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Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries

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Abstract

Sea otter (*Enhydra lutris*) populations experienced widespread reduction and extirpation due to the fur trade of the 18th and 19th centuries. We examined genetic variation within four microsatellite markers and the mitochondrial DNA (mtDNA) D-loop in one prefur trade population and compared it to five modern populations to determine potential losses in genetic variation. While mtDNA sequence variability was low within both modern and extinct populations, analysis of microsatellite allelic data revealed that the prefur trade population had significantly more variation than all the extant sea otter populations. Reduced genetic variation may lead to inbreeding depression and we believe sea otter populations should be closely monitored for potential associated negative effects.

Keywords: ancient DNA, *Enhydra lutris*, genetic diversity, microsatellites, population bottleneck, sea otter

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Introduction

All existing populations of sea otters (*Enhydra lutris*) have suffered at least one population bottleneck due to the commercial depredations of the fur trade in the 18th and 19th centuries (Kenyon 1969; Jameson *et al*. 1982; Riedman & Estes 1990). All extant sea otter populations, remnant and translocated alike, show relatively low levels of genetic variation (Lidicker & McCollum 1997; allozymes; Bodkin *et al*. 1999; restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA); Larson *et al*. 2002, microsatellites and mtDNA sequence variation). The limited genetic variation in modern sea otters may be a result of the fur trade related population bottleneck (Larson *et al*. 2002), but evidence bearing on this suggestion has not been available due to the lack of genetic data from prefur trade sea otters. We test this hypothesis using ancient sea otter remains and both multilocus microsatellite markers and mitochondrial DNA D-loop sequence variation and, for the first time, show evidence that modern sea otters have suffered a loss of genetic variation coincident with the fur trade.

Sea otters once ranged throughout coastal regions of the North Pacific Ocean from the Island of Hokkaido in Japan, north through the Kuril Islands and the Kamchatka Peninsula of Russia, across the Aleutian Islands, and down the west coast of North America to Morro Hermoso in Baja California (Kenyon 1969). They were extirpated from much of this range by hunting associated with the fur trade of the 18th and 19th centuries, and remained in decline until they were protected by international treaty in 1911 (Kenyon 1969). The sizes of remnant populations following protection are unknown, but may have ranged from a few tens to hundreds of individuals (Bodkin *et al*. 1999; Rotterman 1992). By the late 1970s, several sea otter populations had recovered to pre-exploitation levels with the total population numbering approximately 100 000 in 1994 (Bodkin *et al*. 1994). However, throughout much of their historic range, including Southeast Alaska (SEAK), the coasts of Canada (BC), Washington (WA), Oregon (OR) and most of California (CA), sea otters remained absent or below pre-exploitation levels (Kenyon 1969). In the late 1960s and early 1970s several reintroductions from one

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or two remnant populations were made in an effort to reestablish sea otters where they had been extirpated. These efforts were successful in WA, SEAK and BC (Jameson *et al*. 1982). The sources of these translocated populations was predominately Amchitka Island (AM) in the Aleutian Islands, Alaska, because of the desire to move animals away from the vicinity of nuclear tests conducted there in the early 1970s (Jameson *et al*. 1982).

A survey of genetic variation in several extant sea otter populations revealed levels of variation in mtDNA D-loop and nuclear microsatellites that were relatively low in both remnant and translocated populations, and comparable to variation in several mammalian species that have experienced known bottlenecks (Bodkin *et al*. 1999 and Larson *et al*. 2002). However, in the absence of data from prefur trade sea otters, it was unclear whether the current level of genetic variation in sea otters is a consequence of a fur trade-related bottleneck, or of a low long-term effective population size that predates the fur trade. To test the hypothesis that modern sea otter populations have less genetic diversity than prefur trade populations, we examined genetic variation in the bones of sea otters that lived prior to and up to the fur trade, and compared it to previously reported values for extant sea otters (Larson *et al*. 2002).

Materials and methods

Variation at four microsatellite loci (Mvi 57 and Mvi 87, O'Connell *et al*. 1996; Mvis 72, Fleming *et al*. 1999; Lut 453, Dallas & Piertney 1998) was collected in 34 prefur trade (OLDWA) samples. These results were then compared to data from the same four loci found in five extant sea otter populations obtained from: AM ($N = 36$), Prince William Sound (PWS, $N = 33$), SEAK ($N = 24$), WA ($N = 27$) and CA ($N = 54$, Larson *et al*. 2002). The modern populations comprise two subspecies *E. l. kenyoni* (AM, SEAK, PWS and WA) and *E. l. nereis* (CA, Wilson *et al*. 1991). A 320 base pair (bp) stretch of the mtDNA D-loop was also amplified within nine OLDWA prefur trade samples using the universal primers L15926 and H16340 (Cheney 1995). The variation within the mtDNA D-loop sequences in the prefur trade population was then compared to that found within the same five modern sea otter populations mentioned above with the following sample sizes: AM ($N = 34$), CA ($N = 31$), SEAK ($N = 20$), PWS ($N = 29$) and WA ($N = 27$).

Genetic samples of the prefur trade sea otter population (OLDWA) were obtained from archaeological bone samples from the Makah Indian village site of Ozette, near Neah Bay, WA. All excavated materials are currently curated by the Makah Cultural and Resource Center in Neah Bay. Although the Ozette village appears to have been occupied for approximately 2000 years (Daugherty & Fryxell 1967), stratigraphic evidence indicates that the sampled bones represent sea otters that lived during the interval from ~450 YBP to the extirpation of native WA sea otters in the early part of the 20th century (Samuels 1991 and Scheffer 2000). Hence, the prefur trade remains are not related by descent to contemporary WA sea otters, all of which derive from translocated AM otters (Jameson *et al*. 1982).

Caution was used to prevent multiple sampling from the same individual and to prevent sample contamination. To minimize the chances of obtaining more than one sample per individual, three precautions were taken: (1) samples were taken from a wide array of sites; (2) a narrow range of skeletal elements (femur, humerus, mandible, maxilla) was utilized; and (3) after amplification, samples were compared for identical genotypes and if found one was removed. Control of potential contamination of the ancestral bone samples followed aspects of protocols described previously (Hagelberg & Clegg 1991; Hoss & Pääbo 1993; Rosenbaum *et al*. 1997). All materials and equipment that could potentially come into contact with the samples (cotton gauze, tips, tubes, etc.) were treated with UV light for 10 min. Each bone sample was cleaned repeatedly with ethanol, 10% bleach and rinsed with RNA and DNA free water prior to sampling. A variable speed Dremel™ tool was used, with a new UV-treated drill bit for each sample, to collect bone dust. Samples were collected in a sterile 1.5 mL microcentrifuge tube and stored at ambient temperature until extraction. Bone samples were decalcified in 1 mL of 0.5 m EDTA for at least 24 h at 37 °C. Several changes of EDTA supernatant were made to remove pigmented humic acids absorbed from the sediments. Once relatively clear EDTA supernatant was obtained, the EDTA was removed and the resulting bone pellet was rinsed with sterile water, and the DNA was extracted using the DNeasy tissue extraction kit (Qiagen, Valencia, CA). Blank controls during DNA extraction, PCR and fragment visualization were employed to determine potential contamination.

Standard statistical tests for genetic data were performed. Tests for departures from Hardy–Weinberg equilibrium (HWE) were performed for all populations using the probability test in GENEPOP 3.1 (Raymond & Roussett 1995). Sequential Bonferroni adjustments were used to determine significance levels for all simultaneous tests (Rice 1989).

Results and discussion

None of the OLDWA bone samples had identical microsatellite genotypes. Consequently, all 34 samples that had amplified products were included for analysis. Departures from HWE were not significant (corrected $\alpha = 0.001$) for most loci, except for within the OLDWA

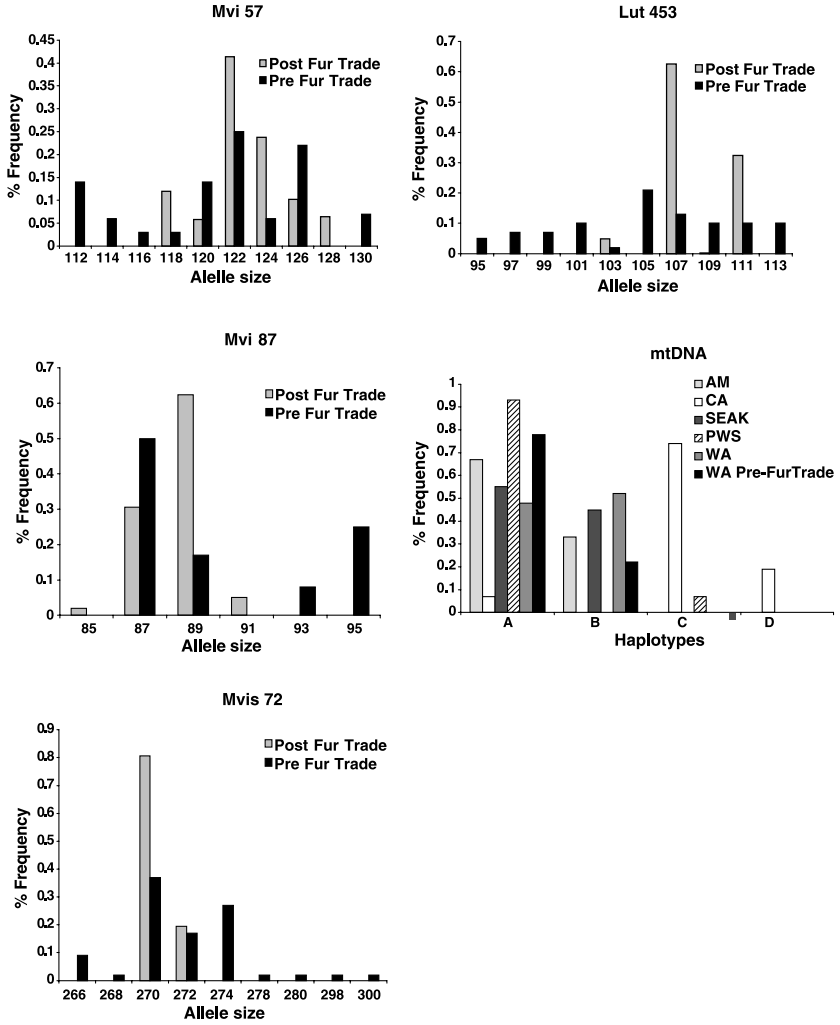

Fig. 1 Four microsatellite loci (Mvi 57, Mvi 87, Mvis72 and Lut 453) and mtDNA haplotype allele frequencies and distribution within the prefur trade WA population (OLDWA) and contemporary sea otter populations (AM, PWS, SEAK, WA and CA).

population at Mvis72 and Lut453, with an excess of homozygotes (Fis = 0.580 and 0.566, respectively). This was due probably to allelic dropout caused by the relatively poor quality of the OLDWA DNA (Hofreiter *et al*. 2001).

The four microsatellite loci amplified within the 34 prefur trade OLDWA bone samples revealed more genetic variation than was detected in 174 sea otters, representing most extant North American populations combined (Fig. 1 and Table 1). Mean heterozygosity in the OLDWA otters was significantly greater than in extant otters in this study ($H_E = 0.823 \pm 0.037$ SE vs. $H_E = 0.466 \pm 0.028$ SE; $P = 0.0004$, *t*-test, Table 1). The total number of alleles across the four microsatellites observed in the OLDWA otters was also significantly greater than the mean number for contemporary populations (32 vs. the mean of 12.8 ± 0.735 SE, $P = 0.004$), and even exceeded the total number (16) of alleles seen in all contemporary sea otters (Fig. 1). The OLDWA sea otters had more alleles per locus than were detected among all contemporary otters at three of the four loci assayed; the exception is locus Mvi 87 in which the number of alleles (four) was the same for both pre- and postfur trade otters (Fig. 1).

Table 1 Pre- (OLDWA) and post (AM, CA, SEAK, PWS, WA) fur trade genetic variation based on the analysis of four microsatellite loci (H_E) and mtDNA d-loop (*h*)

Pop.	AM	CA	SEAK	PWS	WA	OLDWA
H_E	0.508	0.377	0.532	0.486	0.428	0.823
h	0.451	0.414	0.508	0.180	0.509	0.366
N	36	54	24	33	27	34

H_E = microsatellite heterozygosity, *h* = mtDNA haplotype diversity.

Variability of the 320 bp mtDNA D-loop sequence was low in both modern and the extirpated sea otter populations. The haplotype diversity (*h*, equivalent to H_E for microsatellites) was 0.366 in the prefur trade population and a mean of 0.412 in the modern sea otters (Table 1). The relatively low level of genetic variability observed in the

OLDWA otters may be an artefact of the relatively low number of bone samples that had long enough DNA fragments to sequence the control region haplotypes (N = 9 within OLDWA and average N = 28.2 per contemporary population). The smaller sample size within the OLDWA population may have decreased the probability of finding rare mtDNA haplotypes. The relatively low mtDNA variation within the more broadly sampled contemporary populations may nonetheless be due to fur trade associated population bottlenecks as well as contemporary bottlenecks associated with the translocation events of the 1960s and early 1970s (Bodkin *et al*. 1999; Jameson *et al*. 1982).

Two haplotypes (A and B) were found in the OLDWA prefur trade population. One of these (A) occurs within all modern populations and both A and B are found in the contemporary Alaskan sea otter populations (AM, PWS, SEAK and WA; Fig. 1). On the basis of these data, the OLDWA population resembles more closely the recognized northern (Alaskan) sea otter subspecies (*E. l. kenyoni*) rather than the southern (CA) sea otter subspecies (*E. l. nereis*). Therefore, these data support the hypothesis that the natural break (if there was one) between *E. l. kenyoni* and *E. l. nereis* occurred south of the Washington sea otter population. Further analysis of other prefur trade populations (those from OR and CA) will shed more light on the level of gene flow between the northern and southern subspecies before population fragmentation.

The genotypic data gathered on the OLDWA prefur trade sea otters clearly illustrate that modern sea otter populations have experienced a significant loss of diversity, due probably to the great reduction in sea otter numbers following their exploitation for the fur trade (a loss of approximately 99% of the population, Kenyon 1969; Riedman & Estes 1990). Our results show that there has been a loss of at least 62% of the alleles and 43% of the expected heterozygosity in modern sea otters. This estimate is conservative, because we examined only a modest sample of prefur trade otters from a single geographical area. All extant sea otter populations suffered bottlenecks resulting from fur trade hunting, translocation efforts and natural population fluctuations (Kenyon 1969; Simenstad *et al*. 1978; Jameson *et al*. 1982; Estes *et al*. 1998). Our findings are similar to those found in another exploited marine mammal, the northern elephant seal (*Mirounga angustirostris*), where bone samples predating the 19th century blubber hunt had significantly higher levels of mtDNA genetic diversity compared to late 19th and 20th century elephant seals (Weber *et al*. 2000). Our results leave little doubt that sea otter populations historically had greater levels of genetic diversity than we see today and are more similar to the levels found in other vertebrates that have not experienced population bottlenecks (Davis & Strobeck 1998 (American badger, *Taxidea taxus*); Harr *et al*. 2000 (wood mouse, *Apodemus sylvaticus*); Spong *et al*. 2000 (leopard, *Panthera pardus*); Van Hooft *et al*. 2000 (African buffalo, *Syncerus caffer*); Eizirik *et al*. 2001 (jaguar, *Panthera onca*). Population bottlenecks, inbreeding and the associated loss of genetic diversity may result in inbreeding depression, a reduction in fitness and an increased probability of extinction from stochastic events (Wildt *et al*. 1987; Amos & Balmford 2001; Lacy 1997). We recommend continued monitoring of wild sea otter populations to determine if the anthropogenic loss of historic genetic diversity will have a negative impact on long-term population survival.

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