Low Genetic Variation Among Killer Whales (*Orcinus orca*) in the Eastern North Pacific and Genetic Differentiation Between Foraging Specialists

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Killer whales from the coastal waters off California through Alaska were compared for genetic variation at three nuclear DNA markers and sequenced for a total of 520 bp from the mitochondrial control region. Two putative sympatric populations that range throughout this region were compared. They can be distinguished by social and foraging behavior and are known as "residents" and "transients." We found low levels of variation within populations compared to other cetacean species. Comparisons between fish (resident) versus marine mammal (transient) foraging specialists indicated highly significant genetic differentiation at both nuclear and mitochondrial loci. This differentiation is at a level consistent with intraspecific variation. A comparison between two parapatric resident populations showed a small but fixed mtDNA haplotype difference. Together these data suggest low levels of genetic dispersal between foraging specialists and a pattern of genetic differentiation consistent with matrifocal population structure and small effective population size.

Over 1200 killer whales in the eastern North Pacific have been individually identified using photographs of distinguishing congenital and acquired markings (Bigg et al. 1987, 1990; Black et al. 1993; Dahlheim, in press; Dahlheim et al. 1996; Ford et al. 1994; Heise et al. 1991). Long-term studies of killer whale movements and associations in Washington state, British Columbia, and Alaska have indicated the existence of two behavioral strategists, referred to as "residents" and "transients" (Bigg 1982). This distinction was originally based on sighting frequency and the extent of the known range of pods in each category. But there are now known to be a host of characteristics that distinguish pods (social groups) of these two types, including differences in seasonal distribution, predictability of travel routes, pod size, stability and structure, acoustic behavior, respiration behavior, and feeding behavior (Baird and Dill 1995; Bigg 1982; Bigg et al. 1987, 1990). The terms resident and transient have persisted in the literature, and for consistency will be used here. Although sympatric, pods of transients and residents have not been known to travel together. This is based on extensive observational data spanning more than 20 years (see Bigg et al. 1990).

Resident pods vary in size from approximately 10 to 50 whales and are stable over time (Bigg 1982; Bigg et al. 1987, 1990; Ford et al. 1994). They are sighted predictably during the spring through autumn in inland waters from Washington State through Alaska, and in some cases coincide with the principle salmon runs (Heimlich-Boran 1986). Resightings of a given resident pod are typically within a defined range. For example, from about April through October, several pods range from southern British Columbia to southern Puget Sound (see Figure 1), and are seen most often in the waters near the Gulf and San Juan Islands, moving back and forth along predictable routes (Bigg et al. 1987; Olesiuk et al. 1990). Pod composition includes adult females, their offspring, and adult males, some of which are known to be the offspring of females in the pod (see Bigg et al. 1990). In British Columbian and Washington State waters, where resident pods have been studied since the early 1970s, there has been no evidence of dispersal or recruitment by immigration (Bigg et al. 1990; Olesiuk et al. 1990). Predation of fish is often observed, and stranded whales known to be from resident populations were found to have primarily or exclusively fish in their stomachs (Bigg et al. 1990; Ford et al. 1995; Olesiuk et al. 1990). Possible attacks on marine mammals by resident pods are seen very rarely.

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Transient pods are thought to range over at least twice the area of resident pods (though precise data are not available), are seen infrequently in a given area, and are found in smaller (1–15 whales), less stable pods (Baird and Dill 1995; Bigg 1982; Bigg et al. 1987). Pod composition can include adult males and females with calves, just females and calves, just adult males and females, just adult females, or solitary males (Bigg et al. 1987). Whales from these pods are often seen preying on marine mammals, and several stranded transient whales from Washington State and British Columbia were found to have primarily marine mammal parts in their stomachs (Baird et al. 1992; Ford et al. 1995; Morton 1990). Morphological distinctions between transients and residents, including fin shape and pigmentation pattern have been suggested (Baird and Stacey 1988), and a study comparing two transient with five resident killer whales from Washington State and British Columbia indicated a significant level of genetic differentiation at mitochondrial and nuclear loci (Hoelzel and Dover 1991).

Beyond a distinction between transients and residents, there are putative geographic populations. In Puget Sound (Washington) and off Vancouver Island, British Columbia, there are two communities of resident pods that have not been observed to associate with each other over the duration of a 20 year study (Bigg et al. 1990). Within this geographic area, "southern" residents range from lower Puget Sound to the Campbell River estuary (British Columbia), while the "northern" residents range from the Campbell River estuary to southeast Alaska. The range of the Alaskan populations has not been studied as long, but there are at least five putative resident populations—southeast Alaska, Prince William Sound, Kodiak Island, the Aleutians, and in the Bering Sea.

Our long-term goal is to establish the pattern of genetic differentiation within and between these contiguous putative populations from California through to the Bering Sea (and in comparison with populations from other parts of the world). For the purpose of the current study we have assessed the combined level of variation among killer whales across much of this geographic range and have further established the genetic distinction between the transient and resident types.

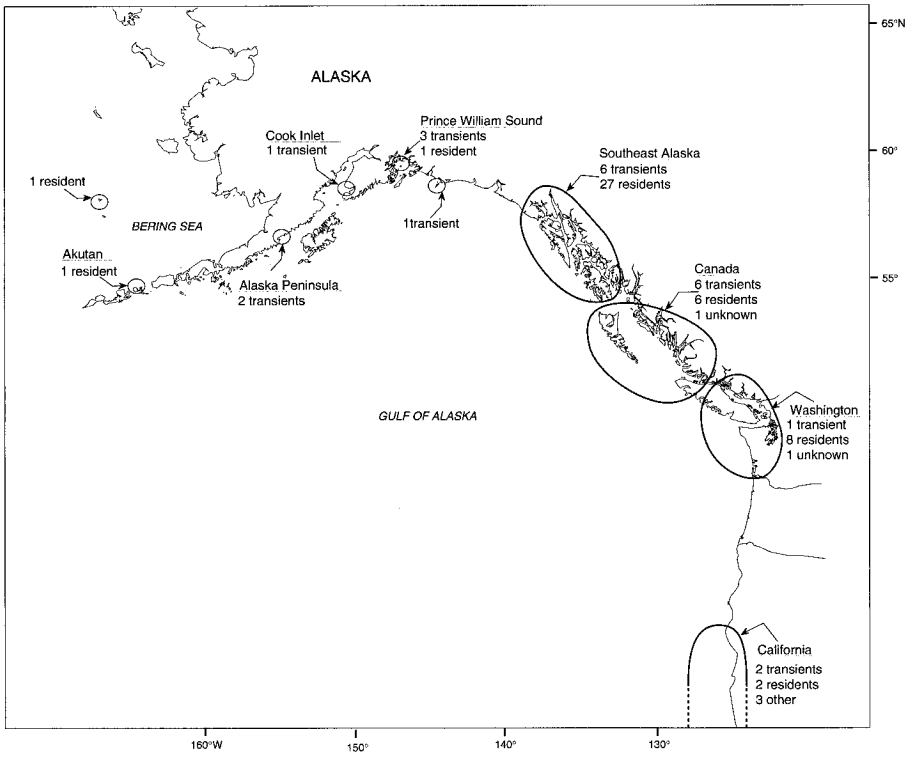

Figure 1. Geographic locations of killer whale samples collected for genetic analysis (total = 73). Three of the samples from north and west of southeast Alaska were classified resident or transient based only on genotype, as were five samples from Canada, five from Washington State, and three from California. Further data on either association behavior, stomach contents, or both were available for all other samples. Three samples from California classified as "other" had a distinct genotype from either residents or transients.

Methods

Sample Collection and DNA Extraction

Samples were collected from stranded (skin and teeth), captive (blood), and free-ranging whales. Free-ranging animals were sampled using a biopsy dart (under NMFS, MMPA permit no. 925). Whales were approached to within a range of 10–30 m on a course that approximated the whale's course and speed. Identification photographs were collected prior to darting. The dart tip is cylindrical, 6 mm in diameter by 2 cm long, attached to a hollow dart, and fired from an air rifle. The sample is retained on two hooks at the center of the dart tip and preserved in the field in a salt/DMSO solution (Amos and Hoelzel 1991; Hoelzel and Dover 1988). A total of 73 samples were available for analysis. Of these, 50 were voucher specimens (classified as either transient or resident types based on photo identification and known associations, or for stranded specimens, on stomach contents). For population comparisons, one of each pair of known first-order relatives (mother/calf pairs collected for paternity testing) was omitted from the analysis. Of the 73 whales sampled, 39 could be assigned to a specific pod and the rest were from stranded whales. These 39 samples represent 3 resident and 3 transient pods in southeast Alaska, 2 resident and 1 transient pod in British Columbia, and 2 resident pods from Washington State. Stranded samples were collected over a number of years and over a broad geographic range. This combined sample set should provide a broad representation of putative populations. Sampling, capture, and stranding locations are given in Figure 1. The condition of samples was variable, and although all samples provided some data, not all could be genotyped for all markers. Teeth samples were screened for mtDNA markers only. For the two mtDNA markers (see below), the 5′ region was haplotyped for 71 samples and the central region for 69 samples. Among the microsatellite markers (see below) locus *BA417* was genotyped for 54 samples, *KWM12a* for 58, and *KWM2a* for 60.

DNA was extracted from blood and skin samples by standard methods (see Hoelzel 1992). Teeth were extracted as follows (after DeGusta D, personal communication). A ¼ in. drill bit was used to remove the outer layers of enamel, followed by a smaller bit to remove 50–100 mg of powdered dentin. The dentin was incubated at room temperature for 48 h in 0.5 M EDTA. The samples were then spun down, resuspended in a digestion solution (0.5 M EDTA, 0.1% sodium sarcoseal, 100 μg/ml

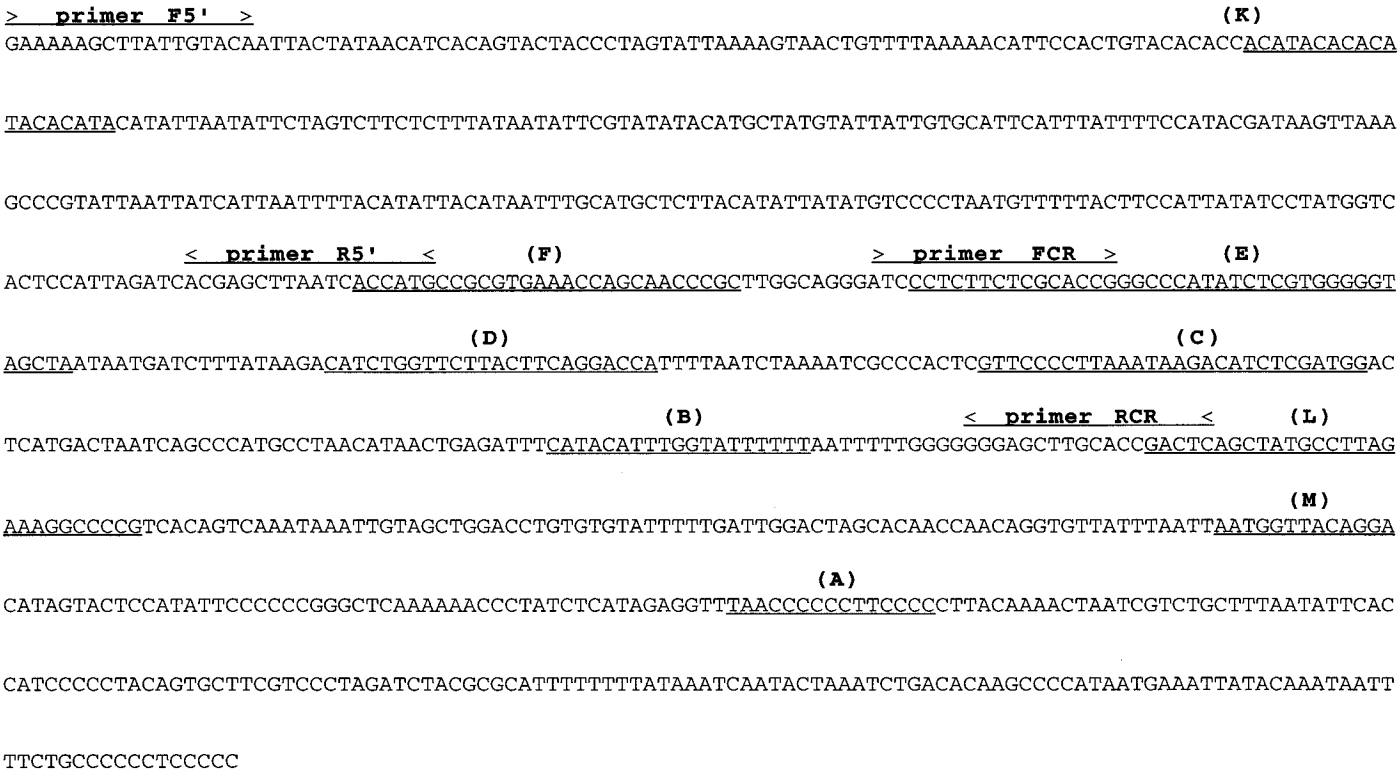

Figure 2. Primer sequence and position in the control region sequence from a resident killer whale (after Hoelzel and Dover 1991). The position of four primers is indicated above the sequence; the 5′ region forward and reverse primers (F5′ and R5′) and the central region forward and reverse primers (FCR and RCR). Lettered, underscored sequences indicate the standard conserved blocks in mammalian mtDNA control regions as defined by Anderson et al. (1982).

protienase K), and incubated at 37°C for 24 h. Samples were then extracted with phenol/chloroform three times and once with butanol.

Microsatellite Analysis

A DNA library was derived in the phagemid, bluescript sk+ (Stratagene). Killer whale DNA was digested with *Hae*III and *Alu*I and size-selected fragments (200–400 bp) were cloned into phosphotased blunt vector. Colonies were screened with a ^{32}P-α-dATP labeled 1 kb $(AC)_n$ fragment. Positive colonies were picked and harvested by standard methods (Perbal 1988) and sequenced by the automated dye-primer method of ABI. Sequences were analyzed for suitable primer sites using the program, Oligo (Rychlik and Rhoads 1989). The following two loci were selected for screening based on quality of amplification product and polymorphism: *KWM2a*—forward primer: 5′-GCTGTGAAAATTAAATGT, reverse primer: 5′-CACTGTGGACAAATGTAA; and *KWM12a*—forward primer: 5′-CCATACAATCCAGCAGTC, reverse primer: 5′-CACTGCAGAATGATGACC. One additional locus based on published primer sequences (*BA417*—forward primer: 5′-TACAGTATTTGTCTTTCTCT, reverse primer: 5′-ATCTGTTTGTCACATATCAT, after Amos et al. 1993) was also screened.

Amplified DNA was analyzed for length variation on a 6% polyacrylamide denaturing gel following incorporation of ^{33}P-α-dATP (PCR reaction conditions: 100 μM dCTP, dTTP, and dGTP, 5 μM dATP, 1.5 mM MgCl, 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 250 pM of each primer, and 0.1 μCi of ^{33}P-α-dATP). The PCR product was denatured at 95°C for 5 min and chilled on ice for 1 min prior to loading.

SSCP Analysis and DNA Sequencing

Two mitochondrial DNA genetic markers were analyzed for single-strand conformational polymorphisms (SSCP; Orita et al. 1989). Primers were designed to amplify from two segments of the control region known to be variable in these populations of killer whales: 300 bp from the 5' region and 220 bp from the central conserved region (see Hoelzel and Dover 1991). Primer sequences and positions relative to the entire killer whale mtDNA control region sequence (from a resident whale from the eastern North Pacific, after Hoelzel and Dover 1991) are shown in Figure 2. The 5′ region is amplified by primers F5′ and R5′, and the central region is amplified by primers FCR and RCR (see Figure 2).

The PCR product was labeled by incorporation of ^{33}P-α-dATP, as described above for microsatellite analysis. Denatured product was then run on a nondenaturing polyacrylamide gel (37.5:1 acrylamide/bis, 4.5%, 10% glycerol run at room temperature).

Unique SSCP bands were sequenced, including a minimum of three individuals to confirm that unique band mobilities represent unique sequences and that different individuals with the same SSCP genotype also had identical sequences. The PCR product was sequenced directly using primers tailed with the universal primer sequences and the ABI dye-primer method or after cloning into the phagemid bluescript sk+.

Population and Phylogenetic Analysis

Allele frequency differences at microsatellite loci were investigated using chi-square comparisons (or the *G* test for small samples in 2 × 2 contingency tests). Analysis of R_{ST} for microsatellite data (Slatkin 1995) was used to assess population differentiation based on allele frequencies using the MICROSAT computer program (Minch et al. 1995). This measure is designed specifically for highly variable markers evolving by a stepwise mutation mechanism, such as microsatellites. For mitochondrial DNA loci, differentiation was assessed using the ϕ_{ST} statistic from the AMOVA computer program (Excoffier et al. 1992). Both ge-

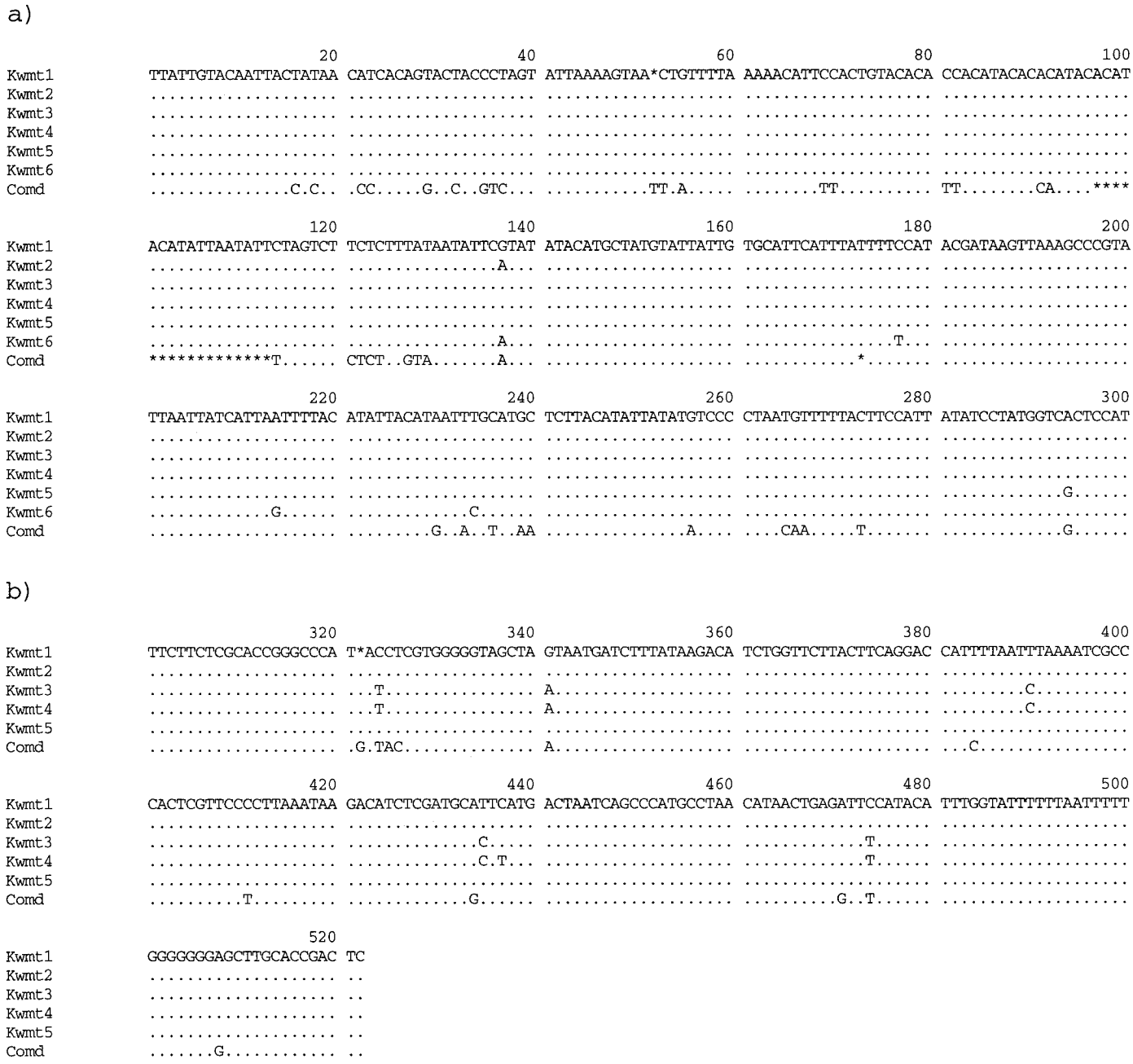

Figure 3. Alignment of mtDNA sequences from the 5′ region **(a)** and the central region **(b)** for five (in b) or six (in a) killer whale haplotypes and the Commerson's dolphin (*Cephalorhynchus commersonii*—Comd) as an outgroup.

netic distance data (percent nucleotide difference) and haplotype frequency were incorporated into the calculation of the mtDNA ϕ_{ST} statistic.

Sequences were compared phylogenetically using the PAUP algorithm for maximum parsimony. Nodes were supported using a bootstrap analysis with 500 replications and a 50% criterion for the retention of nodes. All changes between killer whale sequences were transitions, therefore no transition/transversion weighting was used.

Results

MtDNA Phylogeny

An alignment of the five haplotypes identified from sequencing a combined total of 520 bp (300 bp from the 5′ region and 220 bp from the conserved central region of the mtDNA control region; see Figure 2) is shown in Figure 3. In addition, the sequence for the 5′ region of a sample from California (kwmt6) is aligned with the other haplotypes in Figure 3a. Table 1 shows the frequency of the five complete haplotypes, grouped according to both behavioral strategy and geographic origin. The phylogenetic analysis (Figure 4) distinguishes clearly between two clades. Killer whale haplotypes are compared with an outgroup [Commerson's dolphin (*Cephalorhynchus commersonii*)] using the PAUP maximum parsimony algorithm. All voucher transient samples fall within the clade defined by haplotypes kwmt1, kwmt2, and kwmt5. All voucher resident samples fall within the clade defined by kwmt3 and kwmt4. Both clades are represented in all

Table 1. Haplotype frequency by behavioral category and by location for samples sequenced for two segments of the mtDNA control region

	Haplotype				
	kwmt1	kwmt2	kwmt3	kwmt4	kwmt5
Behavioral and regional grouping					
Alaskan residents	0	0	25	0	0
Northern residents	0	0	4	0	0
Southern residents	0	0	0	5	0
Transients	3	6	0	0	0
Offshores	0	0	0	3	0
Unknown	3	7	5	4	1
Geographic origin					
Alaska	3	8	28	2	0
Canada	0	5	4	2	1
Washington	1	0	0	8	0
California	2	0	2	0	0
Total	6	13	34	12	1

geographic regions (Table 1). The two resident haplotypes differ by only 1 bp, and each is unique to either the Puget Sound, Washington (southern) or the British Columbian and Alaskan (northern) resident populations. The transient central region haplotype (Figure 3b) is 5 or 6 bp different from the resident types, while at the 5′ region (Figure 3a) there are only two haplotypes among the voucher specimens, differing by 1 bp, and unique to either the transient or the resident population.

Three whales thought to be from a population known as "offshore" (see discussion) fall in with the resident clade, and specifically with the southern resident population. These samples were collected from two stranded whales at Barne's Lake in southeast Alaska, and from one free-ranging whale approximately 300 miles west of the Columbia River (at the Oregon/Washington border). Seven whales stranded in California (from San Francisco south to the Channel Islands) were included in the SSCP analysis. Two of these had the same haplotype as the transients (kwmt2), two were the same as the northern residents (kwmt3), and three showed a unique haplotype. The unique haplotype (kwmt6) was sequenced for the 5′ locus and was 4 bp (1.3%) divergent from the transient haplotype, and 5 bp (1.7%) divergent from the resident haplotype (Figure 3a). These individuals were not sequenced for the central region locus, but showed the same SSCP pattern as for kwmt2 (within the transient clade).

Population Differentiation

In addition to comparisons restricted to voucher samples, we used classification by mtDNA haplotype to group individuals of unknown origin (see Table 1) as either resident or transient mtDNA genotypes for further comparisons using nuclear DNA markers (Tables 2 and 3). At locus *BA417* there were four alleles, one of which (A) was unique to the transient samples (Table 2). Chi-square comparisons indicate a significant difference between allele frequencies in the two putative populations (voucher samples: $\chi^2 = 28.8$, $P < .0001$, df = 3; all samples: $\chi^2 = 20.0$, $P = .0002$, df = 3). At the *KWM12a* locus there are seven alleles, two of which (A and B) were unique to transients, and one of which (F) was unique to residents (Table 2). The distribution of allele frequencies was significantly different in the two populations (voucher samples: $\chi^2 = 31.4$, $P < .0001$, df = 3; all samples: $\chi^2 = 45.1$, $P < .0001$, df = 6). At locus *KWM2a* there were also seven alleles, two of which (D and F) were unique to the transients and one of which (G) was unique to the residents (Table 2). Again the difference in allele frequencies was highly significant (voucher samples: $\chi^2 = 53.4$, $P < .0001$, df = 3; all samples: $\chi^2 = 60.1$, $P < .0001$). In these comparisons there is a possible sampling bias (the extent of which is not known due to the use of samples of unknown origin), since there are 24 individuals from just two Alaskan pods in the resident sample, and whales within a pod may be closely related. If we partially correct for this by including only a single representative from each of these two pods, the same pattern is evident (Table 3), and allele frequencies at each locus are still significantly different for the two populations (voucher samples: *BA417*: $\chi^2 = 11.4$, $P = .011$, df = 3; *KWM12a*: $\chi^2 = 12.1$, $P = .05$, df = 6; *KWM2a*: $\chi^2 = 23.9$, $P < .0005$, df = 6; all samples: *BA417*: $\chi^2 = 9.6$, $P = .022$, df = 3; *KWM12a*: $\chi^2 = 23.1$, $P = .0008$, df = 6; *KWM2a*: $\chi^2 = 35.1$, $P < .0001$, df = 6).

An analysis of intra- versus interpopulation variation for the three microsatellite loci (considering all three loci together) indicated that 47.7% (voucher samples only) or 33.5% (all samples) of the variation can be accounted for by differences between resident versus transient populations (voucher samples: for *BA417,* $R_{ST} = 0.521$; for *KWM12a,* $R_{ST} = 0.235$; and for *KWM2a,* $R_{ST} = 0.647$; all samples: for *BA417,* $R_{ST} = 0.220$; for *KWM12a,* $R_{ST} = 0.346$; and for *KWM2a,* $R_{ST} = 0.462$). Using the formulations described by Slatkin (1995), the level of genetic migration (N_m) can be estimated. For example, for an R_{ST} of 0.40, there would be an estimated 0.19 migrants per generation.

A similar comparison using the combined mtDNA markers indicated an even clearer distinction, where 91.9% of the variation is accounted for by variation between transients and residents ($\phi_{ST} = 0.919$). In this case the estimated N_m is much lower at 0.044, and reflects only the movement of females (estimated as $N_m = (1/\phi_{ST} - 1)/2$). Further, all of the mtDNA variation within the resident population could be accounted for by a 1 bp distinction between whales from Alaska and Brit-

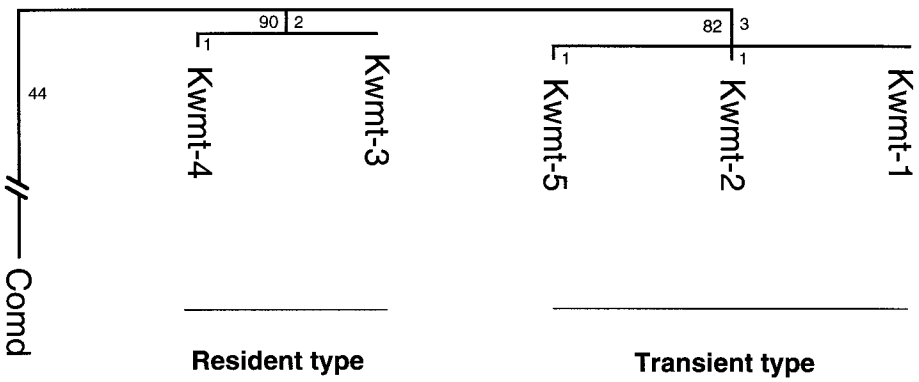

Figure 4. Maximum parsimony tree of five killer whale mtDNA haplotypes and Commerson's dolphin outgroup. Branch length is given to the right and bootstrap support to the left of the branches. Each clade is identified as "transient type" (including haplotypes for all transient voucher samples) or "resident type" (including haplotypes for all resident voucher samples).

Table 2. Allele frequency (%) at three microsatellite loci comparing residents and transients (see text)

		Allele								
	N	A	B	C	D	E	F	G	H_{exp}	H_{obs}
Locus *BA417*										
Transient	16	9.4	28.1	53.1	9.4	—	—	—	62.1	68.7(11.5)
	[8]	[12.5]	[37.5]	[50.0]	[0]				[59.4]	[50.0(17.6)]
Resident	35	0	4.3	82.9	12.8	—	—	—	29.5	34.3(8.0)
	[30]	[0]	[1.7]	[91.7]	[6.7]				[15.4]	[16.7(6.8)]
Locus KWM12a										
Transient	17	5.9	29.4	23.5	23.5	14.7	0	3.0	77.7	82.4(8.5)
	[9]	[5.6]	[22.2]	[22.2]	[27.8]	[16.7]	[0]	[5.6]	[79.0]	[100(0.0)]
Resident	39	0	0	7.7	21.8	61.5	5.1	3.8	56.4	48.7(8.0)
	[34]	[0]	[0]	[4.4]	[22.1]	[67.6]	[1.5]	[4.4]	[49.0]	[50.0(8.6)]
Locus *KWM2a*										
Transient	17	8.8	29.4	8.8	26.5	14.7	11.8	0	79.2	76.5(10.3)
	[10]	[5.0]	[35.0]	[10.0]	[25.0]	[20.0]	[5.0]	[0]	[76.0]	[80.0(12.6)]
Resident	40	2.5	8.8	3.8	0	51.3	0	33.8	61.3	52.5(7.9)
	[35]	[1.4]	[2.8]	[1.4]	[0]	[52.9]	[0]	[41.4]	[54.8]	[48.6(8.4)]

The standard error of heterozygosity is given parenthetically. Data for voucher samples only are in brackets.

ish Columbia versus whales from Washington State.

Polymorphism

Contingency tests indicated no significant difference between observed heterozygosity at each killer whale microsatellite locus and that expected based on predictions from the Hardy–Weinberg rule (Tables 2 and 3). The level of heterozygosity in the resident population was significantly lower than for the transient population at two of three loci (voucher samples: *BA417*: $G = 3.9$, $P = .05$, df = 1; *KWM12a*: $G = 7.4$, $P = .006$, df = 1; *KWM2a*: $G = 3.1$, $P = .08$, df = 1; all samples: *BA417*: $G = 5.3$, $P = .02$, df = 1; *KWM12a*: $G = 5.9$, $P = .019$, df = 1; *KWM2a*: $G = 3.0$, $P = .092$, df = 1). When all but one whale from each of two over-represented pods are removed from the analysis, this effect is no longer seen for the two loci that had shown a significant difference (*BA417* and *KWM12a*), but it becomes significant at locus *KWM2a* (voucher samples: $G = 5.5$, $P = .02$, df = 1; all samples: $G = 6.6$, $P = .012$, df = 1).

In comparison with many other odontocete cetaceans (see review in Hoelzel 1994), the level of mtDNA variation in each killer whale population is very low, with 92% of samples from Washington State through Alaska represented by just three haplotypes, and these are each population specific. There is no indication from the mtDNA data that there is greater variation in the transient population than in the resident population.

Paternity Testing

Samples were collected from two mother/calf pairs in the southeast Alaska pod (designated AG; Dahlheim et al. 1996), from all three adult males within AG, and from three of the four males in AF. AF is the pod with which AG animals associate most frequently (Dahlheim et al. 1996). One additional adult male from AG was present when one of the two calves was conceived. Using the microsatellite genotypes and the allele frequencies given for resident populations in Table 2, we could exclude all but one male (AG2) as the potential father of either calf. The paternal alleles were identified as C, D, and G for loci *BA417, KWM12a,* and *KWM2a,* respectively, for each calf. Based on allele frequencies among presumably unrelated individuals, and using all three microsatellite loci, the probability of misidentification of AG2 as the father was calculated to be 0.061 for either calf (using the multiplicative rule; see Bruford et al. 1992). This suggests that 1 in 16 males could have the matching genotype by chance.

Table 3. Modified resident allele frequencies (%), using only comparisons between pods

		Allele								
Locus	*N*	A	B	C	D	E	F	G	H_{exp}	H_{obs}
BA417	17	0	8.8	64.7	26.5	—	—	—	50.3	70.6(11.0)
	[10]	[0]	[5.0]	[75.0]	[20.0]	—	—	—	[39.5]	[50.0(15.8)]
KWM12a	19	0	0	15.8	34.2	42.1	7.9	0	67.5	68.4(10.7)
	[12]	[0]	[0]	[12.5]	[37.5]	[50.0]	[0]	[0]	[59.4]	[75.0(12.5)]
KWM2a	20	0	17.5	7.5	0	52.5	0	22.5	63.8	35.0(10.7)
	[13]	[0]	[7.7]	[3.9]	[0]	[61.5]	[0]	[26.9]	[54.2]	[30.8(12.8)]

The standard error of heterozygosity is given parenthetically. Data for voucher samples only are in brackets.

Discussion

Hoelzel and Dover (1991) reported that there was as much mtDNA genetic differentiation between killer whales from sympatric resident and transient populations in Puget Sound and off Vancouver Island as between killer whales in different oceans, and that there was limited within-population variation for resident killer whales at nuclear (minisatellite) loci. We now confirm these findings using a much larger sample set and extending the geographic range of the sample in the eastern North Pacific. Mitochondrial DNA haplotypes were nearly fixed in each of three putative populations, which had been proposed based on observational studies (transients, southern residents, and northern residents; see Bigg et al. 1990). The greatest genetic distance was between transients and residents. Alaskan and northern resident (in British Columbia) populations were found to share the same haplotype, while whales captured or stranded in Puget Sound (southern residents) had a fixed 1 bp (0.19%) difference from the resident populations to the north. The latter haplotype was shared with three whales thought to be from a population that has only been encountered in recent years (referred to as the "offshore" population; Ford et al. 1994). These whales travel in pods of 30–60 whales and have been encountered near the Queen Charlotte Islands, off the west coast of Vancouver Island, off the coast of California, and in southeast Alaska (Ford et al. 1994, personal communication). Microsatellite DNA polymorphism at three loci also showed significant genetic differentiation between resident and transient whales identified from field observation, stomach contents (fish versus marine mammal remains), and mtDNA genotype. Variation at these loci was very low within resident pods; lower within resident than within transient populations.

This pattern of little variation within local populations and higher levels between populations is suggestive of a history of founding events. Killer whales form strong social bonds, and it has been suggested that whales within the resident community in the eastern North Pacific remain within their natal pod for life, given that there is no evidence of either male or female dispersal (Bigg et al. 1987, 1990; Olesiuk et al. 1990). These data are based on

expected rates of recruitment and mortality (Olesiuk et al. 1990), as well as observational data of individually recognized whales for over 20 years (Bigg et al. 1990). New pods form by fission when existing pods become large. A gradual division has been seen in several of the pods studied in British Columbian waters (Bigg et al. 1990). The observed pattern of mtDNA genetic differentiation could be explained by such a sequence of founding events and subsequent population growth and pod division. A more thorough analysis of geographic differentiation on a finer scale will be presented when more samples are available, but the lack of mtDNA differences between different pods within regional communities of pods (and for a given behavioral type) is consistent with this model of pod fission and strong matrifocal social structure. Given such a strategy for the colonization of local geographic areas, inbreeding avoidance would require outcrossing outside of the community of pods with shared ancestry, as different pods within a community would be derived from recent divisions along the same matriline. To the extent that this is true, mating between pods within the community may not constitute significantly greater outcrossing than mating within pods. The preliminary paternity testing data we present here does not exclude the possibility of mating within the pod.

Observational data suggest that both males and females are philopatric. If males dispersed and females were philopatric, population genetic structure would be expected at mtDNA markers, but not necessarily at nuclear markers. A comparison of transient versus resident populations showed a total of seven unique alleles at three microsatellite loci and significant allele frequency differences, implying limited genetic dispersal between these populations for either males or females. An estimate of the rate of genetic migration based on R_{ST} also indicates a low rate of exchange, though in comparison with the mtDNA data it may suggest greater dispersal of males than females.

Mitochondrial DNA analysis of samples from Washington state through the Bering Sea showed a consistent sequence difference of about 1% between all known transient and all known resident samples. Further, all unknown samples from this geographic range fell clearly into one of these two clades (Figure 4). A ϕ_{ST} statistic indicates that 91.1% of the mtDNA variation can be explained by differences between resident and transient populations. Together with the microsatellite markers, these data indicate a clear genetic differentiation between pods of the resident and transient types. The limited genetic variation for both nuclear and mtDNA within the putative resident and transient populations (shown here and in Hoelzel and Dover 1991) also suggests that mating occurs primarily within these populations.

The samples from each putative population are representative in that they are from a broad geographic range and from a number of different pods. For example, there is no observational data (based on thousands of hours) to indicate association between resident pods in southeast Alaska and off Washington State (Bigg et al. 1990; Dahlheim et al. 1996), but they differ by only 1 bp out of 520 bp for the mtDNA control region sequence. Similarly, whales from transient pods that have not been seen in association and were sampled in geographically distant locations have the same or very similar haplotypes. Further, when we remove individuals that were sampled from the same pod from the analysis, there is still a significant difference in genotype frequency for comparisons between transients and residents.

The genotype of several samples from California matched that of the transients and residents in British Columbia and Alaska, suggesting that both the resident and transient populations may range farther south. Transients are know to range extensively and individuals have been resighted in California and in Alaska (Goley and Straley 1994). Pods from the "offshore" population (which in our sample of three had the same haplotype as southern residents) have been sighted from California to Alaska, and the "resident" genotypes we detected from two California samples could be representatives from that population. We also found three individuals with a highly divergent haplotype for the 5' mtDNA sequence (Figure 3a), indicating that there is another population found off California and not yet known from waters further north. Their genotype was closest to the transient type.

Genetic differentiation between the sympatric resident and transient populations could have evolved by at least three mechanisms. First, differentiation could have accumulated by drift in allopatry, followed by secondary contact. We consider this unlikely because transients and residents each show unique microsatellite alleles and mtDNA haplotypes. Second, the two populations may represent two different species with physiological or behavioral barriers to interbreeding. However, comparisons between geographically distant populations show as much genetic differentiation as between the residents and transients, and there was no genetic concordance between populations that are allopatric but share similar behavioral strategy (Hoelzel and Dover 1991). Therefore we suggest that these are conspecific populations. Third, local differentiation could be driven by behavioral isolation, based on different foraging strategies. We consider the last possibility the most likely.

The behavioral specializations of killer whales have been studied in some detail (Baird and Dill 1995; Bigg et al. 1990; Hoelzel 1991, 1993; Morton 1990). The differences in social behavior and pod size between resident and transient pods were originally reported by Bigg (1982). A study of the foraging behavior and social group dynamics of the southern resident pods found a relationship between the rate of apparent prey capture and the distance between subgroups within the pod (Hoelzel 1993). The implication was that the search for fish prey occurred while subgroups were dispersed, followed by convergence on a concentration of prey. This type of coordinated foraging behavior may be one factor leading to the stability of pods that pursue fish prey. For pods pursuing marine mammal prey (in various locations in the Atlantic and Pacific Oceans), social group size was on average larger than hunting group size, and individual whales were clearly capable of obtaining sufficient prey to sustain themselves on their own (Hoelzel 1991). This difference in resource exploitation between resident and transient pods in the eastern North Pacific could account for many of the observed differences in social structure and behavior. These could in turn lead to limits on gene flow. For example, group size and composition related to differences in foraging strategy could lead to differences in reproductive strategy, which could lead to isolation, reinforced by differences in habitat use (for further discussion see Hoelzel 1991, 1993, 1994).

The results presented here demonstrate a clear genetic distinction between sympatric populations that differ in foraging behavior and support earlier observations about dispersal behavior (Bigg 1982; Bigg et al. 1990) indicating female philopatry and suggesting a male genetic dispersal pattern limited primarily to within local

populations. These data together with other studies reinforce the need for further genetic analysis of cetacean populations to detect possible sympatric and parapatric differentiation and to facilitate the effective conservation of genetic diversity in these species (cf. Hoelzel 1994).

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