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GENETIC ANALYSIS OF KILLER WHALE (*ORCINUS ORCA*) HISTORICAL BONE AND TOOTH SAMPLES TO IDENTIFY WESTERN U.S. ECOTYPES

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ABSTRACT

Little is known about the historical range of killer whale ecotypes in the eastern North Pacific (ENP). It is possible that ranges have shifted in the last few decades because of changes in availability of food. In particular, the southern resident ecotype, currently found primarily in the inland waters of Washington State, is known to prey extensively on salmon, which have declined in recent decades along the outer coasts of Washington, Oregon, and California. To investigate historical distributions of this and the other ENP ecotypes, samples of teeth and bones were obtained from NMFS and museum collections. We amplified a short section of the mitochondrial DNA control region that contains four diagnostic sites that differentiate between haplotypes found in ecotypes of ENP killer whales. Results did not show any southern resident haplotypes in samples from south of the Washington State inland waterways. One whale genetically identified as a northern resident extends the known southernmost distribution of the population from Oregon to California.

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Items of diet identified from stomach contents of six of the whales genetically identified to ecotype conformed with what is known of the feeding habits of the various ecotypes.

Key words: killer whale, *Orcinus orca*, mtDNA, ecotype, control region, ancient DNA, museum samples.

INTRODUCTION

Three different types of killer whales have been recognized in the eastern North Pacific (ENP), based on morphology (Baird and Stacey 1988), prey preferences (Baird *et al*. 1992, Ford *et al*. 1998, Saulitis *et al*. 2000), vocalizations (Ford 1989, 1991; Ford *et al*. 1994; Barrett-Lennard *et al*. 1996; Ford and Ellis 1999), social behavior (Ford *et al*. 1994, Baird and Dill 1995, Baird 2000), and genetic studies (Hoelzel *et al*. 1998, Barrett-Lennard 2000). Available evidence indicates that the "resident" type eats fish and is possibly a salmon specialist in some areas (Ford *et al*. 1998, Saulitis *et al*. 2000). The "transient" type eats primarily marine mammals (Ford *et al*. 1998, Saulitis *et al*. 2000). The "offshore" type has been observed apparently eating fish, but its prey preferences are not well described (Ford *et al*. 2000).

Three discrete populations of resident-type whales have been described in the ENP, based on individual associations (Bigg *et al*. 1990), vocalizations (Ford 1989, 1991; Yurk *et al*. 2002), and genetics (Hoelzel *et al*. 1998, Barrett-Lennard 2000, Hoelzel *et al*. 2002). In summer, the "southern resident" population is found primarily in Washington and southern British Columbia, the "northern resident" population is found primarily in central and northern British Columbia, and the "Alaska resident" population is found primarily in southeastern Alaska and the Gulf of Alaska. Southern resident killer whales, considered a "population stock" under the U.S. Marine Mammal Protection Act, constitute three pods, defined by individual association patterns (Bigg *et al*. 1990): J pod, K pod, and L pod. Their home range during the spring, summer, and fall includes the inland waterways of Puget Sound, the Strait of Juan de Fuca, and the Strait of Georgia, where they are known to eat Chinook and chum salmon. It has been suggested that they may be Chinook specialists (Ford *et al*. 1998). Their occurrence in the coastal waters off Washington, Vancouver Island, and more recently off the coast of Oregon and central California has been documented (reviewed in Krahn *et al*. 2004). However, given the general lack of sighting data in the winter, southern residents may use outer coastal waters in Washington, Oregon, and California more frequently than is currently recognized. All confirmed sightings from southern Washington, Oregon, and California have been in winter, but sightings have to be considered in light of the sighting effort, and coastal surveys are both rare and biased toward summer months; both summer and winter ranges may be wider and more variable than is currently known.

The southern resident population declined substantially from 1996 to 2001 (Krahn *et al*. 2004) and was listed under the Endangered Species Act (ESA) in 2005. The ESA allows listing of distinct population segments (DPSs) of vertebrates, as well as named species and subspecies. The southern resident DPS has not been seen to associate with northern resident killer whales, they have unique vocalizations that are not shared by other resident killer whales, and they have been found to be genetically different from other resident killer whales in both mtDNA and microsatellite DNA. The population has a distinct mitochondrial haplotype that can be used to distinguish it

from the transient, offshore, and northern resident populations (Hoelzel *et al*. 2002, Krahn *et al*. 2004, LeDuc and Taylor 2004).

The southern resident population may have been significantly larger in the past (Krahn *et al*. 2004), and may have had a wider distribution. Historically, the largest runs of chinook salmon occurred in the Columbia River and California's Central Valley, at latitudes where southern residents are only infrequently seen at present. Substantial declines in native salmon populations have been documented in this region in recent decades, resulting from multiple factors such as hydro-electric dams, habitat destruction, harvest, and competition with hatchery fish (Yoshiyama *et al*. 1998, reviewed in Krahn *et al*. 2004). Salmon-eating killer whales, if formerly present at all of the major salmon runs and dependent on them, may have constricted their range to inland Washington (in summer) subsequent to the salmon population declines.

This information suggests the hypothesis that the southern resident population previously had a larger and more widespread distribution that routinely included the Washington, Oregon, and California coast coincident with the range of Chinook salmon (with a southern boundary at approximately Monterey Bay in California). An evaluation of the DPS status of the southern resident population requires a better understanding, if possible, of the historical distribution of this population and the other ENP ecotypes.

This study focused on 30 historical samples, most of which were from bone and teeth of animals sampled before 1980 south of inland Washington waters (Puget Sound, Strait of Juan de Fuca, Strait of Georgia) (Fig. 1). We have used historical DNA analysis methods to assay diagnostic sites in mitochondrial DNA haplotypes to classify the historical bone and tooth samples, to determine the historical distribution of the ENP haplotypes along the Washington, Oregon, and California coasts. The samples are from animals that were harpooned, live-captured, or stranded on the coast, and as such may present some biases in representation of the different haplotypes relative to their true historical abundance and distributions (*e.g.*, density differences, propensity of animals to strand). This study, however, aims at initial descriptions of historical ecotype distributions, to be added to with additional samples as they become available.

Materials and Methods

Killer whale bones and teeth were sampled using a variety of methods. Sample surfaces were cleaned with 100% ethanol to remove surface contamination before extracting material. Powdered material was obtained by drilling or by crushing portions of the hard material. For drilling, a 1/8 inch bit was used to drill the sample; drill speeds were kept low in order to avoid heating of the sample from friction. About 100–200 mg of powder was collected onto sterile aluminum foil or weighing paper. For crushed samples, a small piece of material was placed in a sterilized mortar and crushed with the pestle, and powder scraped onto aluminum foil. Specimens ranged in age from 20 yr to >150 yr.

In all cases, samples were handled separately with sterile gloves, and all equipment and surfaces were sterilized with 0.25 M HCl or 10% bleach. Drill bits were used only once, then discarded or soaked in concentrated bleach and rinsed with distilled water between uses.

DNA extractions were done using the method of Höss and Pääbo (1993), modified as described in Hofreiter *et al*. (2004). A maximum of six samples were extracted at

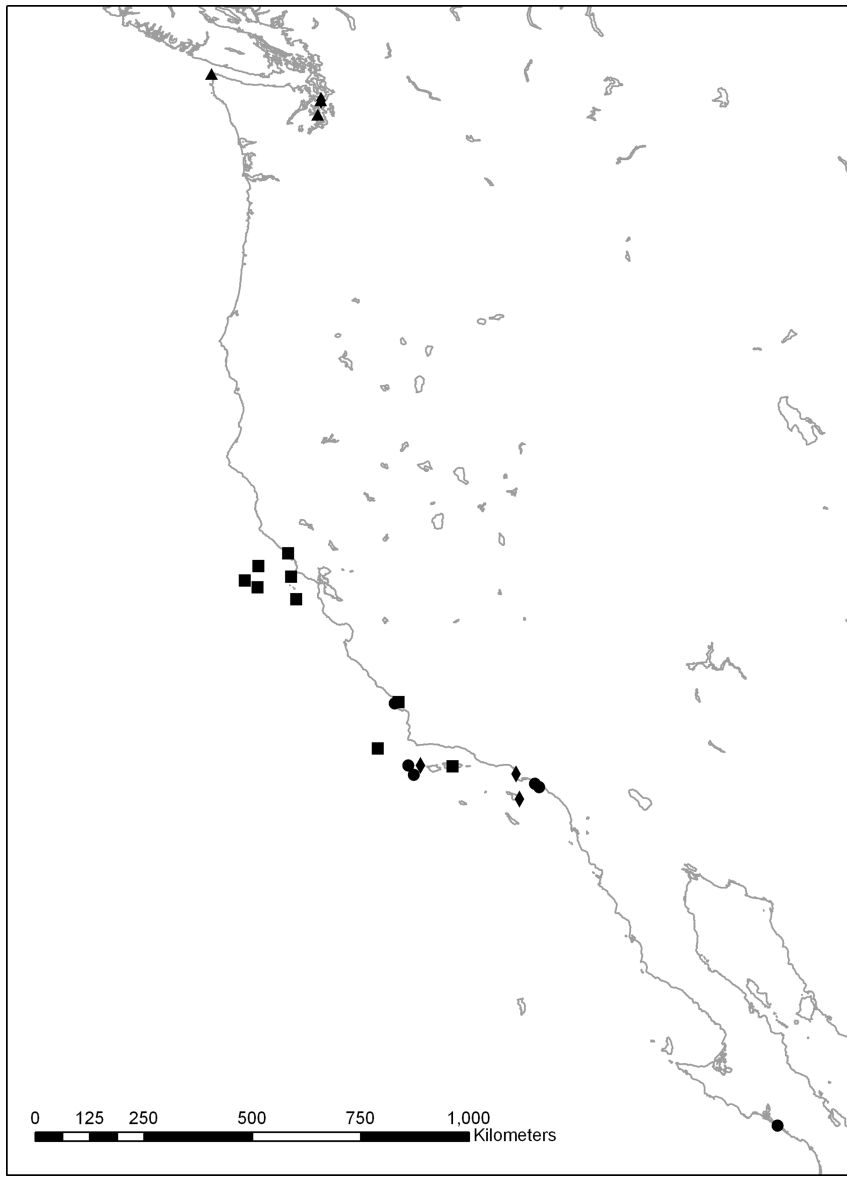

Figure 1. Original sampling locations (when known) for killer whale samples used in this study. Samples with imprecise collection locations (*e.g.*, "California") are not shown, or are shown in general location (*e.g.*, Cape Flattery, WA). Haplotypes are: Square = transient, circle = offshore, triangle = southern resident, diamond = unknown.

one time in a clean room facility that is used only for pre-PCR extraction of historical and ancient DNA samples, with one-way movement of DNA out of the lab for PCR. Sterilization of all equipment and surfaces was performed between each extraction set using 0.25 M HCl and ultraviolet light. Each set of extractions included two

extraction controls, placed at the beginning and end of the set of samples with order maintained throughout the extraction procedure. The extraction controls contained all extraction reagents with the exception of sample material.

All DNA extracts were quantified using 2 μL of template DNA and quantitative PCR (qPCR) assays specifically developed for cetacean mtDNA (Morin and Hedrick in prep). Samples with <40 copies/μL (~140pg/μL) for the mitochondrial assay were re-extracted and sequenced if they produced the same haplotype as any other sample extracted in the same batch, making sure to extract samples with the same haplotype in different re-extraction batches. Samples with low DNA concentrations, but which produced haplotypes that were unique within the group of samples extracted together, were not re-extracted. Sample 37272 had a low DNA concentration, but was not re-extracted, because there was no remaining material. Sample 39063 was also re-extracted and resequenced despite having >40 copy/μL DNA concentration, because it was still in the lower range of extraction concentrations and had the same haplotype as another sample extracted in the same batch . In addition to re-extracted samples, 13 of the 25 positive extracts were resequenced from the initial extraction to verify sequence accuracy.

All pre-PCR and post-PCR laboratory work was completed in separate laboratories at the Southwest Fisheries Science Center. PCR reactions were set up in a clean room area used only for PCR setup for historical and ancient DNA samples, with reagents purchased for and maintained exclusively in that room. Each set of amplifications included a minimum of two no-template controls, and extraction controls were also included in at least one PCR setup per primer pair to be sure that there was no carryover contamination of PCR products or cross contamination between samples. PCR reactions were performed in 50 μl volumes containing 1X NH_4 PCR buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris-Cl (pH 8.8 at 25°C), 0.01% Tween-20; Bioline USA Inc., Randolph, MA), 300 nM of each primer, 150 μM each dNTP (dA, G, C, TTP), 2.5 mM $MgCl_2$, 2.5 units of TAQ polymerase (Biolase, Bioline USA Inc., Randolph, MA), and 4–15 μL of DNA. PCR cycling conditions included initial denaturing for 2 min, 30 sec at 94°C, 50 cycles of 94° for 30 sec, 48° for 45 sec, and 72° for 90 sec, followed by a final extension period of 72° for 10 min. The PCR product was a 160 bp portion of the mitochondrial control region generated using the primers DH6 (H675) 5′ -AAA TAC AYA CAG GYC CAG CTA- 3′ and DL5 (L537) 5′- CCY CTT AAA TAA GAC ATC TCG ATG G- 3′ (primer locations are based on the fin whale sequence (Arnason *et al*. 1991)). PCR products were visualized by 2% agarose gel electrophoresis with ethidium bromide, and visible products were purified using Qiaquick PCR purification columns (Qiagen Sciences, Valencia, CA). Purified products were sequenced in both directions using the PCR primers and Big Dye Sequencing mix v1.1 (Applied Biosystems, Foster City, CA) and an Applied Biosystems 3100 sequence analyzer. Sequences were checked and aligned using Sequencher (v4.1; Gene Codes Corp., Ann Arbor, MI).

The DL5/DH6 portion of the killer whale mitochondrial control region contains diagnostic nucleotide sites that distinguish the killer whale populations found in the higher latitudes of the ENP (Table 2; sites 573–696 in Fig. 2 of Hoelzel *et al*. 2002). Complete control region sequences from approximately 188 samples published to date from coastal Washington, British Columbia, and Alaska fall into seven haplotypes that are clearly divided among the three ecotypes (Hoelzel *et al*. 1998, Barrett-Lennard 2000). Although actual sample sizes for each ecotype across the northern Pacific are still far from exhaustive, the matrilineal social structure together with long-term studies of individual populations has allowed inference of mtDNA haplotypes from

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                                  1 2
37270      O    CTTAAATAAGACATCTCGATGGACTCATGACTAATCAGCCCATGCCTAACATAACTGAGA
34079      T    ........................T.....................................
34080      O    ........................................................
34081      NR   .........................T.....................................
34082      SR   ........................................................
34100      T    ??????????????????????????????????????.....................
34515      O    ?.......................................................
34517      O    ........................................................
34521      O    ........................................................
37271      T    ........................T.....................................
37272      T    ??????????????????????????????.....................
39060      T    ........................T.....................................
39062      O    ........................................................
39063      T    ???????.................T.....................................
39064      T    ........................T.....................................
39065      SR   ........................................................
39066      T    ........................T.....................................
39067      T    ........................T.....................................
39069      O    ........................................................
39070      SR   ........................................................
39071      SR   ........................................................
39075      T    ........................T.....................................
39076      T    ........................T.....................................
39077      O    ........................................................


                    3
37270      O    TTTCATACATTTGGTATTTTTTAATTTTTGGGGGGGAGCTTGCACCGACTCAGCTATGGC
34079      T    ..C.....................................................
34080      O    ........................................................
34081      NR   ........................................................
34082      SR   ........................................................
34100      T    ..C.....................................................
34515      O    ........................................................
34517      O    ........................................................
34521      O    ........................................................
37271      T    ..C.....................................................
37272      T    ..C.....................................................
39060      T    ..C.....................................................
39062      O    ........................................................
39063      T    ..C.....................................................
39064      T    ..C.....................................................
39065      SR   ........................................................
39066      T    ..C.....................................................
39067      T    ..C.....................................................
39069      O    ........................................................
39070      SR   ........................................................
39071      SR   ........................................................
39075      T    ..C.....................................................
39076      T    ..C.....................................................
39077      O    ........................................................


                                        4
37270      O    CTTAGAAAGGCCCCGTCACAGTCAAACAAATTGTAGCTGG
34079      T    ........................T...........
34080      O    ........................................
34081      NR   ........................T...........
34082      SR   ........................T...........
34100      T    ........................T...........
34515      O    ........................................
34517      O    ........................................
34521      O    ........................................
37271      T    ........................T...........
37272      T    ...????????????????????????????????????
39060      T    ........................T...........
39062      O    ........................................
39063      T    ........................T.........?????
39064      T    ........................T...........
39065      SR   ........................T...........
39066      T    ........................T...........
39067      T    ........................T...........
39069      O    ........................................
39070      SR   ........................T...........
39071      SR   ........................T...........
39075      T    ........................T...........
39076      T    ........................T...........
39077      O    ........................................
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Figure 2. Killer whale DNA sequences. Sample IDs and ecotype designations are as in Table 1. The first sequence (37270, offshore) is shown, and all other sequences show only a dot representing the same nucleotide, ? representing unresolved DNA sequence, or the nucleotide that differs from the first sequence. Numbers above the sequence indicate the variable site number.

relatively large numbers of animals (*e.g.*, 81 mitochondrial genotypes inferred from 18 samples, Hoelzel *et al*. 2002), especially for residents, and to a lesser extent for transients. For the offshore ecotype, little is known of the genetic diversity or behavior, but only one haplotype has been found in them so far. Recent expanded sampling of the western North Pacific indicates that the SR haplotype is also found outside of the ENP, and sampling farther south has revealed that there is greater variety of haplotypes among killer whales at low and middle latitudes (where ecotype is usually unknown). The ~1,000 bp of complete control region sequence can be used to identify northern and southern resident and the offshore haplotypes, as well as the four haplotypes associated with transients. In addition, comparable sequence from 265 samples (including published and unpublished sequences) from other areas provide an expanded reference data set from other parts of the ENP. With one exception, the diagnostic sites included in the 160-bp region of the present study are sufficient to diagnose any of the seven published haplotypes associated with known ecotypes. However, there are some haplotypes recorded from the ETP (from animals of unknown ecotype) that share diagnostic character states in this 160-bp region with the SR haplotype.

RESULTS

We obtained DNA sequence data from 25 of 30 samples (83%), with replicate amplification and sequencing from 13 of the extracts for verification (52%). Quantitative PCR of the samples indicated that most samples contained high copy numbers of amplifiable mtDNA, with an average of >18,000 copies/μL (range 11–391,600, Table 1) for those samples with detectable amplification, consistent with other studies showing high levels of amplifiable DNA from recent historical samples (Wandeler *et al*. 2003) and minimizing contamination concerns. A minimum concentration of 60 copies/reaction (~200 pg/reaction) has been shown to produce reliable microsatellite genotypes from noninvasive (hair and feces) samples (Morin *et al*. 2001). For killer whale samples that were extracted together and had the same haplotype, we re-extracted and resequenced (from the new extractions) samples with <40 copies/μL (arbitrarily chosen to exceed the reliable concentration) (Table 1). For all samples, we used at least 4 μL/reaction to ensure that samples amplified from sufficient starting material for reliable products. All re-extraction and resequencing results were consistent, with the exception of sample 39077, which failed amplification from the second extraction. All extraction controls and PCR no-template controls were negative, and extraction controls showed no product in quantitative PCR. In all, 13 samples were amplified and sequenced twice from the same extract, 6 samples were amplified and sequenced twice from different extracts, and 1 sample was sequenced twice from extracts of different tissues (tooth and dried tissue; data not shown). As added support for sequence reliability, eight of the samples in this study were previously analyzed in Hoelzel *et al*. (1998), and consistent results were found in the mtDNA haplotypes.[2]

Of the 25 sequences, 8 were of the offshore haplotype, 12 were of the transient haplotype, 4 were of the southern resident haplotype, and 1 was of the northern resident haplotype (Table 1). In every case except one, diagnostic haplotypes were from animals sampled in the region of their current known distribution (Bigg *et al*. 1990, Ford and Ellis 1999, reviewed in Krahn *et al*. 2004). Specifically, southern resident

[2] Personal communication from Russ Hoelzel, Durham University, South Road, Durham DH1 3LE, England, 20 February 2006.

Table 1. Killer whale sample information. Under "Haplotype ID," O = offshore, T = transient, SR = southern resident, NR = northern resident, as determined from DNA sequences.

Lab ID	Field Museum ID	Tissue type	Amount used (g)	DNA concentration [re-extract] (copies/μL)	Hapotype ID	Source	Yr	Locality
26641	SMI602	tooth	0.2	69		archeological–NMML		CA, San Miguel Is.
34079	USNM13018	muscle	0.15	107	T	USNM	mid-1800s	CA
34080	USNM16487	skull bone	0.19	94	O	USNM	mid-1800s	CA
34081	USNM16488	tooth	0.2	16* [6]	NR	USNM	mid-1800s	CA
34082	USNM16625	tooth	0.19	1,548	SR	USNM	<1883	WA, Clallam co., Cape flattery
34087	USNM49909	periotic bone	0.15	—		USNM		CA, Santa Catalina Is., Avalon
34100	LACM54444	tooth	0.19	802	T**	strand–CA–LACM	1973	CA, Santa Cruz Is., Willows on SE side
34515	LACM22791	tooth	0.12	1,870	O	strand–Mexico–LACM	1951	Mexico, Baja California, Bahia de Sebastian Viscaino
34516	LACM30461	skull bone	0.14	1		strand–CA–LACM	1927	CA, Los Angeles Co., Hermosa Beach
34517	LACM52455	skull bone	0.21	57	O	live-capture–CA–LACM	1961	CA, Orange Co., Newport Beach
34521	LACM72550	skull bone	0.17	4,695	O	strand–CA–LACM	1985	CA, Huntington Beach, Bolsa Chica State Beach
37270	SBMNH979	tooth	0.12	2,108	O	strand–CA–SBMNH	1981	CA, Santa Cruz Is., China Harbor
37271	SBMNH1546	tooth	0.22	258	T	strand–CA–SBMNH	1977	CA, Morro Bay sand spit, 0.4 km north of Haz and Canyon
37272	SBMNH4074	tooth	0.15	0.3	T**	strand–CA–SBMNH	1990	CA, Santa Barbara Co., Santa Cruz Is.

Table 1. Continued.

Lab ID	Field Museum ID	Tissue type	Amount used (g)	DNA concentration [re-extract] (copies/μL)	Hapotype ID	Source	Yr	Locality
37273	SBMNH-NA-CA-125-13C-2	tooth	0.13	11		archeological—CA–SBMNH		CA
39060	NMML0078	tooth	0.18	2,674	T	harpooned-NMML	1961	CA, San Francisco
39061	NMML0079	tooth	0.13	105	T	harpooned-NMML	1963	CA
39062	NMML0080	tooth	0.18	2,377	O	harpooned-NMML	1964	CA
39063	NMML0081	tooth	0.21	224* [139]	T	harpooned-NMML	1965	CA, Pt. Conception
39064	NMML0082	tooth	0.19	3,037	T	harpooned-NMML	1966	CA, Off San Francisco
39065	NMML0083	tooth	0.16	37,060	SR	live capture-NMML	1967	WA, Yukon Harbor, Puget Sound
39066	NMML0084	tooth	0.14	3,495	T	harpooned-NMML	1967	CA
39067	NMML0085	tooth	0.12	28,240	T	harpooned-NMML	1967	CA
39068	NMML0086	tooth	0.19	11		live capture-NMML***	1970	WA, Whidbey Is., Puget Sound
39069	NMML0087	tooth	0.14	—* [18]	O	harpooned-NMML	1966	CA, Morro Bay
39070	NMML0088	tooth	0.23	—* [3,260]	SR	live capture-NMML***	1967	WA, Yukon Harbor, Puget Sound
39071	NMML0089	tooth	0.18	—* [—]	SR	live capture-NMML***	1967	WA, Yukon Harbor, Puget Sound
39075	MVZ-129686	a = tooth, b = muscle	.19 .18	107,800,201	T	harpooned-MVZ	1962	CA, San Francisco CO., 113 km W San Francisco
39076	MVZ-134462	muscle (dry)	0.08	391,600	T	MVZ	1966	CA, Sonoma CO., Goat Rk., mouth of Russian River
39077	MVZ-184169	vertebrae	0.24	38* [4]	O	MVZ	1964	CA, 24 km W San Miguel Is.

*Samples that were re-extracted and re-sequenced from the repeated extraction as well.
**Haplotype based on one of the two diagnostic sites for the transient haplotype.
***Identified by Hoyt (1981) as from K. Pod.

Table 2. Diagnostic sites for eastern North Pacific killer whale sequences. The numbers refer to the variable sites in Figure 2.

Variable site	Offshore	Transient	Southern resident	Northern resident
1	C	T	C	C
2	C	C	C	T
3	T	C	T	T
4	C	T	T	T

haplotypes were from samples collected in Washington, and transient and offshore haplotypes were found in all regions. The one exception was a single northern resident haplotype found from a sample collected somewhere off the coast of California (Table 1). This haplotype was originally identified from the northern resident population found in British Columbia and from Alaska resident killer whales, and has only been found in these populations (Barrett-Lennard 2000, Hoelzel *et al*. 2002). Whales from the northern resident population have been found as far south as Oregon.[3] This sample represents the southernmost sample to date of that population, if it is indeed from California as museum records indicate (it was noted as donated by C. M. Scammon, who whaled along the West Coast in the mid-1800s and reported it to be from California).

Discussion and Conclusions

In California, 12 of the samples had a transient haplotype, 8 of the samples had an offshore haplotype, and 1 had a northern resident haplotype. No samples were obtained from Oregon. All sequenced samples from Washington were of the southern resident haplotype. Most were from inland waters, so this was not a surprising result. However, one sample from the 1800s from the outer coast was of the southern resident haplotype, suggesting the range of southern residents extended to the outer coast of Washington in the 1800s. This is consistent with the current range of southern residents, which have been seen along the outer coast of Washington in fall and winter (Krahn *et al*. 2004). Although it is possible that these stranded animals represent the potentially confounding haplotypes from the ETP or Russia, it is much more likely that they are from the southern resident population known to inhabit these regions.

The northern resident haplotype is interesting. As noted, individuals from the contemporary northern resident population have not been seen south of Oregon, and this haplotype has only been found in the ENP, not the ETP. Northern residents, like southern residents, are thought to be salmon specialists, particularly Chinook salmon (Ford *et al*. 1998), raising the possibility that it was this lineage, rather than that of the southern residents, that frequented these southern salmon runs. Unfortunately, the locality data of this killer whale specimen is too scant to give a more precise location. The large number of offshore samples is interesting, as well, given that some of the samples are from stranded animals. However, offshore type killer whales are occasionally found close to shore and were termed "offshore" type whales only in the sense that they were not found in inland waters of British Columbia and Washington, in contrast to transients and residents (Ford *et al*. 2000).

[3] Personal communication from Jay Barlow, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037, February 2005.

Several of the samples used in this study were associated with other data of interest for killer whale biology and systematics. Three of the samples (IDs 34079–34081, Table 1) were collected by the whaling captain and amateur cetologist Charles M. Scammon during the 1800s (Scammon 1874). These samples have little additional information, but are of historical interest.

Six whales that were genetically identified had stomach content data available as well. The most interesting result was one harpooned whale identified as an offshore (39062) . It was found to have six shark flippers and two teleost skulls in its stomach (Rice 1968). The specimen notes state "The two fish identified as Opah, *Lampris regius,* by Caldwell (in litt., 2 April 1964). Pectoral and pelvic fins of a carchanid shark, possibly blue (*Prionace glauca*) or white-tipped (*Pteralamiops {Charcharhinus} longimanus*) because of long fins" (National Marine Mammal Laboratory (NMML), unpublished data). Another five whales identified as transients had stomach contents. One contained a sea lion, one contained a sea lion and elephant seal remains, one contained California sea lion, elephant seal, and unidentified dolphin remains, one contained a "black and white porpoise," presumably Dall's porpoise, and the fifth contained unidentified baleen and blubber, speculated to be from a minke whale (NMML, unpublished data). All these stomach contents are consistent with what has previously been inferred for diets of each ecotype from stomach content, behavioral, fatty acid, stable isotope, and organochlorine analyses (Ford *et al*. 1998, Herman *et al*. 2005), though there are still uncertainties about the levels of dietary specialization among ecotypes, especially for the little known offshore ecotype.

These data from historical samples, combined with museum and National Marine Fisheries Service (NMFS) records from the original sample collections, provide additional insight into the historical distributions of killer whale ecotypes and their diets. These data provide no evidence to support the hypothesis that the southern resident killer whale ecotype was formerly distributed south of Washington State along the Oregon and California coasts. Only six samples (37271, 39060, 39064, 39069, 39075, 39076) from northern California, in the hypothesized historical range of southern residents, were available, so given the small sample size and the presence of other killer whale ecotypes known to live at least part of the year off of the western U.S. coast, these data do not exclude the possibility that southern residents (or northern residents) also once included these areas in their normal range. Further sampling of museum samples of whales from this region might help to resolve the question with more certainty, and further define the historical ranges of each of the ENP killer whale ecotypes and populations.

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