Genetic differentiation of Hawai‘i insular false killer whales: analyses updated with new samples from the northwest Hawaiian Islands

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The 2010 Hawaiian Islands Cetacean and Ecosystem Assessment Survey (HICEAS) surveyed the near-shore waters of islands and atolls in the northwestern Hawaiian islands (NWHI) resulting in several sightings of false killer whales and the collection of our first skin biopsy samples from the NWHI region of the Hawaiian archipelago. The recent status review of Hawaiian Insular false killer whales concluded that the population met the definition for a distinct population segment (DPS) under the Endangered Species Act (Oleson et al. 2010), but there were no photographs or genetic samples from the NWHI included in studies available at the time. Martien et al. (2011) examine whether the NWHI samples from island-associated animals originate from the Hawaiian Insular stock of the main Hawaiian Islands (MHI) or represent a previously undocumented demographically independent population (DIP). Here we examine the samples collected during the HICEAS cruise in the larger context previously presented in Chivers et al. (2010, aka PSRG-2010-10). Because the important question is whether

these samples change the conclusions of that study with respect to recognition of Hawaiian Insular false killer whales as a DIP and DPS, we present only the updated genetic diversity estimates and population structure analyses for your review.

The methods for generating, reviewing and estimating diversity of the genetic marker data sets are the same as those described in Chivers et al. (2010). The analytical methods for testing for evidence of genetic differentiation are also the same except we calculated F'_{ST} instead of Jost's D for the nuclear data set, because F'_{ST} is more appropriate for demographic analyses (see additional description of statistics in Martien et al. 2011). We also revised the stratification schemes described by Chivers et al. (2010) and added an analysis of the nucDNA data set using STRUCTURE.

For the genetic differentiation analyses, we tested the null hypothesis of panmixia for three *a priori* stratifications of the molecular genetic data sets that now include data for samples collected in 2010 on the HICEAS cruise and during additional field work conducted around the MHI (Table 1). In these analyses, the NWHI stratum only includes samples of island-associated animals, which were identified by having haplotypes identical, or closely related, to those of Hawaiian Insular false killer whales. The stratification schemes we used differ from those of Chivers et al. (2010) in that we defined two broad-scale stratifications and a fine-scale stratification. For each of the broad-scale stratifications we identified three strata: Hawai‘i (HI), central North Pacific (CNP) and eastern North Pacific (ENP). In the first *a priori* stratification, called broad-scale 1, the NWHI and MHI samples are combined in the HI stratum (Figure 1a). In the second stratification, called broad-scale 2, only the MHI samples are in the HI stratum and the NWHI samples are included in the CNP stratum (Figure 1b). In the third stratification, called the fine-scale stratification, we recognized five strata: Hawai‘i insular, NWHI, Hawai‘i pelagic, Mexico, Panama, and American Samoa (Figure 1c). Analyses of the inter-island stratification were omitted, because the most recent social network analyses revealed that structuring within the MHI is defined by social cluster and not geography (Baird et al. 2011). Comparisons of the genetic differentiation among social clusters are presented in Martien et al. (2011).

Table 1. Sample sizes available for the analyses presented in this document compared to those previously available for the mitochondrial DNA (mtDNA) and nuclear DNA (nucDNA) data sets.

	mtDNA		**nucDNA**	
Stratum	**Chivers et al. (2010)**	**2011 update**	**Chivers et al. (2010)**	**2011 update**
	n	***n***	***n***	***n***
Hawai‘i Insular	81	96	76	91
Northwest Hawaiian Islands (NWHI)	0	21	0	21
Central North Pacific Ocean (CNP)	16	27	14	26
Eastern North Pacific Ocean (ENP)	40	40	38	38

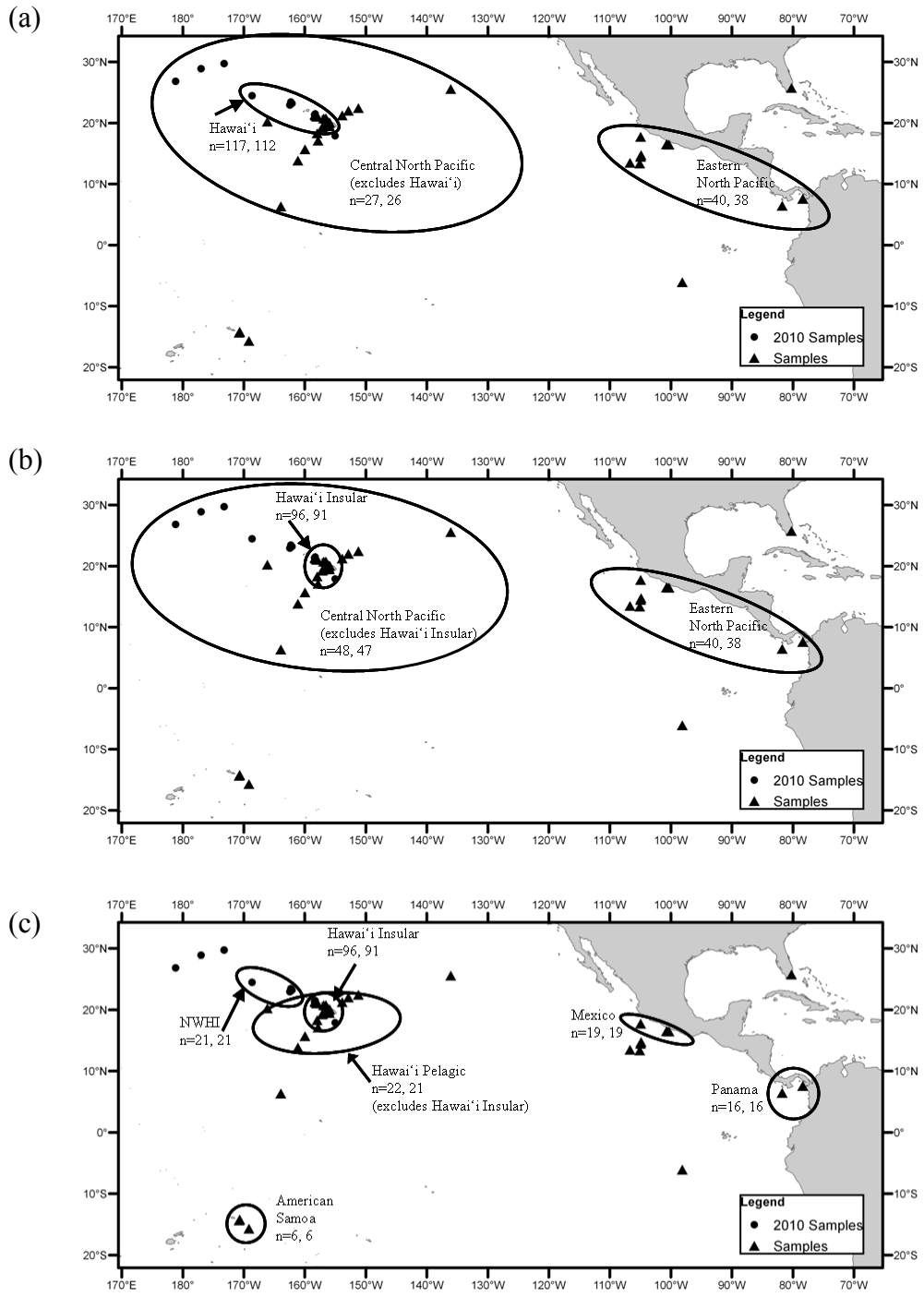

Figure 1. The false killer whale mitochondrial (mtDNA) and nuclear DNA (nucDNA) data sets were analyzed for the two broad-scale stratifications shown in (a) and (b), and the fine-scale stratification shown in (c). Sample sizes for the mtDNA and nucDNA data sets are presented, respectively. NWHI = northwest Hawaiian Islands.

We used the Bayesian clustering program STRUCTURE 2.3.1 (Pritchard et al. 2000, Falush et al. 2003, Hubisz et al. 2009) to cluster the samples on the basis of their microsatellite genotypes (i.e., the nucDNA data set). We used an admixture model with correlated frequencies. We had STRUCTURE cluster the samples into k = 1-5 groups and ran STRUCTURE 3 times for each k. We compared estimates of likelihood and ancestry across runs to confirm convergence. We evaluated support for different values of k by comparing the mean log-likelihood of model runs. We used a burn-in of 50,000 and a run length of 500,000. All other parameters were left at program defaults.

Results

Genetic diversity: Three new haplotypes (i.e., haplotypes 30, 31 and 32) were identified among the samples collected during 2010 field studies (Table 2). Haplotype 31 was found to be closely related to Hawai‘i Insular haplotype 1, and the other two were closely related to other Indo-Pacific Ocean haplotypes (see Figure 4 in Chivers et al. 2010). The new haplotypes together with the new genotype data slightly increased the genetic diversity of the mitochondrial DNA (mtDNA) and nuclear DNA (nucDNA) data sets (Table 3).

Table 2. Haplotype frequencies for false killer whales from eastern Pacific Ocean sampling locales using the fine-scale stratification (Figure 1c). The samples collected during 2010 and added to the analyses presented here are in the Hawai‘i insular, northwest Hawaiian islands (NWHI) and Pelagic strata.

Haplotype ID number	Hawai‘i Insular (n=96)	NWHI (n=21)	Mexico (n=19)	Panama (n=16)	Pelagic (n=22)	American Samoa (n=6)
1	73	20				
2	22					
5	1					
6					1	
7						
9			14		11	
10			2	13		
11			3			
12				3		
17						5
25					2	
26					1	1
30					4	
31		1				
32					3	

Table 3. Summary of genetic diversity statistics by marker: mitochondrial DNA (mtDNA) and nuclear DNA (nucDNA) and stratum for each of three stratifications analyzed for evidence of genetic diversity. Strata abbreviations are: NWHI = northwest Hawaiian islands, CNP = central North Pacific, and ENP = eastern North Pacific. Column headings are: *n* = sample size, *h* = haplotypic diversity, π = nucleotide diversity, *k* = number of alleles/haplotypes per locus, and H_o = observed heterozygosity.

	mtDNA			nucDNA		
Population	*n*	*h*	π	*n*	*k*	H_o
Broad-scale 1 strata:						
Hawai‘i Insular +NWHI	117	0.336	0.0008	112	8.50	0.751
CNP	27	0.712	0.0030	26	7.88	0.764
ENP	40	0.680	0.0024	38	8.63	0.746
Broad-scale 2 strata:						
Hawai‘i Insular	96	0.373	0.0009	91	7.63	0.745
CNP+NWHI	48	0.742	0.0032	47	8.63	0.759
ENP	40	0.680	0.0024	38	8.63	0.746
Fine-scale strata:						
Hawai‘i Insular	96	0.373	0.0009	91	7.63	0.745
NWHI	21	0.095	0.0001	21	6.88	0.735
Mexico	19	0.444	0.0021	19	7.25	0.754
Panama	16	0.325	0.0014	14	5.69	0.693
Hawai'i Pelagic	22	0.719	0.0031	21	7.38	0.762
American Samoa	6	0.333	0.0018	6	4.69	0.711

Genetic differentiation

Analysis of mtDNA data – We found evidence of statistically significant genetic differentiation among strata. Global tests of differentiation revealed significant genetic differentiation between strata overall for both broad- and the fine-scale stratifications (Fisher's exact *P*-value <0.0001 for both stratifications), rejecting the global null hypothesis of panmixia. In both of the broad-scale stratifications, all pairwise comparisons were statistically significant (Tables 4 and 5). Similarly, in the fine-scale stratification, all pairwise comparisons were statistically significant including the comparison of the Hawai‘i Insular and NWHI strata (Table 6). Genetic divergence (Φ_{ST}) between the Hawai‘i Insular stratum and all other strata ranged from 0.1182 to 0.8536 (Tables 5 and 6). The comparison of the Hawai‘i Insular and NWHI strata had the lowest Φ_{ST} : 0.1182.

Table 4. Mitochondrial DNA results for the broad-scale 1 stratification, which has the northwest Hawaiian Island samples combined with the Hawaiian Insular samples (Figure 1a). (a) X^2 P-values, and (b) Φ_{ST} is below the diagonal and F_{ST} is above the diagonal. *P*-values <0.05 are shown in bold.

a)

Putative population	Hawai‘i (*n*=117)	Central North Pacific (*n*=27)	Eastern North Pacific (*n*=40)
Hawai‘i	--		
Central North Pacific	**<0.0001**	--	
Eastern North Pacific	**<0.0001**	**<0.0001**	--

b)

Putative population	Hawai‘i (*n*=117)	Central North Pacific (*n*=27)	Eastern North Pacific (*n*=40)
Hawai‘i	--	0.5434	0.5399
Central North Pacific	0.6907	--	0.1507
Eastern North Pacific	0.7208	0.1117	--

Table 5. Mitochondrial DNA results for the broad-scale 2 stratification, which has samples from the northwest Hawaiian Island combined with the central North Pacific samples (Figure 1b). (a) X^2 P-values, and (b) Φ_{ST} is below the diagonal and F_{ST} is above the diagonal. *P*-values <0.05 are shown in bold.

a)

Putative population	Hawai‘i Insular (*n*=96)	Central North Pacific (*n*=48)	Eastern North Pacific (*n*=40)
Hawai‘i Insular	--		
Central North Pacific	**<0.0001**	--	
Eastern North Pacific	**<0.0001**	**<0.0001**	--

b)

Putative population	Hawai‘i Insular (*n*=96)	Central North Pacific (*n*=48)	Eastern North Pacific (*n*=40)
Hawai‘i Insular	--	0.2049	0.5073
Central North Pacific	0.3097	--	0.2077
Eastern North Pacific	0.6933	0.2463	--

Table 6. Mitochondrial DNA results for analyses of the fine-scale stratification, which has the northwest Hawaiian Island samples combined with the Hawaiian Insular samples (Figure 1c). (a) X^2 P-values, and (b) Φ_{ST} is below the diagonal and F_{ST} is above the diagonal. *P*-values <0.05 are shown in bold. NWHI = northwest Hawaiian islands.

a)

Putative population	Hawai‘i Insular (*n*=96)	NWHI (*n*=21)	Mexico (*n*=19)	Panama (*n*=16)	Hawai‘i Pelagic (*n*=22)	American Samoa (*n*=6)
Hawai‘i Insular	--					
NWHI	**0.0110**	--				
Mexico	**<0.0001**	**<0.0001**	--			
Panama	**<0.0001**	**<0.0001**	**<0.0001**	--		
Hawai‘i Pelagic	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	--	
American Samoa	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	--

b)

Putative population	Hawai‘i Insular (*n*=96)	NWHI (*n*=21)	Mexico (*n*=19)	Panama (*n*=16)	Hawai‘i Pelagic (*n*=22)	American Samoa (*n*=6)
Hawai‘i Insular	--	0.1009	0.6066	0.6394	0.5138	0.6343
NWHI	0.1182	--	0.7372	0.8034	0.5883	0.8486
Mexico	0.7449	0.7677	--	0.5756	0.0765	0.5899
Panama	0.7913	0.8587	0.4106	--	0.4612	0.6734
Hawai‘i Pelagic	0.6517	0.6255	0.0632	0.3320	--	0.4069
American Samoa	0.8536	0.9287	0.6111	0.7585	0.4434	--

Analysis of nuclear marker data – Global tests of differentiation revealed significant differentiation within the nucDNA data set, leading to rejection of the global null hypothesis of panmixia, for all three stratification schemes (χ^2 $p < 0.0001$). We found statistically significant evidence of genetic differentiation among putative populations for all pairwise comparisons in both of the broad-scale stratifications (Tables 7 and 8) and the fine-scale stratification (Table 9). The estimates of divergence between Hawai‘i Insular false killer whales and other strata ranged from 0.0086 to 0.0421 for F_{ST} and from 0.0347 to 0.1533 for F'_{ST} (Tables 8 and 9). The lowest estimates of divergence were for the comparison of Hawai‘i insular and Hawai‘i pelagic strata.

The STRUCTURE results reveal the genetic distinctness of the Hawai‘i insular population of false killer whales, and the limited gene flow between that population and the other putative populations sampled in the eastern North Pacific (Table 10, Figure 2). This analysis uses only the nucDNA data set, and the results indicate that the nuclear genome of the NWHI animals is more similar to that of the other eastern North Pacific animals sampled than to that of the Hawai‘i insular population. However, nearly all the NWHI animals had the most common haplotype of the Hawai‘i insular population. This incongruence between the nucDNA and mtDNA data sets suggests that (1) the NWHI

and Hawai‘i Insular populations were likely founded by closely related individuals, leading them to share a common mtDNA haplotype, and (2) the NWHI has, in recent evolutionary time, experienced higher rates of gene flow with pelagic animals than with the Hawai‘i insular population, leading them to have more nucDNA similarities to pelagic animals. Thus, highlighting the distinctiveness of the Hawai‘i insular population.

Table 7. Pairwise comparisons of the nuclear DNA data set for the broad-scale 1 stratification (Figure 1a). (a) Statistical significance was assessed with a X^2 permutation *p*-values. (b) Divergence was estimated with F_{ST} (above the diagonal) and F'_{ST} (below the diagonal).

a)

Putative population	Hawai‘i (*n*=112)	Central North Pacific (*n*=26)	Eastern North Pacific (*n*=38)
Hawai‘i	--		
Central North Pacific	**<0.0001**	--	
Eastern North Pacific	**<0.0001**	**<0.0001**	--

b)

Putative population	Hawai‘i (*n*=112)	Central North Pacific (*n*=26)	Eastern North Pacific (*n*=38)
Hawai‘i	--	0.0081	0.0205
Central North Pacific	0.0330	--	0.0147
Eastern North Pacific	0.0815	0.0599	--

Table 8. Pairwise comparisons of the nuclear DNA data set for the broad-scale 2 stratification, which has samples from the northwest Hawaiian Island combined with the central North Pacific samples (Figure 1b). (a) Statistical significance was assessed with a X^2 permutation *p*-values. (b) Divergence was estimated with F_{ST} (above the diagonal) and F'_{ST} (below the diagonal)

a)

Putative population	Hawai‘i Insular (*n*=91)	Central North Pacific (*n*=47)	Eastern North Pacific (*n*=38)
Hawai‘i Insular	--		
Central North Pacific	**<0.0001**	--	
Eastern North Pacific	**<0.0001**	**<0.0001**	--

b)

Putative population	Hawai‘i Insular (*n*=91)	Central North Pacific (*n*=47)	Eastern North Pacific (*n*=38)
Hawai‘i Insular	--	0.0154	0.0237
Central North Pacific	0.0621	--	0.0179
Eastern North Pacific	0.0932	0.0724	--

Table 9. Pairwise comparisons of the nuclear DNA data set for fine-scale stratification (Figure 1c). (a) Statistical significance was assessed with a X^2 permutation *p*-values. (b) Divergence was estimated with F_{ST} (above the diagonal) and F'_{ST} (below the diagonal). NWHI = northwest Hawaiian islands.

(a)

Putative population	Hawai‘i Insular (*n*=91)	NWHI (*n*=21)	Mexico (*n*=19)	Panama (*n*=14)	Hawai'i Pelagic (*n*=21)	American Samoa (*n*=6)
Hawai‘i Insular	--					
NWHI	**<0.0001**	--				
Mexico	**<0.0001**	**<0.0001**	--			
Panama	**<0.0001**	**<0.0001**	**<0.0001**	--		
Hawai'i Pelagic	**<0.0001**	**0.0002**	**0.0005**	**0.0002**	--	
American Samoa	**<0.0001**	**<0.0001**	**0.0004**	**<0.0001**	**0.0007**	--

(b)

Putative population	Hawai‘i Insular (*n*=91)	NWHI (*n*=21)	Mexico (*n*=19)	Panama (*n*=14)	Hawai'i Pelagic (*n*=21)	American Samoa (*n*=6)
Hawai‘i Insular	--	0.0312	0.0280	0.0421	0.0086	0.0388
NWHI	0.1209	--	0.0375	0.0543	0.0180	0.0586
Mexico	0.1117	0.1466	--	0.0375	0.0172	0.0339
Panama	0.1533	0.1907	0.1367	--	0.0343	0.0703
Hawai'i Pelagic	0.0347	0.0716	0.0710	0.1271	--	0.0268
American Samoa	0.1453	0.2120	0.1287	0.2346	0.1040	--

Table 10. Summary of log-likelihood values from STRUCTURE runs. The number of groups (*k*) defined by STRUCTURE varied from 1 to 5. Three replicate analyses were run for each value of *k*. The model with the highest mean log-likelihood across replicate runs is shown in bold.

k	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Mean**
1	-9314.2	-9313.5	-9314	-9313.9
2	-9151.2	-9149.2	-9150.6	-9150.3
3	-9110.6	-9117.5	-9115.6	**-9114.6**
4	-9298.2	-9759.5	-9741.9	-9599.9
5	-9480.3	-9662.5	-9465.1	-9536.0

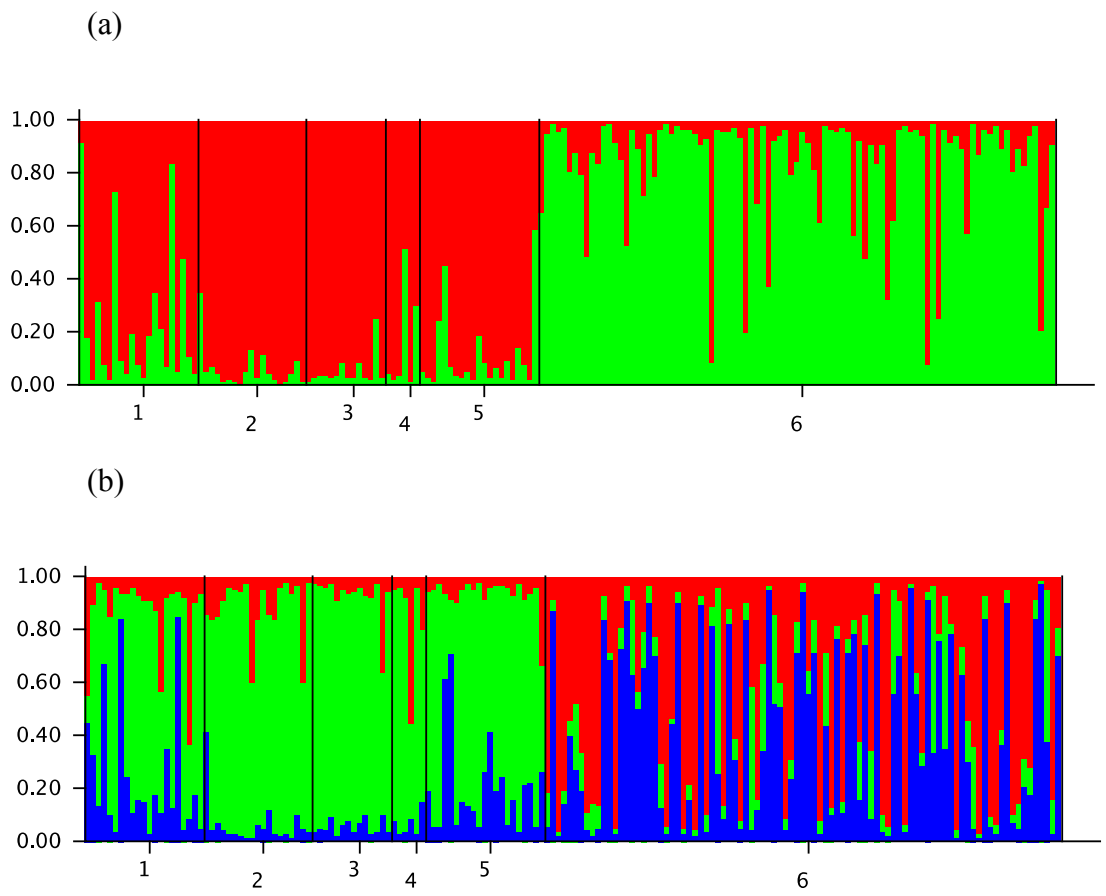

Figure 2. Graphical representation of the results of the STRUCTURE analysis for the models with (a) *k* = 2 and (b) *k* = 3. Bars are shaded as to the proportion of the individual's ancestry that is attributable to each of the groups defined by STRUCTURE. Numbers along the bottom of each graph identify the stratum where a sample was collected: 1=Hawai′i pelagic, 2=Mexico, 3=Panama, 4=American Samoa, 5=NWHI and 6=Hawai′i insular. The numbers are in the middle of their respective sample set, and a black vertical line delineates the sample set for each stratum.

Conclusion and management implications

The results presented here confirm the conclusions of Chivers et al. (2010) with respect to the genetic distinctness of Hawaiian Insular false killer whales, and thus, support recognition of this population as a DIP for management under the Marine Mammal Protection Act (Carretta et al. 2011) and as a DPS under the Endangered Species Act (Oleson et al. 2010). Furthermore, the results indicate that the false killer whales sampled in the NWHI are unlikely to be part of this population. The NWHI is represented by a relatively small number of samples (n = 21) collected from only four groups encountered across an area as large as the range of the Hawai‘i insular population. Further research is needed to determine the relationships among individuals in the NWHI and whether there is additional structure within this part of the Hawaiian island chain.

Post-script: fishery samples

In February 2011, two samples were collected from false killer whales by fishery observers working aboard a longline fishing vessel operating around American Samoa. We generated the mtDNA sequences for these animals and determined their sex. One sample was a male with mtDNA haplotype 26, and the other was a female with haplotype 32. Haplotype 26 was previously observed in a sample collected around American Samoa. Haplotype 32 is a new haplotype that we first identified among the biopsy samples collected from pelagic false killer whales sampled during the 2010 HICEAS cruise. These samples were not included in the analyses presented here, but they will be included in future analyses after the mtDNA and nucDNA data has been reviewed.

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