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POPULATION ECOLOGY - ORIGINAL PAPER

# Foraging segregation and genetic divergence between geographically proximate colonies of a highly mobile seabird

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**Abstract** Foraging segregation may play an important role in the maintenance of animal diversity, and is a proposed mechanism for promoting genetic divergence within seabird species. However, little information exists regarding its presence among seabird populations. We investigated genetic and foraging divergence between two colonies of endangered Hawaiian petrels (*Pterodroma sandwichensis*) nesting on the islands of Hawaii and Kauai using the mitochondrial *Cytochrome b* gene and carbon, nitrogen and hydrogen isotope values ($\delta^{13}$C, $\delta^{15}$N and $\delta$D, respectively) of feathers. Genetic analyses revealed strong differentiation between colonies on Hawaii and Kauai, with $\Phi_{ST} = 0.50$ ($p < 0.0001$). Coalescent-based analyses gave estimates of <1 migration event per 1,000 generations. Hatch-year birds from Kauai had significantly lower $\delta^{13}$C and $\delta^{15}$N values than those from Hawaii. This is consistent with Kauai birds provisioning chicks with prey derived from near or north of the Hawaiian Islands, and Hawaii birds provisioning young with prey from regions of the equatorial Pacific characterized by elevated $\delta^{15}$N values at

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the food web base. $\delta^{15}$N values of Kauai and Hawaii adults differed significantly, indicating additional foraging segregation during molt. Feather $\delta$D varied from −69 to 53‰. This variation cannot be related solely to an isotopically homogeneous ocean water source or evaporative water loss. Instead, we propose the involvement of salt gland excretion. Our data demonstrate the presence of foraging segregation between proximately nesting seabird populations, despite high species mobility. This ecological diversity may facilitate population coexistence, and its preservation should be a focus of conservation strategies.

**Keywords** Gene flow · Niche segregation · Population ecology · Salt load · Stable isotopes

## Introduction

As consumers of widely dispersed marine prey, pelagic seabirds are noted for their remarkably long foraging trips that may extend thousands of kilometers (Warham 1996). However, the same birds that travel great distances to forage may be reluctant to disperse among nearby breeding sites, an apparent inconsistency referred to as the seabird paradox (Milot et al. 2008). For example, Laysan albatross (*Phoebastria immutabilis)* regularly travel >1,500 km away from breeding sites in search of food, but typically breed within 20 km of their hatch site (Fisher 1967, 1976). In association with natal and breeding philopatry, some species show genetic differentiation between breeding colonies (Friesen et al. 2007). Contact between seabird colonies may be further reduced by foraging segregation (Friesen et al. 2007).

Foraging segregation is thought to play an important role in the maintenance of species diversity by reducing competition and allowing seabirds with otherwise similar niches to coexist (Weimerskirch et al. 1986; Ainley et al. 1992; Spear et al. 2007). Sympatric seabird species are known to segregate by feeding niche in a variety of ways, including feeding location, diving depth, and size of targeted prey (Ashmole and Ashmole 1967; Harris 1970; Bocher et al. 2000; Mori and Boyd 2004; Finkelstein et al. 2006). Less is known about foraging segregation between populations, partly due to the difficulty involved in differentiating seabird populations (Abbott and Double 2003; Friesen et al. 2006). However, foraging segregation has been found to occur within some species: between sexes and, in limited cases, between breeding colonies (Phillips et al. 2004; Zavalaga et al. 2007). There is evidence that intra-specific competition for food among pelagic seabirds can be intense, and this competition may drive foraging segregation among colonies (Lewis et al. 2001). Foraging segregation, in turn, may play a central role in producing and maintaining both distinct populations and ecologically specialized morphotypes within a species (Gilardi 1992; Zavalaga et al. 2007).

We investigated both foraging segregation and population divergence between breeding colonies of the Hawaiian petrel (*Pterodroma sandwichensis*, or U'au). This endangered species exemplifies the seabird paradox, sometimes traveling over 10,000 km on individual foraging trips, but breeding no more than 500 km apart on the islands of Hawaii and Kauai in the Hawaiian Archipelago (Adams and Flora 2009; Simons 1985). The Hawaiian petrel foraging range extends throughout most of the North East Pacific, from the Aleutian Islands to the Equator (Fig. 1; Bourne 1965; Bourne and Dixon 1975; Simons and Hodges 1998; Spear et al. 1995). However, there is only one recent study providing colony-level information on foraging range (J. Adams, D. Ainley et al., unpublished data). Hawaiian petrels from different breeding colonies are morphologically indistinguishable, and little is known about population structure or genetic variation within the species (Browne et al. 1997). Because Hawaiian petrels are endangered and logistically difficult to study, a better understanding of their foraging and genetic diversity would be particularly valuable, as it could inform future conservation management decisions.

In the current study, we examined genetic and ecological differentiation among Hawaiian petrels breeding on the islands of Kauai and Hawaii (Fig. 1). We focused on colonies at the endpoints of the Hawaiian petrel breeding

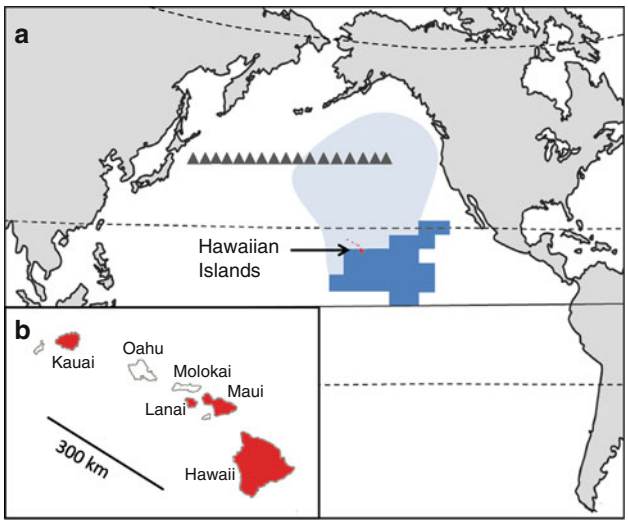


**Fig. 1** Marine distribution (**a**) and breeding distribution (**b**) of the Hawaiian petrel. The *dark blue area* indicates the combined spring and autumn distribution as reported by Spear et al. (1995). The *light blue area* approximates additional areas frequented by the Hawaiian petrel, as inferred from Simons and Hodges (1998). Islands with modern breeding colonies are *shaded red*. *Triangles* mark the origin of black-footed and Laysan albatross in Fig. 3. The *solid line* represents the Equator, while *stippled lines* represent, from south to north, the Tropics of Capricorn and Cancer, and the Arctic Circle

range because we considered them most likely to display genetic and ecological differentiation. To investigate the genetic population structure, we sequenced the mitochondrial *Cytochrome b* gene, which has been widely used in genetic studies of procellariiforms (Austin et al. 2004; Bretagnolle et al. 1998; Nunn and Stanley 1998). To study potential variation in foraging ecology, we examined the stable isotope composition of flight feathers from hatch-year and adult petrels. Specifically, we assembled information on trophic dynamics and foraging location using stable carbon and nitrogen isotopes (Michener and Schell 1994). We also examined the hydrogen isotope composition of Hawaiian petrel feathers. In North American terrestrial environments, hydrogen isotope values of water fluctuate with latitude, and this variation is transferred to organisms such as birds (Hobson et al. 1999). Such variation is not expected in marine organisms that derive their hydrogen from the isotopically homogeneous ocean (Craig 1961; Lecuyer et al. 1997). Thus, an investigation of hydrogen isotopes in the Hawaiian petrel can offer insight into factors other than latitude that may contribute to variation within free-ranging birds in general. Together, we used isotope and genetic techniques to gain a better understanding of Hawaiian petrel ecological and genetic diversity.

## Materials and methods

### Sampling

We sampled a total of 80 Hawaiian petrels, 40 from the island of Kauai and 40 from Hawaii. All individuals were included in genetic analyses, but samples for isotope analysis were only available from 24 Hawaii and 25 Kauai birds. We collected samples from carcasses of birds that were depredated by introduced mammals or that, upon attraction to lights, had collided with human-constructed objects between 1989 and 2009. We also obtained samples from birds rehabilitated after grounding, as well as from two individuals that were collected and prepared as museum skins in 1980 and 1995.

For genetic analyses, we extracted DNA from blood, tissue, feather, toe pad, or bone. For stable isotope analyses, we sampled the same flight feather, primary 1 (P1), from salvaged carcasses of both adult and hatch-year birds (<1 year in age). Flight feathers in hatch-year birds are grown during the late growth stages, from September to December (Ainley et al. 1997; Simons 1985). As in other *Pterodroma*, adult Hawaiian petrel presumably begin molt immediately after cessation of nest attendance (December for breeding adults), with primary molt proceeding distally from P1 (Pyle 2008; Warham 1996). We also sampled P1 from salvaged carcasses of eight adult Newell's shearwaters (*Puffinus auricularis newelli*) collected between 2002 and 2006. We included this species in our analyses for the sake of comparison, as molt location can be constrained to the eastern tropical North Pacific (Spear et al. 1995). Similar to the Hawaiian petrel, Newell's shearwaters molt their remiges at sea following their departure from breeding colonies (Jehl 1982). Molt likely proceeds distally from P1, as in other shearwaters (Pyle 2008).

To enable a comparison of isotope data from Hawaiian petrels and birds foraging in the Transition Zone of the North Pacific (the region bordered by the subarctic and subtropical frontal zones), we reference black-footed albatross (*Ph. nigripes)* and Laysan albatross muscle data taken from birds killed in driftnet fisheries (Gould et al. 1997). We included only breeding-age adult albatross in our data set, all of which died between 40 and 45°N and 145°E and 145°W (Fig. 1). Specifically, we selected individuals that were believed to have obtained their isotope values at sea during the non-breeding season, as opposed to birds that recently migrated or provisioned chicks. To this end, we reference data from adults that died between September and November, at least 3 months after departure from their breeding colonies. Because different tissues may vary in their isotopic enrichment relative to diet, we standardized stable isotope values from muscle to those of feather by correcting for the average offset between muscle and flight feather in hatch-year Hawaiian petrels. Isotope values from both tissues reflect diet during the 4 months of chick growth; most likely the latter 2 months (Hobson and Clark 1992; Simons 1985; Tieszen et al. 1983). We felt this comparison was the best approximation of a difference in tissue-specific fractionation factors for our species.

### Genetic analyses

Genetic analyses assessed levels of divergence and gene flow between petrels breeding on Hawaii and Kauai. We extracted DNA from blood and tissue samples via the Qiagen DNEasy Blood and Tissue kit, or through a standard phenol/chloroform procedure with centrifugal dialysis for toe pad, feather, and bone samples (Fleischer et al. 2000). A 524 bp region of the mitochondrial *Cytochrome b* gene was amplified using the primers CytbL and CytbR9 (Welch et al. 2011), which were designed in Primer3 (Rozen and Skaletsky 2000) from petrel sequences available on Genbank. However, some samples had low DNA concentrations and/or quality, so short overlapping primers were also developed to amplify the same region (Welch et al. 2011). Polymerase chain reaction was performed in 15 μL total reaction volumes containing 1× PCR Gold Buffer (Applied Biosystems), 0.2 mM of each dNTP, 2.5–3.5 mM $MgCl_2$, 1.2 mg/mL BSA (New England

Biolabs), 0.5 μM of each primer, 1 unit AmpliTaqGold DNA Polymerase (Applied Biosystems), and 1–2 μL DNA extract. DNA was initially denatured for 8 min at 94°C, followed by 35–40 cycles of 30 s at 92°C, 30 s at a primer-specific annealing temperature between 50 and 55°C, and 30–60 s extension at 72°C, with a final extension step of 7 min at 72°C. PCR products were cleaned up using a 1/10 dilution of ExoSAP-IT (USB) before cycle sequencing in both directions using the Big Dye Terminator v.3.1 cycle-sequencing kit from Applied Biosystems. Cycle-sequencing reactions were purified using Sephadex G-50 fine columns prior to being electrophoresed in a 3130XL genetic analyzer (Applied Biosystems). Sequences were aligned and visually inspected in Sequencher v.4.8 (Gene-Codes Corporation).

We examined *Cytochrome b* sequences to confirm that they had a mitochondrial origin and were not nuclear copies (Sorenson and Quinn 1998). Amplifications of the same individual with overlapping fragments resulted in identical sequences, and no insertions, deletions, or ambiguous bases were found. After translation in DAMBE 5.1.2, no gaps, non-sense or stop codons were detected, and most substitutions occurred in the third codon position (4, 0, and 10 substitutions for the first, second, and third codon positions, respectively). Additionally, 13 of 14 observed substitutions were transitions, and only two substitutions resulted in nonsynonymous changes. Therefore, it is unlikely that any of these sequences represent nuclear copies.

Haplotype diversity and nucleotide diversity were calculated in Arlequin v3.1 (Excoffier et al. 2005). An analysis of molecular variance (AMOVA) was also performed with significance determined through 1,000 permutations. The Hasegawa–Kishino–Yano model plus a proportion of invariant sites (HKY + I) was selected as the best-fit substitution model under the Akaike information criterion in jModelTest (Posada 2008; Posada and Buckley 2004). However, this model was not available in Arlequin, so the Kimura two-parameter (K80) model, which is very similar, was used instead. A 95% statistical parsimony haplotype network was created in TCS v.1.21 (Clement et al. 2000). Networks graphically depict relationships among haplotypes in closely related populations where ancestral alleles may still be present (Posada and Crandall 2001). Sequence divergence within each population as well as the net divergence between populations were calculated in Mega4 (Tamura et al. 2007) using a K80 distance matrix. Standard error was estimated through 500 bootstrap replicates.

Migration rates were estimated in the coalescent-based program Migrate-n v.3.0.3 (Beerli and Felsenstein 1999). As opposed to summary statistics, coalescent-based analyses take into account the genealogical relationships of the samples to estimate demographic parameters (Kuhner 2009). In Migrate, estimates are compounded by the mutation rate per generation, $\mu$ (i.e., $m = M\mu$, where $M$ is the estimate of migration). The mutation rate was assumed to be $1.89 \times 10^{-8}$ substitutions/year, as estimated for the *Cytochrome b* gene in procellariiform seabirds (Weir and Schluter 2008). The generation time for Hawaiian petrels is unknown; however, age at first breeding is correlated with body size in seabirds of this order (Warham 1990). Petrels similar in size to the Hawaiian petrel begin to breed at approximately 5–6 years of age (Simons 1984), and albatrosses begin to breed regularly by about 12 years of age (Warham 1996). The age at first breeding may not be equivalent to generation time, so demographic estimates were calculated using both 6 and 12 years. Interpretations were consistent regardless of the generation time used, so results are presented for a conservative generation time estimate of 12 years.

For all Migrate analyses, we employed the Bayesian inference method with uniform priors (Holder and Lewis 2003). The analyses utilized a single long chain and three heated chains of 10 million steps each, with the first 10% discarded as burn-in. The static heating scheme was used, with temperatures set to 1.0, 1.5, 3.0 and 6.0. This analysis was conducted concurrently with two additional replicates, and repeated independently so that runs could be compared to assess convergence. The effective sample size (ESS) was greater than 7,000 in all runs. While two intervening Hawaiian petrel populations are not represented in the current study, estimates of migration obtained from Migrate have been found to be robust as long as migration rates from the unsampled populations into the sampled populations are of approximately the same magnitude or lower than migration rates between the sampled populations (Beerli 2004).

The program Bayesian Evolutionary Analysis by Sampling Trees, or BEAST, v.1.5.4 (Drummond and Rambaut 2007) was employed to estimate the divergence time between the Kauai and Hawaii petrel populations. The strict molecular clock model (Arbogast et al. 2002) was implemented with the substitution rate fixed at $1.89 \times 10^{-8}$, as above. The HKY + I substitution model was implemented as selected by the AIC. A corresponding 524 bp *Cytochrome b* sequence (Genbank accession number HQ420337) from the Galapagos petrel (*Pterodroma phaeopygia)*, the sister taxon of the Hawaiian petrel, was aligned to Hawaiian petrel sequences to root the tree. Therefore, a Yule tree prior was utilized for dating analyses. Analyses were conducted for 10 million generations, with the first 10% excluded as burn-in. Stationarity was assessed in Tracer v.1.5 (Drummond and Rambaut 2007) through plots of the –lnL across generations and an examination of the ESS, which was greater than 500 for all parameters. Multiple independent runs were compared to assess convergence.

### Stable isotope analyses

All feathers were washed in solvent (87:13 chloroform:methanol by volume), rinsed with ultrapure distilled water (E-Pure, Barnstead), and dried at 25°C in a vacuum oven. We then took samples representative of the entire feather vanes using the barb or 3-section protocols described in Wiley et al. (2010). For $\delta^{13}$C and $\delta^{15}$N analysis, we weighed 0.8–1.0 mg aliquots of feather into tin capsules and analyzed them with an elemental analyzer (Eurovector) interfaced to an Isoprime mass spectrometer (Elementar). For $\delta$D analysis, we weighed 0.5 mg aliquots of feather vane into silver capsules and allowed them to air-equilibrate under ambient laboratory conditions for a minimum of 2 weeks prior to $\delta$D analysis (Wassenaar and Hobson 2003). Following equilibration to local conditions, samples were pyrolyzed at 1,425°C in a high-temperature elemental analyzer (TC/EA, Thermo Finnigan) interfaced to an isotope ratio mass spectrometer (Thermo Finnigan DeltaPlus XL).

Stable isotope values are expressed in per mil (‰) as

$$\delta X = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1,000$$

where $X$ is $^{13}$C, $^{15}$N, or D and $R$ is the corresponding ratio $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N, or D/H. $R_{\text{standard}}$ is V-PDB, air, and V-SMOW for $\delta^{13}$C, $\delta^{15}$N, and $\delta$D, respectively. For $\delta^{13}$C and $\delta^{15}$N, we analyzed laboratory standards between every five unknowns, with a precision of $\leq$0.2‰ for both $\delta^{13}$C and $\delta^{15}$N. Nonexchangeable $\delta$D values were normalized to in-house keratin standards (LA bear hair, −78.1‰, AK bear hair, −171.5‰) calibrated to BWB-CHS-CFS (Wassenaar and Hobson 2003). Analytical precision and accuracy were $\leq$4‰. Because many of our unknown samples were outside of the calibration range, we performed several experiments in an effort to constrain the extrapolation error (Kelly et al. 2009). Specifically, water standards (V-SMOW, 0‰; V-SLAP, −428‰) were dispensed ($\sim$0.25 μL) into silver capsules, crimp sealed, and analyzed against solid inorganic (IAEA-CH7, −100‰; benzoic acid, −61‰) standards. The experiments confirmed linearity when data are extrapolated 100‰ beyond our calibration range, and as a consequence of this extrapolation our error increases to ±8‰. We recognize that matrix differences confound the interpretation of this test, but complex organic standards with high $\delta$D values are currently lacking, though desperately needed (Kelly et al. 2009). We also analyzed the $\delta$D composition of muscle from a variety of species from the Pacific Ocean. Whereas hydrogen exchangeability in muscle has received little study compared to keratin, the magnitude of exchange appears to be similar (Wassenaar and Hobson 2000; Chesson et al. 2009). To this end, we analyzed lipid-extracted muscle tissue in the same fashion as feather samples, including normalization to keratin standards (Doucett et al. 2007; Finlay et al. 2010).

### Statistical analyses

We assessed differences in isotope values between island and age groups through a multiple analysis of variance (MANOVA) using the general linear model procedure (GLM) in SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). We assessed temporal variation in isotope values in the same manner, with year of feather growth, El Niño-Southern Oscillation (ENSO; cold, neutral, or warm phase as indicated by the National Weather Service Climate Prediction Center), and century (before or after 2001) designated as independent variables in the MANOVA. For the evaluation of ENSO, 2006 and 2009 were considered warm-phase years, and 1989, 1998, and 2000 were categorized cold-phase years. We performed separate MANOVAs for individuals from Hawaii, adults from Kauai, and hatch-year birds from Kauai. Age groups from Hawaii were combined for this temporal analysis because they showed no significant differences in any isotope values (see "Results"). Among Kauai adults, only the effect of year could be incorporated into our statistical model due to the lack of variation in century and ENSO phase. For all statistical models, normal quantile–quantile plots and Levene's tests were used to check assumptions of normality and homogeneity of variances. In subsequent figures and text, isotope values for island and age groups are reported as averages ± standard errors.

## Results

### Genetic analyses

We sequenced 524 bp of the mitochondrial *Cytochrome b* gene in 80 Hawaiian petrels, 40 from each island (Genbank accession numbers JF264905-72, HQ420355-60, HQ420366-68, HQ420372-74). Eleven haplotypes were discovered from 14 polymorphic sites. Six haplotypes occurred in individuals from Hawaii and eight occurred in individuals from Kauai, with three shared between the two islands. However, shared haplotypes were found at different frequencies (0.05 vs. 0.70, 0.28 vs. 0.05, and 0.15 vs. 0.03 for the Hawaii and Kauai populations, respectively; Fig. 2). The majority of Kauai birds shared a single haplotype, whereas two haplotypes were common in Hawaii individuals. Three to four mutational steps separated the most common haplotypes in each population. Gene diversity was significantly higher ($p < 0.05$) in petrels from Hawaii (0.74 ± 0.04) than Kauai (0.51 ± 0.09). Nucleotide diversity was similar between the two populations

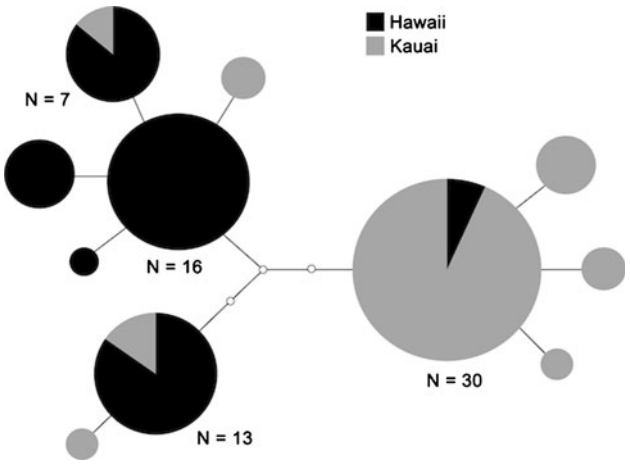


**Fig. 2** Haplotype network of *Cytochrome b* sequences of Hawaiian petrels from Hawaii (*black*) and Kauai (*gray*). *Each circle* represents a unique sequence, and the size of the circle is proportional to the number of individuals possessing that sequence (sample sizes are indicated for the most common haplotypes). *Each line* represents one mutational step, and *small white circles* represent an unsampled intermediate sequence

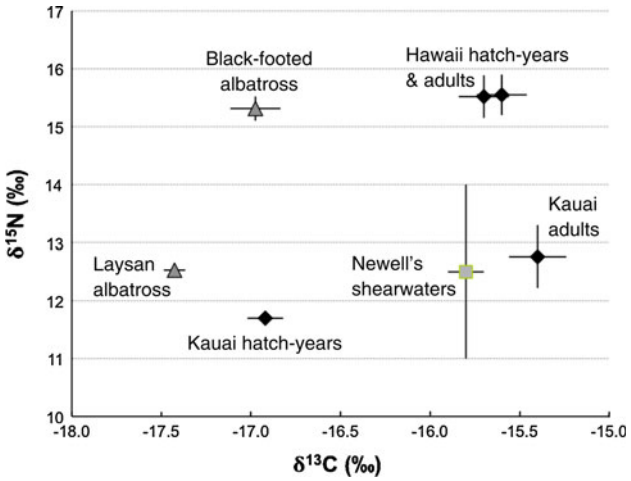


**Fig. 3** Stable carbon and nitrogen isotope values from Pacific pelagic seabirds (group averages ± standard errors). *Black diamonds* represent isotope data from Hawaiian petrel flight feathers. As noted in the graph, *squares* and *triangles* represent data from Newell's shearwater flight feathers and non-breeding adult albatross muscle. Stable isotope values from muscle are standardized to feather isotope values, as indicated in the section "Materials and methods." Sample sizes for Hawaiian petrel data are as follows: Hawaii adults ($N = 14$), Hawaii hatch-year birds ($N = 10$), Kauai adults ($N = 13$), and Kauai hatch-year birds ($N = 12$). Remaining sample sizes are: Newell's shearwaters ($N = 8$), Laysan albatross ($N = 55$), and black-footed albatross ($N = 16$). All albatross derive from 40 to 45°N and 145°W to 145°E (Transition Zone), as shown in Fig. 1

(0.0037 ± 0.0024 for Hawaii, and 0.0031 ± 0.0021 for Kauai).

AMOVA results revealed high levels of differentiation between petrels from Kauai and Hawaii, with $\Phi_{ST} = 0.50$ ($p < 0.0001$). Results from Migrate concurred. All Migrate runs produced distinct, unimodal posterior distributions which overlapped between independent runs. We found migration to be low and roughly symmetrical between populations of petrels on Kauai and Hawaii. Migration of Hawaii individuals into the Kauai population was estimated at $1.2 \times 10^{-4}$ migrants per generation (95% Highest Posterior Density (HPD): $4.5 \times 10^{-6}$ to $4.8 \times 10^{-4}$), while the migration rate from Kauai into the Hawaii population was $6.6 \times 10^{-5}$ migrants per generation (95% HPD: 0.0–$3.9 \times 10^{-4}$). Analyses in BEAST indicated that the mean population divergence time was 118,000 years ago (95% HPD: 61,800–185,000 years ago). Corrected pairwise sequence divergence between the two populations was 0.22 ± 0.40%.

## Stable isotope analyses

Among 49 Hawaiian petrels, $\delta^{13}C$ values varied between −17.7 and −14.6‰, and the range in $\delta^{15}N$ values was 10.6–17.6‰. While adults from the islands of Hawaii and Kauai did not differ in $\delta^{13}C$ ($p = 0.7243$), Hawaii adults had significantly higher $\delta^{15}N$ values as compared with those from Kauai (Fig. 3, $p < 0.0001$). Among Hawaii individuals, hatch-year birds and adults had similar isotope values and showed no significant difference in either $\delta^{13}C$ or $\delta^{15}N$ ($p = 0.5721$ for $\delta^{13}C$ and $p = 0.9581$ for $\delta^{15}N$). In contrast, hatch-year birds from Kauai had significantly lower $\delta^{15}N$ and $\delta^{13}C$ values than adults from the same island ($p < 0.0001$). Kauai hatch-year birds were also lower in $\delta^{13}C$ than adults and hatch-year birds from the island of Hawaii ($p \leq 0.0001$). Eight Newell's shearwaters from Kauai showed a broad range of $\delta^{15}N$ values between 8.5 and 17.6‰, while their $\delta^{13}C$ values ranged from −16.1 to –15.3‰.

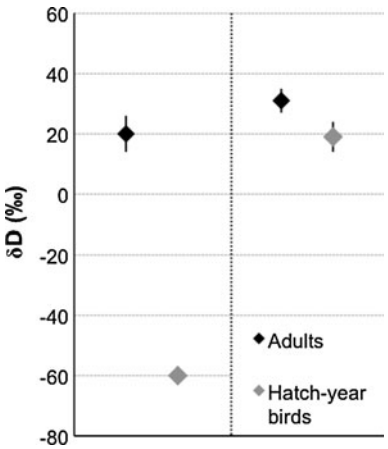


**Fig. 4** Stable hydrogen isotope values in Hawaiian petrel flight feathers. Sample sizes are as follows: Hawaii adults ($N = 14$), Hawaii hatch-year birds ($N = 10$), Kauai adults ($N = 13$), and Kauai hatch-year birds ($N = 12$)

Stable hydrogen isotope values varied extensively among Hawaiian petrels, from −69 to 53‰. Hatch-year birds from Kauai had an average $\delta$D value of −60 ± 3 (SE)‰, at least 79‰ lower than any other group (Fig. 4, $p < 0.0001$ for all comparisons). Kauai adults, Hawaii adults, and Hawaii hatch-year birds had average $\delta$D values of 20 ± 6, 31 ± 4, and 19 ± 5‰, respectively.

We found no significant effect of year, century, or ENSO phase on stable isotope values. Wilks' lambda test for the effects of year and ENSO had values >0.67 ($p > 0.25$) and Hotelling's trace test values for the effect of century were <0.49 ($p > 0.34$).

Among 12 hatch-year Hawaiian petrels, flight feathers were 1.5 ± 0.2 and 0.2 ± 0.4‰ higher in average $\delta^{13}$C and $\delta^{15}$N, respectively, than muscle.

## Discussion

### Genetic analyses

Genetic analyses indicate that Hawaiian petrels nesting on the islands of Hawaii and Kauai are significantly differentiated. While Hawaiian petrels are known to make foraging trips to the Gulf of Alaska during the breeding season, it appears that birds rarely disperse between these two islands, which lie just 500 km apart. Three haplotypes were found to be shared between islands; however, major differences in their frequencies, as demonstrated by the high level of $\Phi_{ST}$, suggest low migration. Sophisticated coalescent-based analyses also indicate that gene flow is very low, with less than one migrant per 1,000 generations. While Hawaiian petrels from other islands were not included in these analyses, migration estimates from Migrate, such as those presented here, have been found to be robust as long as gene flow is low to moderate from the missing populations (Beerli 2004). Even if gene flow from missing populations was high, the migration rates obtained here would represent overestimates (Beerli 2004). Therefore, dispersal between Kauai and Hawaii appears to be low. While these results are based on mitochondrial DNA sequences, and therefore only reflect female dispersal, male dispersal may also be low. Previous analyses of three nuclear intron loci from a subset of Hawaiian petrels from Kauai and Hawaii show similarly low levels of gene flow (Welch et al. 2011). Similarly, using a microsatellite data set, Friesen et al. (2006) found that populations of the Hawaiian petrel's sister taxon, the Galapagos petrel, are also highly differentiated ($F_{ST}$ between 0.07 and 0.26, with $p < 0.01$ for all comparisons).

BEAST analyses indicate that populations on Hawaii and Kauai may have diverged as recently as 100,000 years ago. Therefore, it is likely that the shared haplotypes between Hawaii and Kauai are a result of common ancestry. Corrected pairwise sequence divergence between the two populations of 0.22% is low, but similar to levels of divergence between some petrel and shearwater subspecies (Austin et al. 2004; Techow et al. 2009).

### Stable isotope analyses

Consistent with our genetic analyses, stable carbon and nitrogen isotopes reveal differentiation between Hawaiian petrels nesting on the islands of Kauai and Hawaii. Specifically, there is a disparity in average $\delta^{15}$N values between adults from the two islands, and a difference in $\delta^{15}$N and $\delta^{13}$C between hatch-year birds. To help explain factors that contribute to isotope variation, we present data from other nonbreeding, pelagic seabirds in the North Pacific (Fig. 3). From stable isotope and stomach content data, Gould et al. (1997) showed that black-footed albatross associated with drift net fisheries fed approximately one trophic level higher than Laysan albatross. While the albatross species show a trophic shift in both $\delta^{15}$N and $\delta^{13}$C, a similar difference in $\delta^{13}$C is not observed between adult Hawaiian petrels from Kauai and Hawaii. Thus, trophic level, alone, does not account for the isotopic variation between Hawaiian petrel populations. The likelihood that $\delta^{15}$N is not a simple function of trophic level in the Hawaiian petrel is further suggested by a comparison of Hawaiian petrels from the island of Hawaii and black-footed albatross. While these two groups have similarly high $\delta^{15}$N values, they are unlikely to feed at the same trophic level for several reasons. First, the two species differ greatly in size (e.g., culmen or bill length of 97 cm vs. 33 cm), and are therefore likely to target prey of different sizes and dissimilar trophic levels (Adams and Brown 1989; Ashmole and Ashmole 1967; Frings and Frings 1961; Simons 1985; Spear et al. 2007). Second, these particular black-footed albatross fed heavily on large neon flying squid (*Ommastrephes bartrami*, >40 cm in mantle length; Gould et al. 1997). Unless obtained as a fishery subsidy, the large size of the neon flying squid makes it an unlikely food source for Hawaiian petrels. The use of fishery subsidies is also improbable because Hawaiian petrels have not been reported near fishing vessels, have not been reported to feed on fishing offal, and do not closely associate with fisheries (as evidenced by records from the Burke Museum showing only three Hawaiian petrels associated with the North Pacific driftnet fishery, and P. Gould, personal communication). It is thus improbable that the two species forage at similar trophic levels.

Variation in $\delta^{15}$N between Hawaiian petrel populations is most likely associated with differences in foraging location and spatial variation in biogeochemical cycling at

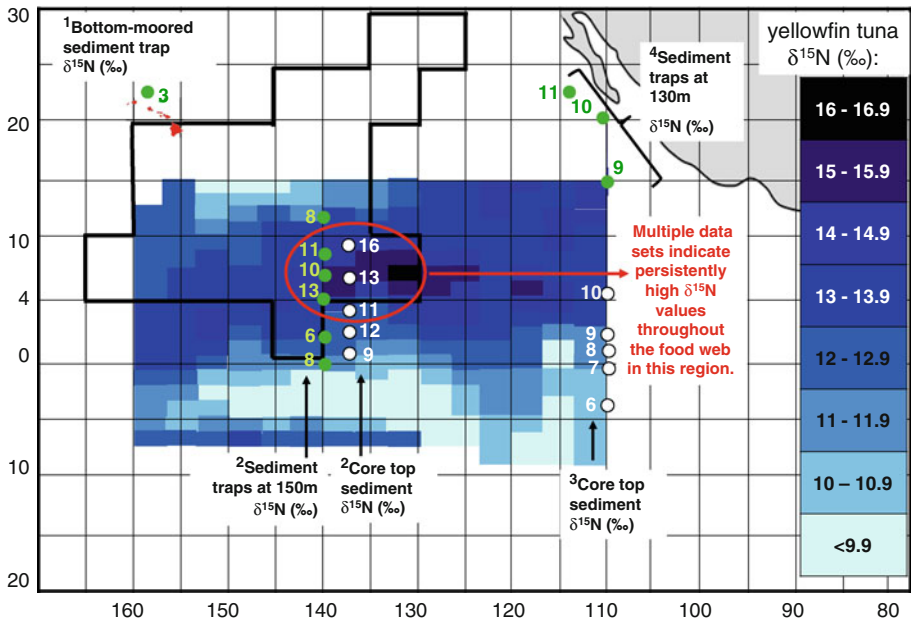


**Fig. 5** Partial distribution of the Hawaiian petrel and local variability in $\delta^{15}$N values. *Black outline* shows the Fall distribution of the Hawaiian petrel, as observed by Spear et al. (1995). *Shades of blue* represent nitrogen isotope values of a mobile consumer, yellowfin tuna (Graham et al. 2010). *Green-labeled points* represent stable nitrogen isotope values of sinking and floating sediments: [1]Karl et al. (1997) (annual mean export pulse), [2] Altabet and Francois (1994), and [4]Voss (2001). *White-labeled points* represent stable nitrogen isotope values of core top sediments: [2]Altabet and Francois (1994), [3]Farrell et al. (1995), and [4]Voss (2001). Latitude is indicated on the *x*-axis (°N) and longitude on the *y*-axis (°W). All points along 140°, 137°, and 110°W are floating or core top sediments, as indicated by *labeled arrows*

the base of the food web. A well-characterized nitrogen isotopic gradient in the eastern tropical North Pacific occurs within the foraging range of the Hawaiian petrel, with the most elevated sedimentary $\delta^{15}$N values observed at 4−10°N and 135–140°W (Fig. 5, location approximated by a red circle). This isotopic pattern arises from spatial variation in nitrogen cycling. In upwelling areas off the coast of Peru, high nitrate concentrations fuel denitrification, which produces a pool of residual nitrate that is markedly enriched in $^{15}$N (Farrell et al. 1995). A further increase in the $\delta^{15}$N of nitrate results from phytoplankton uptake (Altabet et al. 1991; Altabet and Francois 1994). Thus, phytoplankton uptake in the eastern tropical North Pacific results in a conspicuous meridional pattern whereby the $\delta^{15}$N of nitrate increases with distance from the locus of upwelling (Farrell et al. 1995). Such variation in $\delta^{15}$N of nitrate is mirrored in phytoplankton. Consequently, high $\delta^{15}$N values for nitrate and sediments, near 12–15‰, characterize areas where phytoplankton utilize a $^{15}$N-enriched reservoir of residual nitrate, as opposed to values of ≤10‰ for sediments derived from phytoplankton dependent on average marine nitrate (6‰) or fixed $N_2$ (0‰) (Altabet and Francois 1994).

Because isotope values at the base of the food web are passed along to consumers, $\delta^{15}$N values for yellowfin tuna (*Thunnus albacores*) also exhibit spatial trends (Fig. 5; Graham et al. 2010). Like tuna, highly mobile seabirds have the potential to incorporate isotope values unique to their foraging location. Newell's shearwaters grow their feathers in autumn when birds are commonly found southeast of the Hawaiian Islands (highest densities at 4–10°N, 130–165°W) in areas characterized by highly variable $\delta^{15}$N values (Fig. 5; Spear et al. 1995). The high variability in the $\delta^{15}$N among Newell's shearwaters (Fig. 3) may therefore be accounted for by individual variation in foraging location. Similarly, the disparity between average $\delta^{15}$N values of adult Hawaiian petrels from Hawaii and Kauai is likely related to differences in the locations in which these two populations concentrate their foraging efforts. If this is the case, breeding and molting Hawaii birds use a foraging area characterized by higher $\delta^{15}$N values than that used by molting Kauai adults. In this respect, the two populations exhibit segregation in at least one aspect of their marine niche.

Although $\delta^{13}$C values increase with trophic level within a food web from a specific location, there is an inverse relationship between $\delta^{13}$C and latitude for phytoplankton and upper level consumers, including squid and seabirds (Goericke and Fry 1994; Kelly 2000; Takai et al. 2000). The low $\delta^{13}$C values of Transition Zone albatross (from 40 to 45°N) relative to those of Newell's shearwaters, adult Hawaiian petrels, and hatch-year Hawaiian petrels from

Hawaii (Fig. 3) are therefore likely related to the albatross having foraged at more northerly latitudes than the other groups. Indeed, Newell's shearwaters concentrate their foraging between 4 and 10°N near the period of molt (Spear et al. 1995). Similarly, $\delta^{13}$C values in hatch-year Hawaiian petrels from Kauai are significantly lower than those found in other Hawaiian petrels, and likely derive from food resources located farther north.

Our interpretations are consistent with at-sea observations of the Hawaiian petrel. During the breeding season, individuals have been seen between the Hawaiian and Aleutian Islands (Bourne 1965; Bourne and Dixon 1975). However, Spear et al. (1995) also observed Hawaiian petrels to the southeast of the Hawaiian Islands during the breeding season and early nonbreeding season (Figs. 1 and 3). More recently, satellite telemetry revealed that chick-provisioning adults from Maui and Lanai forage extensively throughout the northeastern Pacific, including the Transition Zone and the Subarctic Frontal Domain near the Aleutian Islands (J. Adams, D. Ainley et al., unpublished data). Our $\delta^{13}$C and $\delta^{15}$N data suggest that breeding adults from Kauai also forage north of the Hawaiian Islands. Thus, chick-provisioning adults from Kauai may be among the individuals observed by Bourne and Dixon between the Hawaiian and Aleutian Islands. Our data further suggest that individuals observed southeast of Hawaii by Spear et al. (1995) included chick-provisioning adults from Hawaii, and both Hawaii and Kauai adults during the period of molt.

Surprisingly, Hawaiian petrels showed a very broad range of $\delta$D values (−69 to 53‰), despite the uniform $\delta$D of their ultimate water source (Pacific Ocean: 0‰; Fig. 4; Lecuyer et al. 1997). The variation in $\delta$D between Hawaiian petrel groups mirrors the separation observed in $\delta^{13}$C values (Fig. 3). In general, marine sediments and organisms exhibit $\delta$D values near or below −100‰, reflecting fractionation between plants and their water source during photosynthesis (Estep and Hoering 1980; Hoering 1974; Stuermer et al. 1978). The prevalence of low $\delta$D values in marine ecosystems is further supported by our measurements of neon flying squid, lancetfish (*Alepisaurus* spp.), Pacific herring (*Clupea pallasii*), eulachon (*Thaleichthys pacificus*), and Pacific ocean perch (*Sebastes alutus*), which range from −93 to −130‰. Because seabirds typically derive >80% of their water from prey, low $\delta$D values are also expected in Hawaiian petrels (Goldstein 2001). However, this was not the case for adult Hawaiian petrels from Kauai or any of the Hawaiian petrels from Hawaii.

Water lost through evaporatory processes has a $\delta$D value that is ~50‰ lower than body water in captive rock pigeons and results in an enrichment in body water deuterium (McKechnie et al. 2004). While evaporatory processes may contribute to the $\delta$D variation observed among Hawaiian petrels, it is unlikely to result in the 120‰ range of $\delta$D values in our dataset. Furthermore, variation in evaporative water loss is most likely to impose a difference in $\delta$D values between hatch-year birds (feathers grown while sitting in cool, high-elevation burrows) and adults (feathers grown while flying at sea, where birds are more likely to employ evaporative cooling). However, the difference in $\delta$D between the two age classes of Hawaiian petrels from Hawaii was small (12‰). Similar to water lost through evaporation, we expect water lost through salt glands to have low $\delta$D values relative to body water, and consider it to be a more probable source of elevated $\delta$D values in the Hawaiian petrel. Consumption of prey such as squid and crustaceans that are isosmotic with seawater versus hyposmotic teleost fish can result in a doubling of salt load for seabirds (Goldstein 2001). Increasing salt load, in turn, results in increased salt gland excretion by petrels (Goldstein 2001; Warham 1996). Thus, our data may reflect a greater reliance on fish by Kauai hatch-year birds and a heavier reliance on squid and crustaceans by molting adults and Hawaii hatch-year birds. While Hawaiian petrels are known to feed on both fish and squid (Simons 1985), further research is clearly needed to delineate the causes of $\delta$D variation in pelagic seabirds. In avian research, stable hydrogen isotopes are primarily used to study the migration of terrestrial birds. However, variation in our data demonstrates that factors other than source water can have a large influence on $\delta$D values, at least in some species. The clear segregation of $\delta$D values among Hawaiian petrel age and island groups also suggests that stable hydrogen isotopes may provide useful information about seabird physiology and ecology.

## Causes and implications of genetic and isotopic variation

The genetic and ecological variation we observed between Hawaiian petrels from Kauai and Hawaii reveal unexpectedly complex intra-specific dynamics. Movement between islands is clearly not limited by dispersal ability, as evidenced by the extreme length of foraging trips (often >10,000 km; Adams and Flora 2009; J. Adams, D. Ainley et al., unpublished data). Furthermore, populations on Hawaii and Kauai appear to breed synchronously (S. Judge, unpublished data), and there are no clear morphological differences that separate them. Given the many similarities between Hawaiian petrels from Kauai and Hawaii, the inter-colony variation we observed appears to be a compelling example of cryptic ecological and genetic diversity.

Several factors may have contributed to the genetic differentiation between Hawaiian petrel colonies. Simons (1985) found evidence of strong nest-site and mate fidelity

among Hawaiian petrels, and this philopatry likely decreases dispersal between colonies and contributes to genetic isolation (Friesen et al. 2007). Divergence in foraging locations may have further reduced contact between colonies and imposed distinct selective pressures. Adaptation to local breeding environment is another possible source of divergence. In Kauai, Hawaiian petrels breed in humid areas characterized by dense vegetation receiving over 1,200 cm of precipitation per year. In contrast, Hawaiian petrels on Hawaii breed in open, xeric environments that are 2,000–3,000 m higher in elevation than breeding areas on Kauai (Ainley et al. 1997; Hu et al. 2001). Differences in breeding habitat could result in different selective pressures between islands and reduced fitness among immigrants. However, Hawaiian petrels may have nested in a wide range of habitats on both Kauai and Hawaii in the past, before they were confined to their current range by habitat loss, human harvesting, and introduced mammalian predators (Olson and James 1982; Harrison 1990).

The significant isotopic differences between both adult and hatch-year birds from Hawaii and Kauai result from their unique foraging strategies. Although reminiscent of habitat segregation between closely related seabird species (Fasola et al. 1989; Finkelstein et al. 2006), such differentiation is rarely observed between seabird populations (Navarro et al. 2009). Competition-driven niche segregation, or niche partitioning, may be at work in the Hawaiian petrel, and may have developed as a mechanism to reduce competition and allow the coexistence of geographically proximate colonies. Competition among seabirds is thought to be especially strong during the breeding season, when adults must consistently return to similar nesting locations, and it may account for the particularly large divergence between hatch-year birds from Kauai and Hawaii (Forero et al. 2002; Lewis et al. 2001).

Genetic and ecological variation between Hawaiian petrel populations has important conservation implications. Procellariiform seabirds are a highly endangered group and face numerous threats from fishing, habitat loss, and introduced predators (Bartle et al. 1993; Bell and Merton 2002). While further studies of the Lanai and Maui populations are required, it appears that distinct management units exist within the Hawaiian petrel (Moritz 1994, 2002). Conservation measures should be considered on an island-to-island basis, as opposed to working under the assumption that populations are homogeneous. Inter-colony diversification in additional seabird species may be unrecognized due to the logistical difficulties of studying cryptic and highly mobile marine organisms, particularly when they are rare. Genetic and isotopic methods, such as those reported here, can be used to help overcome this barrier and increase knowledge of the diversity and ecology of marine vertebrates.

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