Genetic variation and evidence for population structure in eastern North Pacific false killer whales (*Pseudorca crassidens*)

Susan J. Chivers, Robin W. Baird, Daniel J. McSweeney, Daniel L. Webster, Nicole M. Hedrick, and Juan Carlos Salinas

Abstract: False killer whales (*Pseudorca crassidens* (Owen, 1846)) are incidentally taken in the North Pacific pelagic long-line fishery, but little is known about their population structure to assess the impact of these takes. Using mitochondrial DNA (mtDNA) control region sequence data, we quantified genetic variation for the species and tested for genetic differentiation among geographic strata. Our data set of 124 samples included 115 skin-biopsy samples collected from false killer whales inhabiting the eastern North Pacific Ocean (ENP), and nine samples collected from animals sampled at sea or on the beach in the western North Pacific, Indian, and Atlantic oceans. Twenty-four (24) haplotypes were identified, and nucleotide diversity was low (π = 0.37%) but comparable with that of closely related species. Phylogeographic concordance in the distribution of haplotypes was revealed and a demographically isolated population of false killer whales associated with the main Hawaiian islands was identified (Φ_{ST} = 0.47, $p < 0.0001$). This result supports recognition of the existing management unit, which has geo-political boundaries corresponding to the USA's exclusive economic zone (EEZ) of Hawai'i. However, a small number of animals sampled within the EEZ but away from the near-shore island area, which is defined as <25 nautical miles (1 nautical mile = 1.852 km) from shore, had haplotypes that were the same or closely related to those found elsewhere in the ENP, which suggests that there may be a second management unit within the Hawaiian EEZ. Biologically meaningful boundaries for the population(s) cannot be identified until we better understand the distribution and ecology of false killer whales.

Résumé : Les fausses orques (*Pseudorca crassidens* (Owen, 1846)) sont à l'occasion capturées dans les pêches à la palangre dans le Pacifique Nord; on connaît cependant trop peu la structure de la population pour pouvoir évaluer l'impact de ces captures. Des données de séquençage de la région de contrôle de l'ADN mitochondrial (mtDNA) nous ont permis de mesurer la variation génétique chez cette espèce et d'évaluer la différentiation génétique entre les strates géographiques. Nos données comprennent 124 échantillons, dont 115 prélèvements de biopsie de la peau chez des fausses orques de l'est du Pacifique Nord (ENP) et neuf échantillons provenant d'animaux capturés en mer ou sur la plage dans l'ouest du Pacifique Nord, l'Atlantique et l'océan Indien. Il est possible d'identifier 24 haplotypes; la diversité des nucléotides est basse (π = 0,37 %), mais semblable à celle d'espèces fortement apparentées. Il y a une concordance phylogéographique dans la répartition des haplotypes; une population isolée démographiquement de fausses orques est associée avec les îles principales d'Hawai'i (Φ_{ST} = 0,47, $p < 0,0001$). Cette observation vient appuyer la reconnaissance de l'unité de gestion actuelle qui possède des frontières géopolitiques qui correspondent à la zone économique exclusive des É.-U. (EEZ) à Hawai'i. Cependant, un petit nombre d'animaux capturés dans l'EEZ, mais loin de la zone à proximité des îles (celle située à <25 milles nautiques (1 milles nautiques = 1.852 km) des rivages) possèdent des haplotypes identiques ou presque à ceux trouvés ailleurs dans l'ENP; il peut donc y avoir une seconde unité de gestion au sein de l'EEZ d'Hawai'i. Il n'est pas possible de définir des frontières de signification biologique pour la ou les populations tant que la répartition et l'écologie des fausses orques ne seront pas mieux comprises.

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Introduction

Understanding how cetacean populations are structured within their environment is essential for developing effective conservation and management plans. Pelagic cetaceans, like the false killer whale (*Pseudorca crassidens* (Owen, 1846)), often have large nearly continuous distributions with no obvious barriers to movement. Such distributions taken together with a low cost of locomotion for cetaceans (Williams 1999) suggest the potential for little population structure. However, patterns of structure are becoming increasingly evident in coastal, pelagic, and migratory populations of cetaceans as a result of genetic, morphological, and tagging studies. These patterns are likely the result of animal movements established in response to the distribution of preferred habitat, prey availability, and social structure. For example, relatively fine-scale population structure has been documented for coastal harbor porpoise (*Phocoena phocoena* (L., 1758); e.g., Walton 1997; Chivers et al. 2002), pelagic Dalls' porpoise (*Phocoenoides dalli* (True, 1885); e.g., Escorza-Treviño and Dizon 2000) and pantropical spotted dolphin (*Stenella attenuata* (Gray, 1846); Escorza-Treviño et al. 2005), and migratory humpback whales (*Megaptera novaeangliae* (Borowski, 1781); e.g., Palsbøll et al. 1997; Calambokidis et al. 2001). While cetaceans are generally considered vulnerable to exploitation as a result of their late age at maturation and low reproductive rates, population structure likely increases their vulnerability. However, our knowledge about structure for most wide-ranging, pelagic cetacean species, like the false killer whale studied here, is limited because these species are inherently difficult to study.

Information about the population structure of false killer whales is needed to assess the impact of the pelagic long-line fishery on their population(s) within the central Pacific Ocean. Beginning in 1994, biological technicians were placed aboard long-line fishing vessels after a number of protected species, including false killer whales, were documented among the incidental take of the fishery (Nitta and Henderson 1993). Only one stock of false killer whales is recognized for management by the USA within the fishery's operating area. The stock's boundaries are geo-political and correspond to the USA's exclusive economic zone (EEZ) of the Hawaiian archipelago (Carretta et al. 2006). The current abundance estimate for the "Hawaiian" stock is 236 animals (CV = 1.13; Barlow 2006), and the estimated mortality owing to the fishery results in a "strategic" designation for the stock (i.e., more animals are estimated to be incidentally killed in the fishery than the population can sustain and maintain desired population levels; Wade 1998), as defined by the US Marine Mammal Protection Act (MMPA) (Carretta et al. 2006). However, fishing effort by the long-line fleet also occurs outside the Hawaiian stock boundaries. Because the MMPA regulates incidental mortality by US fisheries on the high seas and within US territorial waters, and this long-line fleet operates in both, our investigation considers whether the Hawaiian stock boundaries should be extended to include these waters or whether whales caught in these areas belong to a different stock.

Within the eastern North Pacific Ocean (ENP), our primary study area, the distribution of false killer whales has been well documented and indicates an essentially continuous distribution between approximately 15°S and 40°N latitude and west to 150°W longitude (Fig. 1; Wade and Gerrodette 1993). Additionally, observations collected by the biological technicians aboard long-line fishing vessels show that their distribution extends farther west (NMFS, PIRO[2]). These data are consistent with other information that false killer whales are a largely pelagic species widely distributed in tropical and subtropical waters worldwide (Kasuya 1975; Miyazaki and Wada 1978; Stacey and Baird 1991; Stacey et al. 1994). However, data are insufficient to infer population structure on the basis of distributional patterns.

To further our understanding of population structure in false killer whales and to evaluate the appropriateness of recognizing the Hawaiian stock for management, we used the mitochondrial DNA (mtDNA) control region to quantify genetic variability in false killer whales and to investigate patterns of genetic differentiation. The mtDNA marker has an evolutionary rate that makes it useful for reconstructing phylogenetic relationships, and because it is maternally inherited, for revealing genetic differentiation between demographically independent populations when female dispersal is limited (i.e., negligible movement of breeding females). An objective of the MMPA is to maintain demographically independent populations, which means that the internal population dynamics of the group are essential to its persistence and not influenced by animal movements among neighboring groups. The resolution of the mtDNA marker is consistent with meeting this MMPA objective.

Materials and methods

Samples

The mtDNA control region was sequenced from tissue samples collected from false killer whales biopsied at sea (n = 118, including 5 sampled by observers during long-line fishing operations) or stranded on the beach (n = 6) between 1983 and 2005 (Fig. 1). Within our primary study area, the ENP, multiple individuals were sampled from 15 groups of animals (n = 106). Photographs of individuals sampled within a group, the size of the false killer whale groups sampled (Table 1), and our movements within animal groups during sampling events allowed us to feel confident that the likelihood of sampling individual animals more than once was low. Using available photographs, duplicate samples of three individuals were removed from our data set prior to sequencing. All tissue samples (i.e., skin or muscle) were preserved frozen or in a 20% dimethylsulphoxide solution saturated with NaCl (Amos and Hoelzel 1991; Amos 1997) and archived at SWFSC (for information contact S.J. Chivers).

DNA extraction, PCR amplification, and sequencing

The 5′ end of the hypervariable mtDNA control region was amplified from extracted genomic DNA (lithium chloride protocol: Gemmell and Akiyama 1996; Qiagen DNeasy

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Fig. 1. Collection locations of skin-biopsy samples within our primary study area are shown here together with all recorded sightings of false killer whales (*Pseudorca crassidens*) within the region. The sighting data were collected on aerial and shipboard surveys conducted between 1974 and 2005 (Mobley et al. 2000; Baird et al. 2005; Gerrodette and Forcada 2005; Barlow 2006), as well as by observers working aboard long-line fishing vessels between 1994 and 2004 (NMFS, PIRO[2]). See legend for guide to symbols; all sample collection locations are indicated by a solid triangle or star.

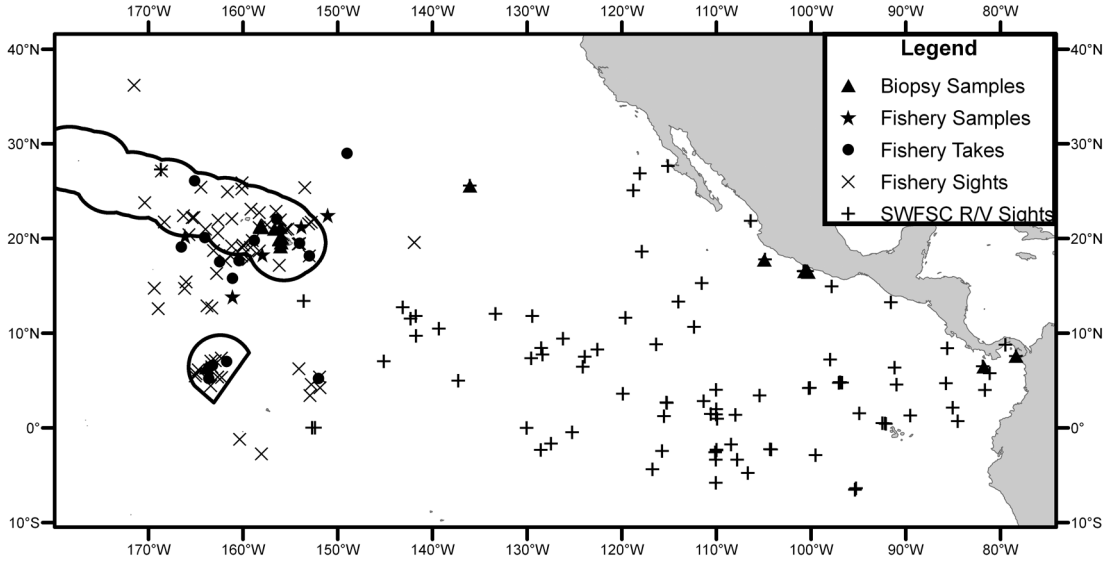


Table 1. Summary of group statistics from which >1 sample was collected.

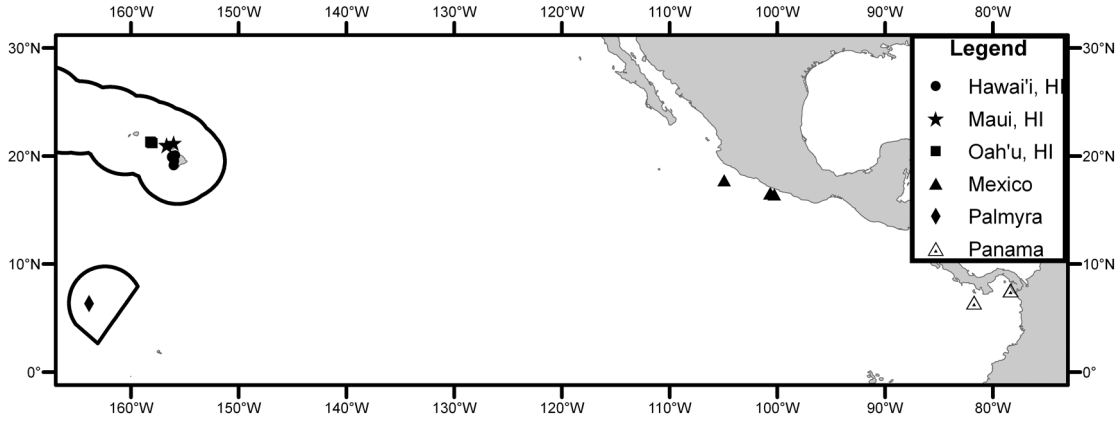
	Group	Mean group size (range)	No. of samples	No. of haplotypes	Sex (females, males, unknown)
Hawaiian islands					
Maui	1	18 (15–24)	4	2	2, 2, 0
Maui	2	14 (11–17)	3	3	2, 1, 0
Maui	3	35 (30–50)	6	2	3, 3, 0
O‘ahu	4	5 (5–5)	4	1	4, 0, 0
Hawai‘i	5	41 (38–50)	7	2	4, 3, 0
O‘ahu	6	35 (30–40)	22	1	11, 11, 0
Hawai‘i	7	41 (38–50)	2	2	1, 1, 0
Hawai‘i	8	30 (12–70)	10	4	6, 3, 1
Palmyra Atoll	1	17 (11–50)	6	4	2, 1, 3
Eastern North Pacific					
Mexico	1	20 (18–29)	5	1	4, 1, 0
Panama	2	26 (21–32)	13	2	5, 8, 0
Panama	3	17 (15–20)	3	1	1, 2, 0
Mexico	4	51 (32–64)	8	2	2, 6, 0
Mexico	5	8 (5–14)	7	2	6, 1, 0
Mexico	6	10 (7–13)	6	2	5, 1, 0

Note: The name of the putative population (see Fig. 2) to which each group belongs is identified under each regional heading.

#69506; Sigma-Aldrich X-tractor Pack 2 cat# 045K6158) using the polymerase chain reaction (PCR) and then sequenced using standard techniques (Saiki et al. 1988; Palumbi et al. 1991). The sequence was generated in two parts. For the first segment, we used primers H16343[3] (5′-CCTGAAGTAAGAACCAGATG-3′) (Rosel et al. 1994) and L15812 (5′-CCTCCCTAAGACTCAAGG-3′) (Chivers et al. 2005); for the second segment, we used primers H516 (5′-AAGGCTAGGACCAAACCT-3′) and L16195 (5′-TGGCCGCTCCATTAGATCACGAGC-3′) (both developed at SWFSC). Both strands of the amplified DNA product for each specimen were sequenced independently as mutual controls using standard four-color fluorescent protocols on the Applied Biosystems Inc. model 377 and model 3100 sequencers (Applied Biosystems Inc., Foster City, Calif.). The second segment of approximately 573 base pairs included an approximately 20 base pair section of overlap with the first 395 base pairs of the control region to ensure all sequences were complete. The final sequences were 945 base pairs long and were aligned using the software pro-

[3] All primer names reference their position in the mtDNA sequence of the fin whale (Árnason et al. 1991).

Fig. 2. Locations of skin-biopsy samples collected and used in the analyses of putative populations of false killer whales within the main Hawaiian islands and eastern tropical Pacific. See legend for name and location of each putative population.



grams SEQED version 1.0.3 and Sequencher version 4.1 (Genecodes; Applied Biosystems Inc. 1992).

Sex determination

Sex was genetically determined by amplifying the zinc finger gene: ZFX and ZFY for the X and Y chromosomes, respectively. The gene was amplified with PCR using primers ZFY0097 and P2-3EZ, and the zinc finger gene (i.e., ZFX/ZFY) was used as an internal control to confirm PCR conditions. PCR products were either separated by electrophoreses or visualized using a real-time PCR detection system (MX3000p; Stratagen Inc., La Jolla, California). Sex was determined by the amplification pattern: males had two products and females had one (Morin et al. 2005).

Data analyses

Genetic diversity

We identified the unique haplotypes in our data set using MacClade version 3.08a (Maddison and Maddison 1992), and quantified genetic variability in terms of haplotypic diversity (h) and nucleotide diversity (π) (Nei and Tajima 1981; Nei 1987) using Arlequin version 2.0 (Schneider et al. 2000).

Genetic differentiation

To examine the concordance between the distribution of mtDNA control region haplotypes and geographic region for evidence of the role evolutionary processes may have played in the patterns revealed, a minimum spanning network was generated using Arlequin version 2.0 (Schneider et al. 2000). Optimal minimum spanning networks incorporate information about haplotype frequency to obtain the most parsimonious network for haplotype evolution. For example, haplotypes that are "rare" or occur at low frequencies would be most likely to have been derived from haplotypes that are "common" or occur in high frequencies rather than from another rare haplotype for a given series of mutation events (Excoffier and Smouse 1994; Excoffier et al. 1992).

We further analyzed our data for ENP samples using conventional analyses for detecting population structure by quantifying genetic differentiation among putative populations. We tested the null hypothesis of panmixia against our a priori data stratification that equated sampling sites to putative populations, and applied the distance- and frequency-based statistics Φ_{ST} and F_{ST}. To calculate Φ_{ST}, we used the number of homologous nucleotide differences between two individuals as the measure of genetic distance. Both statistics tested significance with a nonparametric permutation method in an analysis of molecular variance (AMOVA) framework implemented in Arlequin version 2.0 (Schneider et al. 2000). We analyzed two a priori stratifications of our ENP data. In the first a priori stratification, we recognized two putative populations: ENP and Hawai'i. In the second a priori stratification, we recognized six putative populations for animals sampled off the coasts of Mexico and Panama, within the Palmyra Atoll, and in Hawai'i off the coasts of O'ahu, Maui, and the island of Hawai'i (Fig. 2). Both stratifications were analyzed using all of the available sequence data for each population and using only one haplotype/sex per group sampled. The latter analyses was done because multiple animals were sampled from many of the groups encountered and the relatedness among individual animals in a group is unknown. By using the haplotype and sex data for each animal sampled, we were able to conduct analyses using the minimum number of individuals sampled.

A multiple test correction factor was not applied to the results of our analyses. Two important considerations to be made when applying correction factors to analyses of population structure, in particular, are that they (1) are only appropriate when all the null hypotheses being tested are true simultaneously and (2) effectively reduce the critical value (α), or type I error rate, at the expense of the type II error rate (Perneger 1998). In conservation management applications, reducing the type I error rate means that one is more willing to commit an under-protection error (i.e., incorrectly pooling strata) than an over-protection error (i.e., incorrectly subdividing strata). Because within an analyses each pairwise comparison tested a different hypothesis and the results of our analyses have implications for the management of false killer whales, which may be affected by the acceptance of particular type I and type II error rates (Dizon et al. 1995; Taylor et al. 1997), we did not apply a correction factor to our analyses and interpreted our results with $\alpha = 0.05$.

We used the maximum likelihood coalescence based method implemented in MIGRATE version 2.1.3 (Beerli 1997–2004; Beerli and Felsenstein 1999, 2001) to estimate the dispersal rates between the Hawaiian island and ENP strata. This program estimates dispersal rate as the long-

Table 2. The polymorphic sites (vertical notation) of 24 haplotypes identified for false killer whales (*Pseudorca crassidens*).

Haplotype	12222222 2233333333 4455556779 5600555678 9900002699 7804454193 1687023765 0725679045 7171292910
Hawaiian islands	
1 (EF601197)	ATTCACCACC TCGGCCTCCC TCCGTCATGT
2 (EF601198)	....T.T.. ....
3 (EF601199)	....
4 (EF601200)	....
5 (EF601201)	.....T.... ..A..T.... ..T..T....
Palmyra Atoll	
7 (EF601202)	.....T.... ..A..T..T. .....T....
8 (EF601203)	.........T ..A..T.... ....
Eastern North Pacific Ocean	
6 (EF601204)	.....T..T. ...A.T.... ....
9 (EF601207)	.........T ..A..T.... .....T....
10 (EF601208)	.... .TA..T.... .....T....
11 (EF601209)	....T...T .TT..TG...
12 (EF601210)	.....T.... ..A.TT..T. .....T....
13 (EF601211)	.... .TA..T.... G....T....
14 (EF601212)	.........T ..A..T.... ....GT....
15 (EF601213)	T......... .....T...T .TT..TG...
16 (EF601205)	......T..T ..A..T.... .....T....
Central Indian Ocean	
21 (EF601214)	....A..T..T. ..T..T....
22 (EF601215)	....A..T..T. ..T..T...A
Western North Pacific Ocean	
17 (EF601206)	.....T..TT .....T.... ..T..T....
18 (EF601216)	.....T.... .....T.... ..T..TG.C.
19 (EF601217)	....GT.... C.A..T.... ..T..T....
20 (EF601218)	.....T.... ..A..T.... ..T..T.C..
Western North Atlantic Ocean	
23 (EF601219)	.C..G.TG.. C.......T. ...A.T....
24 (EF601220)	.CCTG.TG.. C.....C.T. .....T....

Note: The haplotype numbers correspond to the numbers used in Fig. 3; their GenBank accession numbers are in parentheses. Indels are not shown.

term mean number of migrants (i.e., females) moving between populations each generation. We ran the program twice with each run generating the final estimates from five replicates. We compared the estimates from MIGRATE to the estimated gene flow calculated by using Φ_{ST} and F_{ST} in Wright's formula: $Nm = [(1/F_{ST} - 1)/2]$, where Nm is the effective number of females that disperse between populations per generation (Wright 1931; Takahata and Palumbi 1985).

Results

There were 24 haplotypes identified among the 124 samples sequenced. The mean number of pairwise differences between haplotypes was 3.53 (SD = 1.81), and there were 35 polymorphic sites including 30 substitutions (25 transitions and 5 transversions) and 5 indels in the 945 base pair sequences (Table 2). The observed nucleotide diversity was low (i.e., π = 0.37%) compared with other delphinids (i.e., 1%–2%) but comparable with estimates of nucleotide diversity for sperm whales, *Physeter macrocephalus* (= *Pyseter catodon* L., 1758), (0.38%; Lyrholm et al. 1996) and other closely related species (e.g., killer whales, *Orcinus orca* (L., 1758), 0.54% (Hoelzel et al. 1998*a*); short-finned pilot whales, *Globicephala macrorhynchus* Gray, 1846, 0.28% (Chivers et al. 2003)). Overall, haplotypic diversity was 0.788 (±0.028). The sequence characteristics for the false

Table 3. Sequence statistics for false killer whale mitochondrial DNA control region sequences.

		Indian Ocean and Pacific Ocean regions		
Sequence characteristics	Atlantic Ocean (n = 2)	Eastern Pacific Ocean, excluding the Hawaiian islands (n = 55)	Hawaiian islands (n = 62)	Western North Pacific Ocean and Indian Ocean (n = 5)
Unique haplotypes (n)	2	13	6	5
Polymorphic sites (n)	5	18	10	9
Observed indels (n)	1	0	3	0
Haplotypic diversity (h; mean ± SE)	na	0.750±0.044	0.359±0.071	1.000±0.126
Nucleotide diversity (π; mean ± SE)	na	0.0030±0.0018	0.0009±0.0007	0.0042±0.0030

Note: The sequence statistics for the Atlantic Ocean samples are not presented, because there were only two samples. na, not available.

killer whale samples in our data set are presented by geographic region in Table 3.

The minimum spanning network shows the relationship between haplotypes, their frequency, and geographic distribution. Phylogeographic concordance is evident in the distribution of haplotypes (Fig. 3). That is, there is a unique set of haplotypes for each oceanic region, and within the ENP, the animals sampled from the near-shore waters of the main Hawaiian islands (i.e., <25 nautical miles (1 nautical mile = 1.852 km) from shore) also had a unique set of haplotypes. There were few shared haplotypes between sampling sites in the ENP. Specifically, one animal sampled off the island of Hawai'i and three animals sampled within the Palmyra Atoll had a haplotype identified as a common haplotype among animals sampled off Mexico, and one additional animal sampled within the Palmyra Atoll had a haplotype also identified among animals sampled off the islands of Hawai'i (Table 4). Four of the five samples collected by fishery observers aboard long-line fishing vessels >75 nautical miles from the main Hawaiian islands had the most common ENP haplotype (i.e., haplotype 9), and the fifth animal revealed a haplotype that was a minimum of 4 base pair changes different from the Hawaiian island haplotypes (i.e., haplotype 6; Fig. 3). A minimum of 2 base pairs separated the most closely related haplotypes from each region — the main Hawaiian islands, ENP, western Pacific Ocean, and central Indian Ocean, whereas a minimum of 10 base pair changes separated the most closely related Indo-Pacific Ocean and Atlantic Ocean haplotypes.

As expected from the phylogeographic concordance in haplotype distribution evident within the ENP, we found statistically significant evidence of genetic differentiation among the putative populations. Comparing the ENP and Hawai'i putative populations in our first a priori stratification, Φ_{ST} was 0.59 ($p < 0.0001$) and F_{ST} was 0.38 ($p < 0.0001$) for the entire data set, and Φ_{ST} was 0.47 ($p < 0.0001$) and F_{ST} was 0.25 ($p < 0.0001$) for the data set using only 1 haplotype/sex per group sampled. Comparing the putative populations in our second a priori stratification using all data, the overall Φ_{ST} was 0.67 ($p < 0.0001$) and F_{ST} was 0.56 ($p < 0.0001$). All pairwise comparisons were statistically significant for both statistics Φ_{ST} and F_{ST}, except for the comparisons of Maui to the island of Hawai'i, and Mexico to Palmyra Atoll. In these cases, sample sizes for the a priori strata were relatively small and were dominated by a single haplotype (Table 5). When the analyses were conducted using only 1 haplotype/sex per group, the overall Φ_{ST} was 0.51 ($p < 0.0001$) and F_{ST} was 0.33 ($p < 0.0001$), and the results differed from the analyses using all data in that the three putative populations around Hawai'i were not significantly different from each other. However, all three of the Hawai'i putative populations were significantly different from Mexico and Panama. Mexico and Panama were also significantly different from each other but neither was significantly different from Palmyra Atoll (Table 6).

Estimated dispersal rates (i.e., number of female migrants/generation) were 0.637 (95% CI = 0.05–4.05) from the ENP to Hawai'i and 0.476 (95% CI = 0.029–1.930) from Hawai'i to the ENP. All sequence data from the ENP and Hawai'i were used for the analysis, because when the data set was restricted to 1 haplotype/sex per group sampled, the program MIGRATE was highly variable between runs. The indirect estimates of gene flow calculated using Φ_{ST} and F_{ST} in Wright's formula were approximately 1.2 and 2.1 migrants/generation, respectively.

Discussion

The phylogeographic concordance observed in the distribution of false killer whale haplotypes suggests limited female dispersal among sampled regions. Worldwide, the distinct sets of haplotypes identified for each region, and particularly the 10 base pair minimum difference observed between Indo-Pacific Ocean and Atlantic Ocean haplotypes, suggests that there is at least ocean-basin-scale population structure. While these results are consistent with the evidence of population structure indicated by morphological differences between false killer whales sampled off Australia and Scotland (Kitchener et al. 1990), resolving the scale of population structure for false killer whales will require analyses of data that much better represent the whales' distribution. Similarly, conclusions about population structure within our ENP study area are limited but informative with respect to the central question of our study, which was whether the current boundary for the Hawaiian stock of false killer whales is appropriate for management.

The genetic distinctness evident among the animals sampled around the main Hawaiian islands indicates that they represent a demographically independent population (i.e., Φ_{ST} = 0.47 and F_{ST} = 0.25 when only 1 haplotype/sex per group sampled was included in the analyses). Concordant with these large genetic differences, the estimates of migrants/generation were low (i.e., <1 migrant/generation). While these estimates provide only relative information about animal movement because the methods used make several as-

Fig. 3. Minimum spanning tree for the 24 haplotypes identified for false killer whales in this study. Each haplotype is identified by number with its observed frequency in parentheses if >1. Each connecting branch is labeled with the minimum number of base pair changes if >1. Broken lines indicate alternate links.

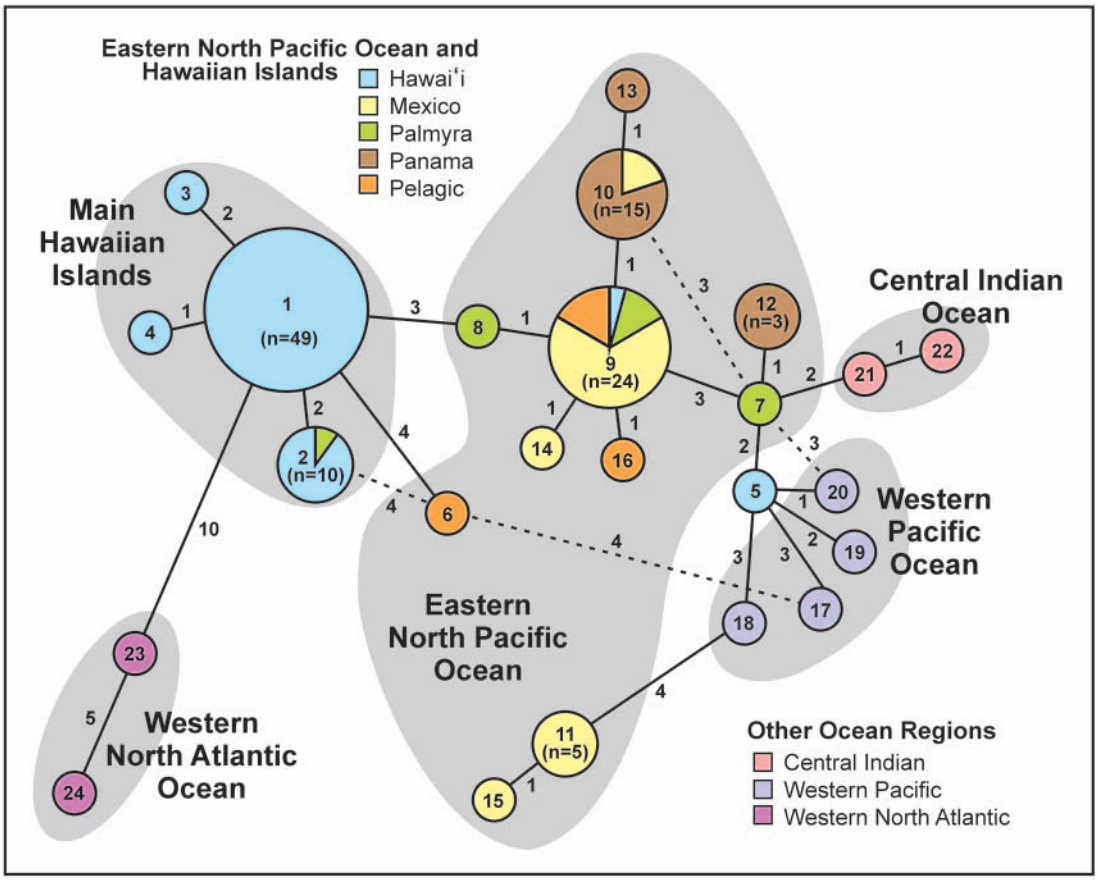


Table 4. Haplotype frequencies for eastern North Pacific Ocean putative populations.

	Hawai'i					
Haplotype ID number	O'ahu (*n* = 27)	Maui (*n* = 14)	Hawai'i (*n* = 21)	Mexico (*n* = 26)	Panama (*n* = 16)	Palmyra Atoll (*n* = 6)
1	27	9	13			
2		4	5			1
3		1				
4			1			
5			1			
7						1
8						1
9			1	16		3
10				3	12	
11				5		
12					3	
13					1	
14				1		
15				1		

Note: Haplotype numbers not listed were not identified among these samples.

sumptions about population histories and migration patterns that cannot be verified (Takahata and Palumbi 1985; Beerli and Felsenstein 1999, 2001), they can be interpreted to indicate that gene flow is demographically insignificant for false killer whales around Hawai'i but likely sufficient to prevent the accumulation of differences that could lead to speciation (Wright 1931; Mills and Allendorf 1996). All of the false killer whales in this putative population were sampled relatively close to shore (i.e., <25 nautical miles from the coast). When compared with the animals sampled by biological technicians aboard long-line fishing vessels within the Hawaiian EEZ, which had haplotypes identical

Table 5. Genetic differentiation quantified for eastern North Pacific Ocean putative populations, or sampling sites.

	Hawai'i					
Putative population	O'ahu (n = 27)	Maui (n = 14)	Hawai'i (n = 21)	Mexico (n = 26)	Panama (n = 16)	Palmyra Atoll (n = 6)
Hawai'i						
O'ahu	—	0.352 (0.0036)	0.270 (<0.0001)	0.709 (<0.0001)	0.836 (<0.0001)	0.835 (<0.0001)
Maui	0.282 (0.0020)	—	–0.049 (0.9997)	0.431 (<0.0001)	0.521 (<0.0001)	0.326 (0.0053)
Hawai'i	0.193 (0.0007)	–0.031 (0.6509)	—	0.396 (<0.0001)	0.491 (<0.0001)	0.294 (0.0026)
Mexico	0.709 (<0.0001)	0.558 (<0.0001)	0.507 (<0.0001)	—	0.433 (<0.0001)	0.017 (0.2949)
Panama	0.878 (<0.0001)	0.716 (<0.0001)	0.649 (<0.0001)	0.348 (<0.0001)	—	0.440 (<0.0001)
Palmyra Atoll	0.872 (<0.0001)	0.569 (0.0003)	0.471 (0.0010)	0.056 (0.1712)	0.402 (0.0013)	—

Note: Φ_{ST} values are in the lower diagonal and F_{ST} values are in the upper diagonal with p values in parentheses.

Table 6. Genetic differentiation quantified for eastern North Pacific Ocean putative populations, or sampling sites, using only 1 haplotype/sex per group when multiple animals were sampled from a group.

	Hawai'i					
Putative population	O'ahu (n = 4)	Maui (n = 10)	Hawai'i (n = 14)	Mexico (n = 13)	Panama (n = 5)	Palmyra Atoll (n = 5)
Hawai'i						
O'ahu	—	0.008 (0.5154)	0.125 (0.2592)	0.508 (0.0007)	0.558 (0.0102)	0.505 (0.0109)
Maui	–0.058 (0.6760)	—	–0.028 (0.5253)	0.385 (<0.0001)	0.375 (0.0030)	0.304 (0.0215)
Hawai'i	0.032 (0.2883)	–0.002 (0.4936)	—	0.268 (<0.0001)	0.260 (0.0036)	0.144 (0.0717)
Mexico	0.538 (0.0023)	0.553 (<0.0001)	0.426 (<0.0001)	—	0.208 (0.0172)	–0.007 (0.4248)
Panama	0.687 (0.0083)	0.672 (0.0007)	0.519 (0.0003)	0.245 (0.0119)	—	0.150 (0.0926)
Palmyra Atoll	0.570 (0.0159)	0.532 (0.0007)	0.312 (0.0093)	0.038 (0.2978)	0.195 (0.1435)	—

Note: Φ_{ST} values are in the lower diagonal and F_{ST} values are in the upper diagonal with p values in parentheses.

to others found in the ENP or distantly related to those from Hawai'i, the evidence suggests that there are at least two populations within the currently recognized Hawaiian stock: an island-associated population and a population that is more pelagic in distribution.

Results from analyses of the second a priori data stratification indicated no additional population structure within the islands when the data set was limited to 1 haplotype/sex per group (Table 6), but there may be if all data were used (Table 5). The mark–recapture data from photographic identification of individual animals show that animals move among the main Hawaiian islands, particularly between O'ahu and Hawai'i (Baird et al. 2005). While these data suggest no inter-island structure, additional genetic analyses will be needed to determine whether the observed movements are demographically significant and to resolve whether the apparent discrepancy in the genetic results is due to sample size or social structure.

False killer whales are generally considered a wide-ranging pelagic species not typically associated with coastal or island habitats. However, evidence of false killer whales occupying coastal and island habitats has been documented in a photographic study off Costa Rica (Acevedo-Guitiérrez et al. 1997), and the effects of island biogeography are well known. The Hawaiian archipelago is the most isolated island group in the world, and in addition to its remoteness, is noteworthy for having relatively high endemism (i.e., accumulated evolutionary changes) (Briggs 1961, 1966; Carlquist 1966). The geographic isolation of the Hawaiian island chain may also provide favorable habitat for false killer whales that effectively limited their movements and thus gene flow. The oldest islands in the archipelago are estimated to date back to the Miocene, and the volcano that erupted to create the youngest island in the chain has been estimated to have emerged 460 000 years ago. On the basis of these approximate dates, one could hypothesize that there has been sufficient time for genetic differences to accumulate in a founding population of false killer whales in the Hawaiian archipelago. However, the genetic differences observed in this study may be the result of a more recent founding event, a bottleneck, or a sudden significant decrease in population abundance. The demographic history of false killer whales around the Hawaiian islands is essentially unknown and limits the exploration of potential hypotheses, but our results suggest that at least female dispersal has been limited for a long time.

There are several examples of cetacean species generally considered capable of large-scale movements that have morphologically and genetically differentiated populations occupying coastal habitats and genetically distinct populations within the near-island habitats of the Hawaiian archipelago. Species that have distinct coastal populations include the pantropical spotted dolphin (Douglas et al. 1984; Escorza-Treviño et al. 2005), common dolphin (genus *Delphinus* L., 1758; Rosel et al. 1994), and bottlenose dolphin (genus *Tursiops* Gervais, 1855; Walker 1981; Mead and Potter 1995; Hoelzel et al. 1998*b*). For the common dolphin and bottlenose dolphin, species-level differences in morphological and genetic characteristics have accumulated so that two species of common dolphin are recognized (*Delphinus delphis* L., 1758 and *Delphinus capensis* Gray, 1828; Rosel et al. 1994) and the current consensus is that there are at least two spe-

cies of bottlenose dolphin (*Tursiops truncatus* (Montagu, 1821) and *Tursiops aduncus* (Ehrenberg, 1833); Wang et al. 1999; LeDuc et al. 1999; Rice 1998). Within the near-shore habitats of Hawai'i, studies of spinner dolphins (*Stenella longirostris* (Gray, 1828)) suggest that there are local island populations throughout the archipelago (Norris et al. 1994; Galver 2002; Andrews et al. 2006), and bottlenose dolphins appear to also have demographically independent populations at each of the main islands (Martien et al. 2005; Baird et al. 2006). Additionally, Bryde's whales (*Balaenoptera edeni* Anderson, 1878) (R.G. LeDuc, unpublished data) and short-finned pilot whales (S.J. Chivers, unpublished data) sampled around the Hawaiian islands are genetically distinct from animals sampled from the surrounding pelagic waters, suggesting that they also have demographically independent island-associated populations (Chivers et al. 2003).

The low genetic diversity we observed appears to be typical of species that form groups of closely related individuals like sperm whales (Lyrholm et al. 1996; Richard et al. 1996), killer whales (Hoelzel et al. 1998*a*), and long-finned pilot whales (*Globicephala melas* (Traill, 1809); Amos et al. 1993). Several hypotheses have been proposed as explanations for the comparatively low genetic diversity observed for these species, including low effective population size, cultural selection of maternally inherited characteristics, and ongoing natural selection on the mitochondrial genome (Lyrholm et al. 1996; Whitehead 1999). Available population abundance estimates for our study area and observations about social structure enable us to discuss the first two hypotheses with respect to false killer whales.

Estimates of current population abundance for the Hawaiian archipelago indicate that it is a relatively small population. Three survey methods (aerial, shipboard, and mark–recapture photo-identification surveys) produced abundance estimates of 121 (CV = 0.473; Mobley et al. 2000), 236 (CV = 1.13; Barlow 2006), and 123 (CV = 0.72; Baird et al. 2005), respectively. Populations of this size may be expected to have distinctive genetic characteristics, but there is no information about historic trends in abundance or exploitation to evaluate how the population's demographic history may have influenced the genetic diversity we see. Certainly, small populations would be expected to accumulate genetic differences relatively quickly primarily as a result of the effects of genetic drift. The available abundance estimate for the ENP is for the *Stenella* spp. study area to the east of Hawai'i (see SWFSC *R/V* Sights in Fig. 1). Shipboard surveys conducted between 1986 and 1990 produced an estimate of 39 800 (CV = 0.636) false killer whales (Wade and Gerrodette 1993). The observed differences in genetic diversity between Hawai'i and ENP are consistent with the differences in population abundance (Table 3). However, results of the pairwise comparison of the putative populations of Mexico and Panama suggests that there is additional structure within the region (Tables 5, 6), which needs to be better understood to evaluate the potential influence of effective population size on genetic diversity.

Social structure has also been suggested to influence patterns of population structure and genetic diversity. False killer whales are known to have strong social bonds among group members (e.g., Porter 1977), and mass-stranding events are fairly typical as they are for other species that exhibit strong social bonds between group members (Stacey and Baird 1991). No long-term studies have been published on false killer whales to document group dynamics or individual association patterns within groups. However, the social organization for false killer whales has been presumed to be matrifocal as has been observed in sperm whales (Mesnick 2001; Mesnick et al. 2003) and pilot whales (Amos et al. 1993). Within our data set, two or more haplotypes were present in 11 of the 15 groups with multiple individuals sampled, suggesting that multiple matrilines are present within groups (Table 1). Although analyses of social organization for false killer whales is beyond the scope of this paper, its influence may need to be carefully considered in the future, especially if population structure analyses on a finer scale are warranted.

False killer whales inhabiting the near-shore waters of the main Hawaiian islands have scars consistent with injuries caused by long-line fishing gear (Baird and Gorgone 2005). However, none of the samples collected by biological technicians aboard fishing vessels were identified as belonging to the near-shore island-associated population. That is, four of the five animals sampled had a common ENP haplotype (i.e., haplotype 9) and the fifth animal had a haplotype distantly related to the Hawaiian haplotypes (i.e., haplotype 6; Fig. 3). The age and accumulation rate of scars in the Baird and Gorgone (2005) study is unknown, and the long-line fishery is closed within 50 nautical miles of the islands to protect monk seals and within 75 nautical miles in some areas to prevent gear conflicts with smaller fishing vessels (Carretta et al. 2006). This apparent discrepancy in population(s) of animals affected by the fishery will require additional research to resolve.

Management implications

Our results document significant genetic differentiation of false killer whales inhabiting the near-shore waters of the Hawaiian islands, and thus support recognition of a Hawaiian stock. However, the genetic data suggest that there is more than one stock within the Hawaiian EEZ. Knowledge about false killer whales within the Hawaiian EEZ is insufficient to identify biologically meaningful geographic boundaries at this time, and development of a management plan to provide appropriate protection for island-associated false killer whales, as well as those affected by the long-line fishery, will require additional research to better understand their population structure, distribution, and ecology.

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