Conserv Genet
DOI 10.1007/s10592-007-9422-z

SHORT COMMUNICATION

Ancient DNA reveals genotypic relationships among Oregon populations of the sea otter (*Enhydra lutris*)

Kim Valentine · Deborah A. Duffield · Lorelei E. Patrick · David R. Hatch · Virginia L. Butler · Roberta L. Hall · Niles Lehman

Received: 6 February 2007 / Accepted: 3 September 2007

Abstract The sea otter has experienced a dramatic population decline caused by intense human harvesting, followed by a century of recovery including relocation efforts to reestablish the species across its former range in the eastern Pacific. Although the otter was historically present along the coast in Oregon, there are currently no populations in this region and reintroduction efforts have failed. We examined the mtDNA genotypes of 16 pre-harvest otter samples from two Oregon locations in an attempt to determine the best genotypic match with extant populations. Our amplifications of a 222 base-pair portion of the control region from otters ranging in age from approximately 175–2000 years revealed four genotypes. The genotypic composition of pre-harvest otter populations appears to match best with those of contemporary populations from California and not from Alaska, where reintroduction stocks are typically derived.

K. Valentine · D. A. Duffield · L. E. Patrick
Department of Biology, Portland State University, P.O. Box 751, Portland, OR 97207, USA

D. R. Hatch
Elakha Alliance, 704 SE Umatilla, Portland, OR 97202, USA

V. L. Butler
Department of Anthropology, Portland State University, P.O. Box 751, Portland, OR 97207, USA

R. L. Hall
Department of Anthropology, Oregon State University, Corvallis, OR 97331, USA

N. Lehman (✉)
Department of Chemistry, Portland State University, P.O. Box 751, Portland, OR 97207, USA
e-mail: niles@pdx.edu

Keywords *Enhydra lutris* · Ancient DNA · Mitochondrial DNA · Sea otter · Historical distribution

Introduction

The sea otter (*Enhydra lutris*) was historically distributed throughout coastal regions of the North Pacific Rim from Baja California, Mexico, up the western coast of North America, across the Aleutian Islands, through the Kamchatka Peninsula and Kuril Islands of Russia, to the island of Hokkaido, Japan (Kenyon 1969; Scribner et al. 1997). Commercial exploitation in the 18th and 19th centuries of otters along the western coast of North America led to the extirpation of this species from much of this range. Hunting was banned in 1911 by international treaty and the sea otter was later granted legal protection in the US and Canada. However, the species was probably reduced to less than 1% of an estimated pre-harvest abundance of several hundred thousand individuals (Riedman and Estes 1990). Eleven populations survived in California, southeastern Alaska, the Aleutian, Commander, and Kuril Islands, and in Kamchatka. The recovery of this species has its nuclei in these few individuals (Scribner et al. 1997).

Recently, efforts have been made to reintroduce the otter into much of its original range. Some of these have been productive, such as the movements of hundreds of animals from Amchitka Island in the Aleutians to locations in southeastern Alaska, British Columbia, and Washington in the late 1960's (Jameson et al. 1982). Other attempts have not been as successful, including laborious undertakings to establish healthy populations on the Channel Islands in southern California and along the Oregon coast. In 1970 and 1971, 93 otters from Amchitka were introduced at two

Oregon locations, Port Orford and Cape Arago, but these did not establish breeding populations. By 1982, otters were absent from Oregon, and the reintroduction was declared a failure (Jameson et al. 1982). However, some of the California populations have taken root.

It has been suggested that the efficacy of otter establishment efforts may rely on the genetic features of the stock population. A debate currently exists over the number and distribution of subspecies of *E. lutris* (e.g., Davis and Lidicker 1975; Wilson et al. 1991; Cronin et al. 1996), with the implication that certain genotypes may fare better in certain physical environments. Several population-genetic studies have been performed on extant otter populations, and the consensus is that geographically separated populations are genetically differentiated (Rotterman 1992; Cronin et al. 1996; Scribner et al. 1997; Bodkin et al., 1999; Tul'skaya et al. 1999; Gorbics and Bodkin 2001). Recently, Larson et al. (2002a) confirmed partitioning by showing significant microsatellite allele heterogeneity among eastern Pacific populations. A parallel study comparing microsatellite and mtDNA allele frequencies found in extant otters with those obtained from the bones of pre-harvest individuals suggested that there was significantly more genetic variability prior to the human-induced population bottleneck (Larson et al. 2002b). In terms of mtDNA control-region variation, Larson et al. (2002b) detected four closely related genotypes among one pre-harvest and five extant populations. They designated these genotypes A, B, C, and D, which differ by an average of 0.6% of the 320 b.p. genotyped. From extant samples, the A and B genotypes were found in 98% of otters among more northern populations from Amchitka Island, southeast Alaska, Prince William Sound (Alaska), and Washington, while the C and D genotypes were found in 94% of otters in California populations. All nine pre-harvest samples were from Native American excavations near Neah Bay, WA (Fig. 1), and these all possessed genotypes A ($n = 7$) or B ($n = 2$).

Our interest was to expand the range of known pre-bottleneck sea otter genotypes into Oregon in an attempt to assess the historical genetic make-up of these populations. This information should help guide subsequent reintroduction strategies south of the Columbia River. We have examined 16 pre-harvest sea otters from two different Native American middens in Oregon for mtDNA genotypic variation. Our data suggest that the Oregon populations were closer in mtDNA genotypic composition to modern Californian populations than to modern Alaskan populations, and that indeed a genotype X environmental component may determine the success of future otter reintroduction efforts.

Materials and methods

Sea otter tissue samples

Tissue samples were obtained from archaeological specimens housed in the Archaeology Department at Oregon State University, Corvallis, Oregon. Bones or

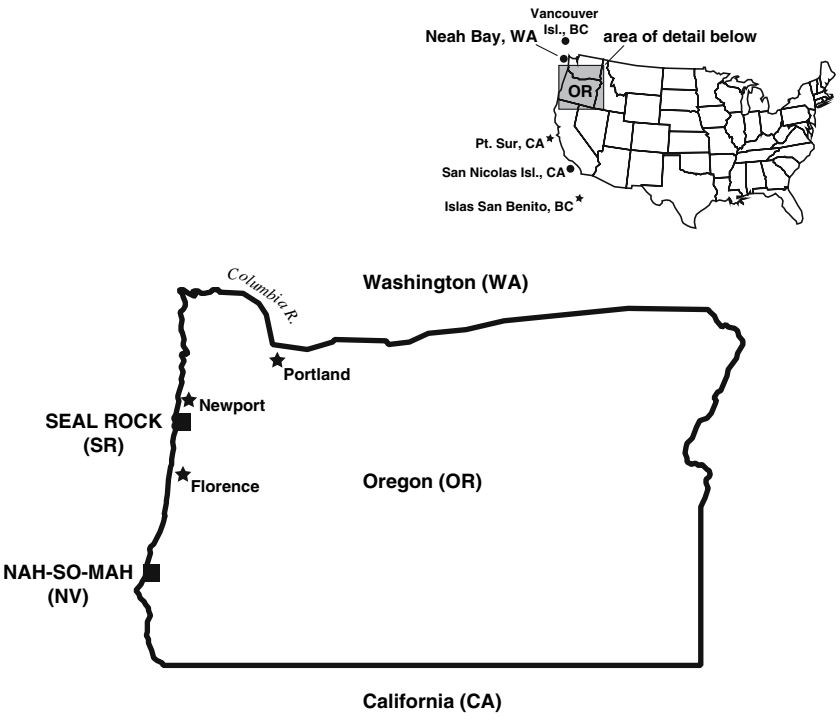

Fig. 1 Map of sea otter distribution and sampling sites. The pre-harvest distribution of otters included the entire Pacific Rim from northern Japan around to Baja California. In the upper map, the stars indicate remnant colonies existing at the time of a hunting ban in 1911, and the dots indicate successful sea otter translocated colonies

teeth from Indian middens were sampled from two separate Oregon sites (Fig. 1) spanning four time units ranging in age from 170–2000 years before present (ybp) as determined by radiocarbon dating. The Seal Rock (SR) location in north-central Oregon contained faunal remains ~170–400 ybp (Clark 1991) from which specimens were genotyped from a single time unit. The larger Nah-So-Mah Village (NV) location in southern Oregon near Bandon contained faunal remains ~700–2000 ybp (Hall 1995). Specimens from this site were genotyped from three distinct time strata (NV-old: 1800–2000 ybp; NV-medium: 980–1430 ypb; NV-young; 695–1060 ybp). One contemporary otter bone from Alaska was also processed as a control.

DNA extraction

DNA was extracted from bone or teeth material using a spin-column technique (Yang et al. 1998) as described previously (Butler and Bowers 1998; Weber et al. 2000; Weber et al. 2004). Briefly, outer surfaces of bone and teeth were cleaned with ethanol and then 10% bleach. Powdered tissue was obtained either by the use of a mortar and pestle or a Dremel™ tool. Approximately 0.3 g of each sample was subject to proteinase K digestion in buffer ATL (Qiagen) at 55°C overnight. The resulting solution was then purified through spin-columns using the DNeasy tissue extraction kit (Qiagen).

PCR amplification and genotyping

PCR primers targeting the same portion of the mtDNA control region assayed by Larson et al. (2002b) were designed by homology to known sea otter and other marine mammal sequences. Amplification of ancient DNA was performed using a nested technique as described previously for seals (Weber et al. 2004). This approach increases the probability of success for weathered samples but also mandates extraordinary contamination controls, described below. First, primers ELD-3 (5′–TATAGCTACCCCCA CATGCGG–3′) and ELD-6 (5′–TCCTCACCCTATTGTCC –3′) were employed to achieve amplification of a ~300 b.p. fragment using 1–6 µl of the undiluted DNA extract as a template. Next, the resulting products were diluted 1/10 and 1–2 µl was used to seed a second semi-nested reaction in which primers ELD-2 (5′–GAAGAGGTACACGTCAA–3′) and ELD-6 were employed to achieve amplification of a 222 b.p. fragment. All amplifications were carried out in 25 µl volumes using 10 pmol of each primer, employing 0.2 mM each dNTP and 1.5 mM $MgCl_2$, contained within a single puReTaq DNA polymerase bead (Amersham) containing a reaction buffer provided by the manufacturer. Initial cycling conditions utilized a 95°C initial denaturation step for 5 min, followed by 10 cycles of 93°C for 1 min, 52°C for 1 min, and 72°C for 50 s. Nested cycling conditions utilized 25 cycles of 90°C for 45 s, 60°C for 1 min, and 72°C for 1 min. Amplification products were assayed by 2% agarose gel electrophoresis.

Successful PCR amplifications were purified using the Qiaquick purification kit (Qiagen). Bidirectional sequence determination was achieved using BigDye (v. 2) chemistry and capillary gel electrophoresis on an Avant 3100 DNA sequencer (Applied Biosystems). Resulting sequences were aligned by eye with the aid of the SeqEd program (Lasergene). Allelic variation statistics and haplotype diversity values were obtained using the DnaSP (version 4.10.8) program (Rozas and Rozas 1999), and a minimum spanning tree of genotypes was constructed using the MJ method of Bandelt et al. (1999).

Contamination controls

Strict controls were employed to ensure the amplification of ancient otter DNA uncontaminated with material either from modern otter material or other potential templates. These controls were based on previous contamination-free projects on marine mammals (Weber et al. 2000; Weber et al. 2004). Four separate rooms were dedicated to: (*i*) the handling of pre-harvest tissue, (*ii*) DNA extraction, (*iii*) setting up the PCR reaction, and (*iv*) the actual PCR amplifications. Extraction tools were thoroughly cleaned between each sample using bleach. Dummy extraction samples consisting of pure water were performed using no tissue inputs, and these were processed alongside genuine otter samples through the entire amplification procedure. All PCR reaction set-ups utilized sterile barrier pipet tips and were performed with no-template negative controls. All PCR equipment, including vials and pipettors, was sterilized with ultraviolet light prior to use. To check for reproducibility, all PCR products were sequenced twice, once from each of the two primers. Additionally, all samples were processed multiple times independently, starting at various points in the genotyping pathway (see Appendix). One bone and one tooth from the same individual were processed independently, and all of the remaining samples were processed twice from the extracted DNA through sequence analysis. Although these replicates were not performed in a completely independent lab, we strove to match the other recommendations of Gilbert et al. (2005) and based our conclusions on general trends and not single sample results.

Results

From a total of 17 pre-harvest tissue samples, we obtained mtDNA genotypes from 16. All replicate sample controls produced the same DNA sequence, including two that revealed new genotypes (see below), although only one sample could be replicated from two independent bones due to limitations on sample availability.

We found the single modern-era sample from Alaska to have the A mtDNA genotype as reported by Larson et al. (2002a). Among the pre-harvest Oregon samples, we detected both the A ($n = 2$) and C ($n = 11$) genotypes, plus two new ones that we designated W ($n = 2$) and X ($n = 1$). We did not detect genotypes B or D (Table 1). All of these genotypes are similar, differing typically by a single nucleotide. A parsimony network (Fig. 2) shows all of the relationships among these genotypes; the single X genotype was the most distinct, but even it differed by at most 8 b.p. from any other genotype within the 222 b.p. assayed. Of the two new genotypes, only W could possibly be the result of spurious recombination in the PCR (cf., Yu et al. 2006), but we found this genotype in two individuals, both of which were processed twice independently from the DNA extract. While either novel genotype could be the result of post-mortem change, this seems less likely for the W genotype, as it was sequenced from two individuals.

Our sample sizes were too small to allow all possible tests of genotype frequency changes among our two geographical sites or our four time periods. However, it is clear that the constellation of pre-harvest genotypes in Oregon matches more closely that of existing California otters than those of either pre-harvest Washington samples or of existing otter populations north of Oregon (Table 1). While genotype C dominates both pre-harvest Oregon samples (11 of 16) and those of contemporary California (23 of 31), genotypes A and B dominate all other samplings (117 of 119)(Larson et al. 2002b). While the Oregon/California genotype difference is not statistically different by a *G*-test of independence ($P = 0.6$), the frequency of C genotypes in these two locations is highly significantly different from that in all other locations ($P < 0.001$). Thus our data suggests that pre-harvest Oregon otter populations were genotypically distinct from populations from more northern latitudes.

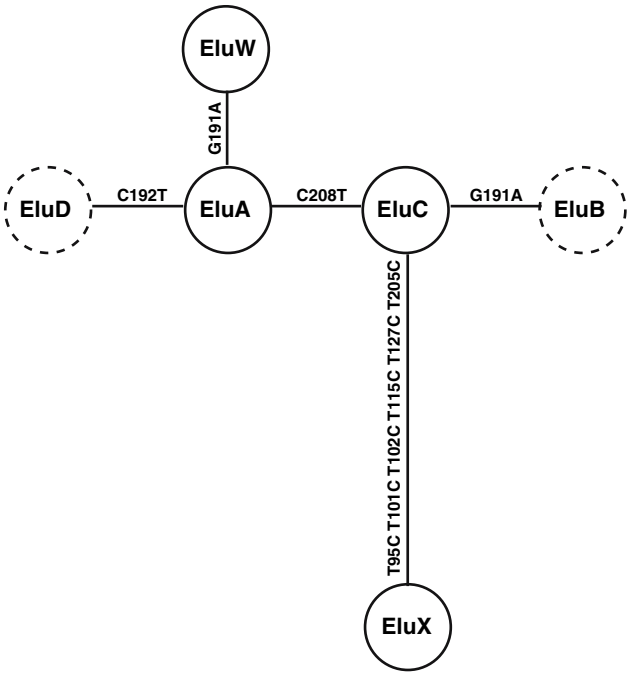

Fig. 2 Parsimony network of sea otter mtDNA control-region genotypes. Circles represent known genotypes; circles in solid lines denote genotypes found in ancient samples from Oregon locations, while circles in dashed lines denote genotypes found in extant populations (Larson et al. 2002a; 2002b) but not among the pre-harvest Oregon samples in this study. Designations above lines represent single nucleotide substitutions with the letters closest to a particular circle denoting that present in that genotype (numbering as per Larson et al. 2002a)

Table 1 Mitochondrial DNA variation in sea otters

mtDNA genotype	Modern: north of OR[a, c]	Modern: south of OR[b, c]	Modern (this study, AK)	WA[c] 100–450 ybp	SR 170–400 ybp	NV 695–1060 ybp	NV 980–1430 ybp	NV 1800–2000 ybp
A	74	2	1	7	0	0	1	1
B	34	0	0	2	0	0	0	0
C	2	23	0	0	5	4	2	0
D	0	6	0	0	0	0	0	0
W[d]	0	0	0	0	0	2	0	0
X[d]	0	0	0	0	1	0	0	0

[a] Includes Aleutian, SE Alaskan, and Washington samples

[b] Includes California samples

[c] Data from Larson et al. (2002a; 2002b)

[d] Genotypes W and X were new genotypes detected in this study and the sequences have been deposited in GenBank (accession numbers EF640383 & EF640381)

Discussion

The mtDNA genotypes of pre-harvest sea otters in Oregon may differ from those of pre-harvest Washington genotypes, and from the composition of extant populations in Alaska and California. However, the extant southern subspecies (in which the C genotype is most common followed by the A genotype, Table 1) may be a stronger genetic match to pre-harvest Oregon populations than any of the extant northern subspecies.

Variations at nuclear loci related to phenotypic characteristics may be correlated with mtDNA variation, regardless of sequence identity in the latter (Avise et al. 1987). Auspiciously, skulls from much of the eastern Pacific sea otter range show various character gradients from north to south. Wilson et al. (1991) reviewed these assessments and added a morphometric analysis. They concluded that there are currently three subspecies: *E. l. lutris* from the northwestern Pacific Ocean, *E. l. kenyoni* from the Aleutian Islands east and south to Oregon, and *E. l. nereis* from California. Lyman (1996, 1988)examined prehistoric otter remains recovered from archaeological sites on the Oregon coast in the middle latitudes of the species' historically documented range. Prehistoric Oregon otters do match modern Alaskan otters in a few characters, but in most, they match modern California otters. In particular, the prehistoric Oregon otters possessed upper and lower molars (M1) and upper pre-molars (P4) that are the same size as modern California otters. The widths of the prehistoric Oregon and California teeth are statistically smaller than modern Alaskan teeth (Lyman 1988). Our genetic data are in agreement with a match with California stocks, although more genetic and morphological data from historic samples would be useful in seeking a more powerful correlation.

The subspecies debate concerning which sea otters were locally present in Oregon is of significance because national legislation relies on accurate delineations of various taxonomic levels, including populations (O'Brien and Mayr 1991; Lyman 1996). Lyman (1996) noted that this directly relates to the transplanting of sea otters because by law, only distinct forms native to an area can be translocated there during populational reestablishment, as it is probable that such forms are the products of adaptation. The original transplantation efforts to the Oregon coast were attempted using Alaskan otters. If the genetic attributes of transplanted otters are not matched with those of pre-harvest genotypes of their target environments, adaptation could be impeded. Our study indicates that the California (southern subspecies) sea otter may be more suitable to the Oregon environment, and awaits a formal test using pre-harvest nuclear loci surveys.

Acknowledgements We thank Rebekah Hull and Klaus Koepfli for technical assistance, and the Oregon Sea Grant program and EcoTrust for funding. We also acknowledge the support of the Confederated Tribes of Siletz Indians and the Coquille Indian Tribe.

Appendix

Sea otters genotyped in this study

Sample no.	Tissue	Location –stratum	Est. age (ybp.)	mtDNA genotype
Elu-mo29	Tooth-molar	AK	Contemporary	A
Elu-anc10	Tooth-molar	SR	170–400	C[b]
Elu-anc11	Left tibia	SR	170–400	C[b]
Elu-anc12	Left tibia	SR	170–400	C[b]
Elu-anc13	Mmolar	SR	170–400	C[b]
Elu-anc14	Left tibia	SR	170–400	C[b]
Elu-anc15	Molar	SR	170–400	X[a]
Elu-anc16	Femur	NV-old	1800–2000	Failed
Elu-anc17	Molar/cranium	NV-old	1800–2000	A[c]
Elu-anc19	Night tibia	NV-medium	980–1430	A[b]
Elu-anc20	Molar	NV-medium	980–1430	C[b]
Elu-anc21	Premolar	NV-medium	980–1430	C[a]
Elu-anc22	Canine	NV-young	695–1060	C[a]
Elu-anc23	Right humerus	NV-young	695–1060	W[a]
Elu-anc24	Left humerus	NV-young	695–1060	W[b]
Elu-anc25	Left humerus	NV-young	695–1060	C[b]
Elu-anc26	Molar	NV-young	695–1060	C[b]
Elu-anc27	Molar	NV-young	695–1060	C[b]

[a] Samples for which DNA extract was amplified by PCR and sequenced multiple times

[b] Samples for which DNA was extracted from bone powder and processed multiple times

[c] Samples for which two bones from the same individual were processed independently

References

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel J, Reeb C, Saunders N (1987) Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annu Rev Ecol Syst 18:489–522

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Bodkin JL, Ballachey BE, Cronin MA, Scribner KT (1999) Population demographics and genetic diversity in remnant and translocated populations of sea otters. Conserv Biol 13:1378–1385

Butler VL, Bowers NJ (1998) Ancient DNA from salmon bone: a preliminary study. Anc Biomol 2:17–26

Clark L (1991) Archaeology of seal rock (35Lnc14). In: Lyman RL (ed) The prehistory of the oregon coast: The effects of excavation strategies and assemblage size on archaeological inquiry. Academic Press, New York, pp 175–240

Cronin MA, Bodkin J, Ballachey B, Estes J, Patton JC (1996) Mitochondrial-DNA variation among subspecies and populations of sea otters (*Enhydra lutris*). J Mammal 77:546–557

Davis J, Lidicker WZ Jr. (1975) The taxonomic status of the southern sea otter. Proc Calif Acad Sci 40:429–437

Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. Trends Ecol Evol 20:541–544

Gorbics CS, Bodkin JL (2001) Stock structure of sea otters (*Enhydra lytris kenyoni*) in Alaska. Mar Mamm Sci 17:632–647

Hall RL (1995) People of the Coquille Estuary. Words and Pictures Unlimited, Corvallis, OR

Jameson RJ, Kenyon KW, Johnson AM, Wight HW (1982) History and status of translocated sea otter populations in North America. Wildl Soc Bull 10:100–107

Kenyon KW (1969) The sea otter in the eastern Pacific Ocean. North Am Fauna 68:1–352

Larson S, Jameson R, Bodkin J, Staedler M, Bentzen P (2002a) Microsatellite DNA and mitochondrial DNA variation in remnant and translocated sea otter (*Enhydra lutris*) populations. J Mammal 83:893–906

Larson S, Jameson R, Etnier M, Flemings M, Bentzen P (2002b) Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. Mol Ecol 11:1899–1903

Lyman RL (1988) Zoogeography of Oregon coast marine mammals: the last 3,000 years. Mar Mamm Sci 4:247–264

Lyman RL (1996) Applied zooarchaeology: the relevance of faunal analysis to wildlife management. World Archaeol 28:110–125

O'Brien SJ, Mayr E (1991) Bureaucratic mischief: recognizing endangered species and subspecies. Science 251:1187–1188

Riedman ML, Estes JA (1990) The sea otter (*Enhydra lutris*): behavior, ecology, and natural history. U.S. Fish & Wildlife Service Biology Report 90,126 pp

Rotterman LM (1992) Patterns of genetic variability in sea otters after severe population subdivision and reduction. Ph.D. dissertation, University of Minnesota, Minneapolis, 227 pp

Rozas J, Rozas R (1999) DnaSP version 3: an integrated program for moleulcar population genetic and molecular evolution analysis. Bioinformatics 15:174–175

Scribner KT, Bodkin J, Ballachey B, Fain SR, Cronin MA, Sanchez M (1997) Population genetic studies of the sea otter (*Enhydra lutris*): a review and interpretation of available data. Mol Gen Mar Mamm 3:197–208

Tul'skaya OL, Derenko MV, Malyarchuk BA (1999) Low level of mitochondrial DNA variation in sea otter populations from Kamchatka and Komandor Islands. Russ J Genetics 35:17–21

Weber DS, Stewart BS, Garza JC, Lehman N (2000) An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. Curr Biol 10:1287–1290

Weber DS, Stewart BS, Lehman N (2004) Genetic consequences of a severe population bottleneck in the Guadalupe fur seal (*Arctocephalus townsendi*). J Hered 95:144–153

Wilson DE, Bogan MA, Brownell RL Jr, Burdin AM, Maminov MK (1991) Geographic variation in the sea otters, *Enhydra lutris*. J Mammal 72:22–36

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Improved DNA extractions from ancient bones using silica-based spin columns. Am J Phys Anthropol 105:539–543

Yu W, Rusterholtz KJ, Krummel AT, Lehman N (2006) Detection of high levels of recombination generated during PCR amplification of RNA templates. BioTechniques 40:499–507