) and Hall (1981).

Additional support for the conclusion that distance may not be the only mechanism affecting gene flow among populations is the observation that although the straight-line distance between KEFJ and KATM is shorter (160 km) compared with PWS and KATM (325 km), river otters from the latter clustered with those from PWS, estimated migration rates between these two populations were slightly higher, and

Fig. 3. Cavalli-Sforza chord distance tree (as calculated by program PHILYP) with southern Vancouver Island, British Columbia (BC), rooted as an outgroup, indicating strong divergence of Kenai Fjords National Park (KEFJ) and Prince William Sound (PWS) from the Kodiak Island Archipelago (KOD) and Katmai National Park and Preserve (KATM), high support for divergence between KEFJ and PWS, and weak support for divergence of KOD from KATM. Genotypes were obtained from 383 fecal samples representing unique individuals. Samples were collected between 2004 and 2007.



the pairwise F_{ST} value was slightly lower than those between KEFJ and KATM. Finally, within KOD, migration rates were unrelated to distance and were higher from north to south as would be expected if dispersing animals are carried by the GOACC through Shelikof Strait.

Our results for KATM should be interpreted with caution because of the small sample size from that location. Although we surveyed much of the coastline in KATM, we found relatively few active river otter latrines and collected few fresh fecal samples. Low habitat quality (e.g., few sections with large intertidal rocks and abundant cover; Ben-David et al. 1996; Bowyer et al. 2003) and indirect evidence of predation attempts by Katmai brown bears may explain some of the low levels of otter activity along the KATM coast (Ben-David and Golden 2009). Nonetheless, it is possible that the low river otter activity we observed stems from habitat availability. The KATM coast is characterized by a limited number of small bays, which are separated from each other by large sections of shoreline exposed to the currents of Shelikof Strait. Indeed, during our surveys, the only otter latrines we found occurred within protected bays; we found no river otter latrines on the outer coast of KATM.

Fig. 4. Posterior probability assignments of river otters (*Lontra canadensis*) to two genetic clusters inferred by STRUCTURE for five river otter populations sampled along the Pacific coast between 2004 and 2007. No individuals from the Kodiak Island Archipelago (KOD) or British Columbia (BC) were misassigned to any other population. Animals from Katmai National Park and Preserve (KATM) largely clustered with those from Prince William Sound (PWS).

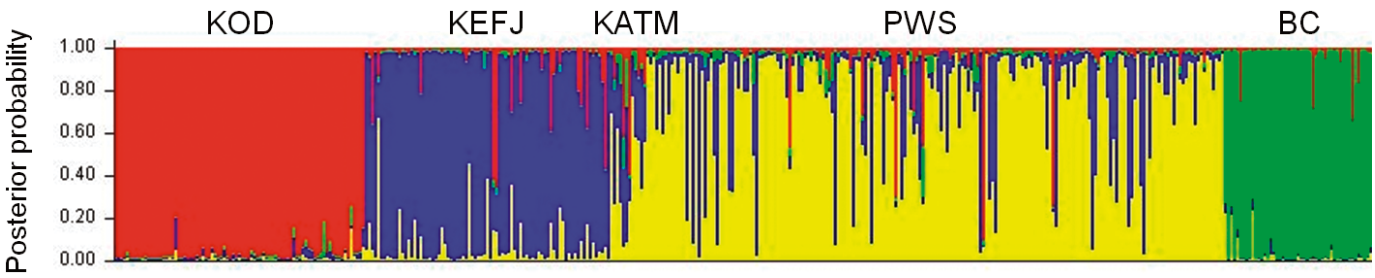


Table 4. Pairwise mean effective number of migrants per generation (*Nm*) between inferred subpopulations ($Nm = [1/(4F_{ST})] - (1/4)$; upper diagonal) and migration rates as estimated with BAYESASS version 1.3 (lower diagonal) for river otters (*Lontra canadensis*) along the Pacific coast (Fig. 1).

Location	KOD	KATM	KEFJ	PWS	BC
KOD	—	0.7	0.8	1.1	0.4
KATM	0.013	—	**3.3**	**4.0**	1.4
KEFJ	0.039	0.041	—	**3.0**	1.3
PWS	0.003	0.005	**0.356**	—	1.3
BC	0.005	0.006	0.035	0.011	—

Note: Genotypes were obtained from 383 fecal samples representing unique individuals. Samples were collected between 2004 and 2007. Values in boldface type suggest meaningful gene flow among populations. KOD, Kodiak Island Archipelago; KATM, Katmai National Park and Preserve; KEFJ, Kenai Fjords National Park; PWS, Prince William Sound; and BC, Vancouver Island, British Columbia.

Despite the relatively small number of samples successfully genotyped from KATM, this location provides important insights into processes leading to the isolation of KOD river otters. First, Cavalli-Sforza chord distance and Jost's *D* analyses indicated that KOD and KATM animals are genetically less distinguishable from each other compared with the other sampled populations. This is likely a result of the relative high occurrence of several alleles (such as 228 and 232 in LUT-829 and 330 and 333 in RIO-05) in these two populations and their scarcity in all others. Thus, KATM and KOD otters likely differentiated from one ancestral stock, which inhabited the Pleistocene southwestern shores of Alaska, and was isolated from other more eastern populations by distance.

Presently, however, KATM river otters are more connected to conspecifics in PWS and KEFJ than they are to those in KOD. This observation points to glacial retreat and the formation of ocean currents as barriers to otter dispersal. For river otters to disperse directly from KEFJ to the Alaska Peninsula (and vice versa), they would need to cross Cook Inlet and Shelikof Strait, some of the largest and most treacherous bodies of water in the Gulf of Alaska, with only the Barren Islands, north of KOD, as potential staging areas. Shelikof Strait is notorious for high variance in yearly tidal cycles, strong currents, and violent storms (Mysak et al. 1981). Thus, it appears that gene flow would more likely occur along the mainland coast of Cook Inlet (Fig. 1). For PWS otters to reach Cook Inlet along the mainland coast, they would either need to circumnavigate the Kenai Peninsula, including KEFJ, or cross into Turnagain Arm overland, likely via the isthmus located at the north end of Kenai Peninsula (in the vicinity of Portage Glacier and Blackstone Bay) in western PWS (Fig. 1). As implausible as such a crossing may seem, during telemetry flights in PWS in the 1990s, river otter tracks crossing both Portage and Tiger glaciers were observed (Blundell et al. 2002; Bowyer et al. 2003). In addition, Ben-David et al. (2002) reported that a radio-instrumented otter crossed overland from Eaglek Bay to Unakwik Inlet in northwestern PWS (Ben-David et al. 2002), suggesting that river otters in the region are capable of such relatively short overland movements, which may facilitate limited gene flow between PWS and KATM. It is unfortunate that our survey of the shoreline of Cook Inlet in LACL did not produce any viable samples, as this area may be an important corridor for gene flow among river otter populations in southwest Alaska.

Whether ocean currents alone, rather than a combination of ocean currents and predation by marine mammals such as sea lions and killer whales (Estes et al. 2006), may be the main mechanism for divergence of river otter populations is unclear. In PWS, where marine predators are abundant and most subpopulations occur in protected waters, river otter gene flow is largely determined by proximity. Blundell et al. (2002) arrived at similar conclusions after investigating genetic structuring using DNA derived from live-captured individuals. Similarly, the suggestion of recent bottlenecks in the different populations in KOD points to a potential increase in predation by marine mammals. Estes et al. (2006) observed a recent increase in predation of sea otters in southwestern Alaska following overharvest of great whales in the late 1800s. However, the most convincing evidence for the role of ocean currents in influencing gene flow of coastal river otters is the directional migration rates in KOD. It is unlikely that marine predators would preferentially attack river otters dispersing from south to north. Rather, the predominant north to south direction of currents through Shelikof Strait is more likely responsible for the pattern of migration rates we observed. Furthermore, the only pair of locations where there was no directionality in migration rates occurred on Afognak Island, where river otters can disperse overland rather than by venturing into the surrounding ocean currents. It is possible that despite their advanced swimming capability, gene flow in these semiaquatic mammals could be influenced by ocean currents.

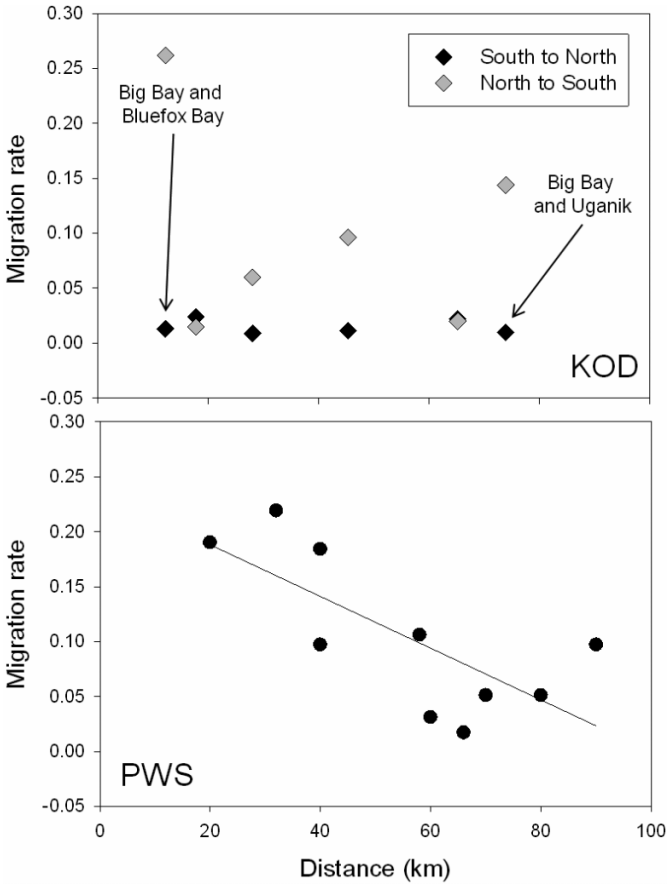
Table 5. Pairwise F_{ST} values (upper diagonal) and 95% confidence intervals (lower diagonal) for river otters (*Lontra canadensis*) along the coast of the Kodiak Island Archipelago (KOD; Fig. 1) and Prince William Sound (PWS; Fig. 1).

KOD				
	Big Bay	Blue Fox Bay	Paramanof Bay	Uganik Bay
Big Bay		0.085	0.133	0.026
Blue Fox Bay	0.003–0.159		0.068	0.052
Paramanof Bay	0.053–0.226	0.008–0.132		0.054
Uganik Bay	0.002–0.126	0.023–0.094	0.027–0.091	

PWS					
	Knight Island	Jackpot Bay	Unakwik Inlet	Valdez Arm	Olson Bay
Knight Island		0.046	0.033	0.065	0.072
Jackpot Bay	0.026–0.065		0.043	0.057	0.097
Unakwik Inlet	0.008–0.061	0.019–0.077		0.039	0.039
Valdez Arm	0.034–0.095	0.010–0.101	0.016–0.068		0.046
Olson Bay	0.037–0.103	0.054–0.142	0.013–0.066	0.000–0.101	

Note: Genotypes were obtained from 80 fecal samples representing unique individuals in KOD collected in 2007 and 137 in PWS collected in 2004 and 2006.

Fig. 5. Migration rates (estimated by program BAYESASS) as a function of distance for four sites on KOD and five in PWS sampled between 2004 and 2007. In KOD (top panel), migration rates are asymmetrical between sampling localities, with the higher rates occurring from north to south and lower rates from south to north for the same pairs of locations. In PWS (bottom panel), migration rates between pairs of locations showed no trend in directionality but a significant isolation by distance ($R^2 = 0.38$, $p = 0.05$).



Ocean currents are one of three main forces influencing long-distance dispersal in oceanic systems (with the other two, winds and birds, more important to the dispersal of plants than animals; Gillespie et al. 2012). The best documented effect of ocean currents and rafting is the facilitation of long-distance dispersal. The distribution of animal species with a larval phase, such as marine fishes and invertebrates, is strongly influenced by ocean currents (Gillespie et al. 2012). This effect on distribution also determines patterns of gene flow. Examples of species influenced by current mediated gene flow include the rosethorn rockfish (*Sebastes helvomaculatus* Ayres, 1859; Rocha-Olivares and Vetter 1999), Antarctic icefish (*Chionodraco rastrospinosus* DeWitt and Hureau, 1979; Papetti et al. 2012), planktonic copepods (*Calanus finmarchicus* (Gunner, 1765); Unal and Bucklin 2010), various species in deep-sea hydrothermal vents (Vrijenhoek 2010), broadcast spawning corals (*Acropora millepora* (Ehrenberg, 1848); van Oppen et al. 2011), and many more. Although rafting with the aid of ocean currents has been documented in semiaquatic vertebrates, such as the Neotropical water rat inhabiting offshore islands in Brazil (Almeida et al. 2005), it is less common to observe the obstruction of gene flow by directionality of ocean currents as we report here.

The early Holocene isolation of river otters on KOD resulted in lower allelic diversity compared with the other sampled localities (including KATM). The mean numbers of alleles across all four areas sampled at KOD were comparable to those reported for Tasmanian devils (4.1; Jones et al. 2004) and Neotropical water rats (3.9 and 4.0; Almeida et al. 2005). The mean number of alleles per location, however, was markedly lower (2.11–3.89) and more comparable to those of Galápagos Lava Lizards on isolates (1.8–3.5; Jordan and Snell 2008) and Pacific martens on Admiralty Island (1.3; Small et al. 2003). In addition, the lower genetic diversity in Big Bay on Shuyak Island further reflects the limited levels of dispersal among KOD river otters. Bailey et al. (2007) demonstrated, by comparing wild and captive fish populations, that genetic diversity may not rapidly decline as long as some dispersal is maintained. The northerly location

of Shuyak Island in KOD and the north to south dispersal pattern on the west coast of KOD may have contributed to genetic isolation of this subpopulation. Thus, it appears that the low genetic diversity in KOD, especially on Shuyak Island, resulted from an initial founder effect during the early Holocene followed by more recent limited gene flow among the different islands within the Archipelago.

Differentiation of river otter populations along the Alaska coast, which likely resulted from glacial retreat, rising sea levels, and formation of the GOACC in the early Holocene, has implications for potential speciation events related to current climate change. Increasing global temperatures are correlated with trends of decreasing glaciers and ice caps, increasing sea levels, and higher variation of climatic events (Patz et al. 2005), resulting in large shifts in location and direction of ocean currents (Harley et al. 2006, Di Lorenzo et al. 2008). These changes in ocean currents could result in further isolation of coastal populations of semiaquatic and terrestrial animals, especially in low-elevation island archipelagos and heavily fragmented and urbanized coastal regions. The rise in sea levels and changes to ocean currents in recent years (Di Lorenzo et al. 2008), in conjunction with isolation and declines in abundance from habitat loss and fragmentation (Crooks 2002; Henle et al. 2004), pollution (Bowyer et al. 2003; Guertin et al. 2010), and emergent diseases (Gaydos et al. 2007; Siddle et al. 2007), may result in accelerated loss of genetic diversity compared with the early Holocene. Many populations of coastal terrestrial animals could become extinct under such conditions, given the expected extirpation of 18%–35% of extant species by 2050 (Thomas et al. 2004). Others, like KOD river otters, may maintain large enough populations to survive. How such rapid loss of genetic diversity and isolation may influence the rate of genetic differentiation in coastal terrestrial animals merits further investigation.

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