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ORIGINAL ARTICLE

Population divergence and gene flow in an endangered and highly mobile seabird

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Seabirds are highly vagile and can disperse up to thousands of kilometers, making it difficult to identify the factors that promote isolation between populations. The endemic Hawaiian petrel (*Pterodroma sandwichensis*) is one such species. Today it is endangered, and known to breed only on the islands of Hawaii, Maui, Lanai and Kauai. Historical records indicate that a large population formerly bred on Molokai as well, but this population has recently been extirpated. Given the great dispersal potential of these petrels, it remains unclear if populations are genetically distinct and which factors may contribute to isolation between them. We sampled petrels from across their range, including individuals from the presumably extirpated Molokai population. We sequenced 524 bp of mitochondrial DNA, 741 bp from three nuclear introns, and genotyped 18 microsatellite loci in order to examine the patterns of divergence in this species and to investigate the potential underlying mechanisms. Both mitochondrial and nuclear data sets indicated significant genetic differentiation among all modern populations, but no differentiation was found between historic samples from Molokai and modern birds from Lanai. Population-specific nonbreeding distribution and strong natal philopatry may reduce gene flow between populations. However, the lack of population structure between extirpated Molokai birds and modern birds on Lanai indicates that there was substantial gene flow between these populations and that petrels may be able to overcome barriers to dispersal prior to complete extirpation. Hawaiian petrel populations could be considered distinct management units, however, the dwindling population on Hawaii may require translocation to prevent extirpation in the near future.
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INTRODUCTION

Populations are fundamental units in evolutionary biology and exist in a balance between genetic isolation and gene flow (Allendorf and Luikart, 2007). The mechanisms involved in reducing gene flow and promoting genetic differentiation between populations of highly mobile seabirds, which can travel hundreds to thousands of kilometers during a single foraging trip, can be difficult to identify *a priori*. Several mechanisms have been hypothesized (Friesen *et al.*, 2007a). Physical barriers, such as the Isthmus of Panama and continental landmasses, can isolate populations in different ocean basins (Steeves *et al.*, 2005). Geographic distance between colonies may also be important and seabirds may preferentially disperse to neighboring colonies over more distant ones (Burg *et al.*, 2003). Strong philopatry may also be an important mechanism. Once seabirds have selected a breeding site, they are known to return there over multiple years to breed (Warham, 1990). These species may also exhibit philpatry to natal sites. Given the widespread and patchy distribution of suitable breeding habitat, birds may return to their natal colony to breed, as they know from experience that a chick can be successfully raised there (Friesen *et al.*, 2007a). However, seabirds may disperse if habitat quality decreases, for example, due to density-dependent factors (Kildaw, 2005). Finally, population-specific nonbreeding or foraging distribution may be important in reducing contact between individuals from different colonies (Burg and Croxall, 2001). The relative importance of these factors remains unclear. It is also unclear if these mechanisms are strong enough to prevent dispersal from colonies at risk of becoming extirpated, for example, due to the introduction of exotic mammalian predators.

Investigating levels of gene flow and the factors promoting genetic differentiation in seabirds has important implications for their conservation. Today, many seabird species, and in some cases nearly entire genera, are threatened by extinction (Gangloff *et al.*, 2011). Information about population genetic differentiation and levels of dispersal can be useful for identifying management units. Such knowledge can also be useful for informing translocation strategies to promote demographic stability, prevent inbreeding and preserve genetic

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diversity (Frankham *et al*., 2002). Preventing the further decline of seabird populations is important because these species have broad ecological impacts. As marine predators, seabirds have an essential role in transferring marine nutrients to terrestrial ecosystems on oceanic islands, and therefore they can influence ecosystem productivity at multiple levels (Croll *et al*., 2005; Fukami *et al*., 2006).

The Hawaiian petrel (*Pterodroma sandwichensis*) is a highly mobile, endangered, pelagic seabird (Simons and Hodges, 1998; Pyle and Pyle, 2009). It is known to make foraging trips in excess of 10 000 km during the breeding season, ranging from the equator to the Aleutian Islands (Spear *et al*., 1995; Simons and Hodges, 1998; Adams and Flora, 2010). The Hawaiian petrel is currently known to breed on the islands of Hawaii, Maui, Lanai and Kauai; however, the population on Hawaii appears to be declining at a severe rate and is at risk for extirpation (Hu *et al*., 2001). On the basis of subfossil and historical evidence it is clear that the Hawaiian petrel was much more widespread in the past. Range contractions have occurred on islands with contemporary breeding colonies, and this species appears to have been extirpated completely from Oahu and Molokai (Pyle and Pyle, 2009). Subfossil records indicate that a large population on Oahu may have been extirpated prior to or shortly after European contact in 1778 AD (Olson and James, 1982). Historical records (Munro, 1955) indicate that until recently a large population also bred on the island of Molokai, where petrels were so abundant that they 'darkened the sky.' Radar surveys suggest a small number of birds (presumably petrels) visit the island (Pyle and Pyle, 2009), but reports from recent surveys indicate that only five to ten birds have been heard calling, and no burrows or breeding colonies have been located so far (Birdlife International, 2011).

Little information is available about movement among Hawaiian petrel populations. These birds are difficult to study because they spend most of their life at sea and return to land nocturnally to breed in underground burrows. In addition, extant colonies are located at the highest elevations on each island in remote and very rugged terrain (Simons and Hodges, 1998). Banding studies in a colony on Maui have shown that adults are highly philopatric, returning to the same burrow to breed for multiple years (Simons, 1985). However, delayed maturity (these petrels do not begin to breed until ~6 years of age), coupled with the difficulty of conducting thorough surveys for burrows in rugged terrain, mean that banded chicks have not been resighted (Simons, 1984) and direct evidence of natal philopatry is lacking. Genetic information on dispersal is also limited. Browne *et al*. (1997) analyzed 13 allozyme loci from blood to study the taxonomy of Galapagos (*P. phaeopygia*) and Hawaiian petrels. Hawaiian petrels were sampled from a single colony on Maui and all individuals were found to be monomorphic at all loci. Recent evidence from mitochondrial and nuclear DNA sequences obtained from a limited number of individuals indicates that population structure may be present among colonies of these birds on different islands (Welch *et al*., 2011). However, a more extensive analysis using larger sample sizes and additional population level data, for example, analysis of microsatellite genotypes, is needed.

We have conducted a comprehensive, range-wide population genetic study of the Hawaiian petrel. We obtained samples from all islands where these birds have been known to breed in the past two centuries and developed three genetic data sets, consisting of mitochondrial and nuclear DNA sequences as well as genotypes from 18 microsatellite loci. These data were analyzed to investigate population differentiation and gene flow among all extant populations and a presumably extirpated population from the island Molokai. Furthermore, we discuss the factors promoting population divergence in this highly mobile species.

MATERIALS AND METHODS

Samples

A total of 322 Hawaiian petrel samples were obtained from across the current and historical breeding range of this species (Table 1, Figure 1). Modern samples (N=294) consisting of blood, muscle or other tissue, bone and feather were salvaged from carcasses of birds depredated in breeding colonies on Hawaii, Maui, Lanai and Kauai, between 1990 and 2010, or collected from birds that were handled during conservation management procedures, such as rehabilitation following power-line strikes or crashes due to disorientation caused by artificial light sources (Podolsky *et al*., 1998). Crashed birds and carcasses were assumed to be breeders (or the offspring of breeders) on the island where they were discovered, as nonbreeders depart during the first half of chick-rearing (Simons, 1985; Simons and Hodges, 1998). Blood samples of chicks previously collected from Haleakala National Park, Maui, were also obtained (Browne *et al*., 1997). Toe pads were sampled from historical Hawaiian petrel museum specimens (N=28), originally collected on Molokai in 1907 and 1914 and deposited at the Bernice P. Bishop and the Los Angeles County Natural History Museums (Supplementary Table 1). It is possible that genetic differentiation could occur among different colonies on the same island (Friesen *et al*., 2007b), such as between east and west Maui; however, sample sizes are not sufficient to address those questions here, and therefore samples were grouped according to island for all analyses.

Molecular techniques

Genomic DNA was extracted from blood and tissue samples using the DNEasy tissue kit (Qiagen, Valencia, CA, USA). DNA was obtained from bone, feather and toe pad samples via phenol/chloroform extraction and centrifugal dialysis

Table 1 Sample sizes for Hawaiian petrels (*P. sandwichensis*) obtained from all islands where this species is currently, or was historically, known to breed

Island	*Time period*	*Sample size*		
		mtDNA	*nuDNA*	*Micros*
Hawaii	Modern	71	51	48
Maui	Modern	122	54	114
Lanai	Modern	38	25	28
Molokai	Historic	28	0	0
Kauai	Modern	63	34	42
Total		322	164	232

Abbreviations: mtDNA, mitochondrial DNA sequences; nuDNA, nuclear intron DNA sequences.

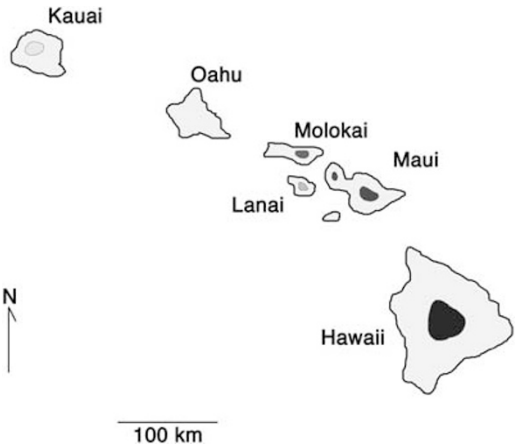

Figure 1 Map of the main Hawaiian Islands with approximate locations of modern (Hawaii, Maui, Lanai and Kauai) and historically known (Molokai) breeding colonies shaded.

(Fleischer *et al*., 2000). All extractions for historical samples were performed in a physically separate, dedicated ancient DNA laboratory, and a sample from a different species was extracted in between each Hawaiian petrel sample to detect cross-contamination. Multiple extraction and negative PCR controls were also used to detect contamination.

Three data sets were generated for this study. First, a 524-bp fragment of the mitochondrial *Cytochrome b* gene was amplified using the primers CytbL and CytbR9 according to Welch *et al*. (2011). The control region is more variable than *Cytochrome b*, and often used for population level studies, but this region has been duplicated in procellariiform seabirds (Abbott *et al*., 2005). To minimize the potential for error and uncertainty, especially in ancient DNA sequences, we utilized the variable 5′ region of the *Cytochrome b* gene instead. Second, a set of three nuclear intron loci were sequenced, including *α-Enolase* (Enol) intron 8, *Lamin A* (Lam) intron 3 and *Ribosomal Protein 40* (RP40) intron 5 (Friesen *et al*., 1997, 1999). For both sequence data sets, Primer3 (Rozen and Skaletsky, 2000) was used to design primers to amplify small, overlapping fragments for historic and degraded samples (Supplementary Table 2). Third, a set of 18 polymorphic microsatellite loci were amplified (Welch and Fleischer, 2011). For historic samples, a minimum of two independent amplifications were conducted for each mitochondrial and nuclear intron primer set. For microsatellites, loci were amplified and assayed between three and five times per individual for all samples (Taberlet *et al*., 1996). The genotyping error rate was found to be 0.05%.

Polymerase chain reactions were carried out in 15 μl (for modern) or 25 μl (for historical samples) total volumes. Reactions consisted of 1× colorless GoTaq Flexi buffer (Promega, Madison, WI, USA) or PCR Gold Buffer (Applied Biosystems (ABI), Carlsbad, CA, USA), 2.0–4.0 mM $MgCl_2$, 0.2 mM each dNTP, 1.2 mg ml^{-1} bovine serum albumin, 0.5 μM each primer, 1 unit of Promega GoTaq Flexi or AmpliTaq Gold DNA polymerase and 1–3 μl DNA extract. Thermocycle profiles consisted of a denaturation step of either 95 °C for 2 min (for GoTaq Flexi) or 94 °C for 8 min (for AmpliTaq Gold), followed by 35–45 cycles of 95 °C for 30 s, a primer-specific annealing temperature for 30 s, 72 °C for 30–45 s, proportional to the length of the fragment, and a final 72 °C extension step for either 7 min for sequences or 30 min for microsatellites. For sequencing, PCR products were cleaned up using a 1:10 dilution of ExoSAP-IT (USB, Cleveland, OH, USA), cycle-sequenced in both directions using the Big Dye Terminator v3.1 Cycle-Sequencing kit (ABI), and then purified through Sephadex G-50 fine columns (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). All fragments were electrophoresed on an ABI 3130xL Genetic Analyzer. Sequences were assembled, aligned and visually inspected in Sequencher v 4.9, and genotypes were assigned manually in GeneMapper v 4.1.

Data analyses

Prior to any analyses, the program GeneCap (Wilberg and Dreher, 2004) was used to identify any individuals that may have inadvertently been sampled multiple times (for example, individuals banded during rehabilitation and later depredated in the breeding colony). The probability of identity (Sib P[ID]) was calculated from modern microsatellite genotypes and three duplicated individuals were identified ($(P<0.05)$: one individual from Maui and two from Kauai). In each case, the bird was sampled first as a chick or rehabilitated fledgling, and then later as a carcass (for example, a set of wings and tail or a cluster of feathers) collected on the same island.

Sequences

Mitochondrial DNA sequences were investigated to determine whether they could represent a nuclear copy. Sequences were characterized using MacClade v 4.08 (Maddison and Maddison, 2008) and translated in Dambe v 5.1.2 (Xia and Xie, 2001). To visualize relationships among haplotypes at the population level, a statistical parsimony network for *Cytochrome b* sequences was constructed using TCS v. 1.21 (Clement *et al*., 2000) with a 95% connection limit. For both mitochondrial and nuclear sequence data sets, pairwise F_{ST} was calculated in Arlequin v 3.1 (Excoffier *et al*., 2005) from a Kimura two parameter distance matrix. This substitution model was selected using jModelTest (Posada, 2008) and the Akaike information criterion. Statistical significance of F_{ST} values was determined through 1000 permutations. Two methods were used to correct for multiple tests: the sequential Bonferroni method (Rice, 1989) and the Benjamini–Hochberg false discovery rate (Benjamini and Hochberg, 1995). Both were found to yield the same results. Arlequin was also used to determine whether there was a correlation between geographic and genetic distances. For all data sets, Mantel tests were conducted using Slatkin's linearized F_{ST} and a matrix of distances between each of the islands, with significance determined through 1000 permutations.

We also used the coalescent-based program Migrate v 3.2.6 (Beerli and Felsenstein, 2001; Beerli, 2006) to estimate migration rates between islands. We used the Bayesian mode with uniform priors and substitution model parameters set to values estimated in jModelTest: the transition/transversion ratio=14.0 for mitochondrial data and 15.0, 3.0, 8.0 for the nuclear introns Enol, Lam and RP40, respectively, with rate heterogeneity for the mitochondrial locus modeled by a gamma distribution with $\alpha=0.083$. Three simultaneous replicate analyses were run with a single long chain of 20 million steps, of which the first 10% were discarded as burn-in. A static heating scheme with four chains was used to increase searching effectiveness, and heating parameters were set to 1.0, 1.2, 3.0 and 6.0. Convergence was assessed through examination of results from independent runs, and the effective sample sizes for all parameters were $\geqslant 1000$. Effective population size (θ) and gene flow (m) estimates from Migrate are compounded by the mutation rate (that is, $\theta=x\mathrm{N}e\mu$ and $m=M/\mu$, where x is a scalar dependent on the ploidy and inheritance mode of the locus). Therefore, to avoid making an assumption about mutation rates (particularly for the microsatellite data set), the effective number of migrants per generation ($\mathrm{N}eM/x$) was calculated.

Microsatellites

The microsatellite data were screened for the presence of large-allele dropout and null alleles using the program Microchecker v. 2.2.3 (van Oosterhout *et al*., 2004). Null alleles are genotyping artifacts resulting from the differential amplification success of alleles (for example, due to genetic variability in priming sites) and can lead to an apparent excess of homozygotes. However, inbreeding, which can be an important issue for insular populations of endemic species (Frankham, 1998), results in the same pattern. If null alleles are present in the data set in high frequencies, they can bias estimates of population differentiation, such as F_{ST}. Therefore, we used the program INEst (Chybicki and Burczyk, 2009) to simultaneously estimate the inbreeding coefficient and null allele frequency. The data were also checked for departure from Hardy–Weinberg expectations and the presence of gametic disequilibrium using the program GenePop v 4.0 (Rousset, 2008). The program Convert (Glaubitz, 2004) was used to create infiles for further population genetic analyses. Simulations were conducted in Powsim (Ryman and Palm, 2006) to determine whether the microsatellite data set contained sufficient power to detect low levels of population genetic differentiation. In the simulated data sets, the effective population size was set to 1000 and divergence time to 10, so that the overall F_{ST} of the simulated populations was 0.005. A total of 100 simulations were performed, with sample sizes from the simulated populations drawn corresponding to those utilized here, and significance determined through Fisher's exact test.

To investigate the levels of differentiation between Hawaiian petrels breeding on different islands, we calculated an estimate of F_{ST}, G'_{ST} and D for the microsatellite data set. F_{ST} was originally derived for biallelic data and depends on the variation of the loci used. In the case of highly polymorphic markers, such as microsatellites, F_{ST} may therefore underestimate genetic differentiation (Meirmans and Hedrick, 2011). Several alternative measures have been suggested, including G'_{ST} and D. Both of these correct for maximum possible differentiation (Hedrick, 2005; Jost, 2008), but differ according to the aspect of genetic diversity examined: G'_{ST} examines heterozygosity, whereas D takes into account the effective number of alleles (Meirmans and Hedrick, 2011). An estimate of D was calculated using the package DEMEtics (Gerlach *et al*., 2010) implemented in R (R Development Core Team, 2009), and G'_{ST} was calculated using DEMEtics and the program RecodeData (Meirmans, 2006), where $G'_{ST}=G_{ST}/G_{ST(max)}$ (Meirmans and Hedrick, 2011). The unbiased estimator was used for both statistics, and *P*-values were determined using 1000 bootstrap replicates (Gerlach *et al*., 2010). Correction for multiple tests was conducted as indicated above. Gene flow was also estimated using the program Migrate, as described above, except that the Brownian motion approximation of the stepwise substitution model was used instead.

The number of genetic populations was investigated using the Bayesian clustering program STRUCTURE v. 2.3.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). This program uses multilocus genotypes to build genetic clusters that are in Hardy–Weinberg and linkage equilibrium. We conducted analyses for 1–8 genetic clusters (*K*) using the admixture ancestry and the correlated allele frequency models, with sampling location information included as part of the prior (Hubisz *et al.*, 2009). The model including sampling location information has been found to be sensitive to weak population structure, but unbiased when no structure exists. Eight independent replicates were performed, and runs were conducted for three million generations with the first 10% discarded as burn-in. Results from STRUCTURE were input into the program STRUCTURE HARVESTER (Earl and vonHoldt, 2011), to calculate the *ad-hoc* ΔK statistic suggested by Evanno *et al.* (2005), which takes into account the change in the log probability of the data between increasing numbers of clusters.

RESULTS

Mitochondrial sequences

Mitochondrial DNA sequences were obtained for a total of 322 modern and historical Hawaiian petrel samples (Table 1). Sequences were deposited in the GenBank database under accession numbers HQ420351–HQ420378 and JN015638–JN015862. DNA was successfully amplified from 100% of the historical samples from Molokai, with a mean combined sequence length of 479 bp. There were no gaps present in the alignment, and after translation no nonsense or stop codons were found. Six amino-acid substitutions were detected: three were valine/methionine, and there was one each of tyrosine/histidine, alanine/threonine and asparagine/aspartic acid, although the last two substitutions occurred at very low frequency in the data set. The majority (~72%) of substitutions occurred in the third codon position, and 34 out of 35 were transitions. This evidence indicates that a nuclear origin of the sequences is unlikely. A total of 35 haplotypes were found with 11, 16, 7, 9 and 9 haplotypes from Hawaii, Maui, Lanai, Molokai and Kauai, respectively. In the statistical parsimony network (Figure 2), haplotypes tended to cluster according to island of origin. While a few common haplotypes were shared between islands, they occurred at different frequencies on each. In addition, each island had its own set of private haplotypes that did not occur on any of the other islands.

Overall, mitochondrial DNA sequences revealed significant differentiation among petrels breeding on different islands (global F_{ST}=0.425, $P<0.001$). Pairwise F_{ST} values ranged between 0.037 and 0.633 (Table 2) and were significant in all comparisons involving contemporary populations. The highest F_{ST} occurred between the islands of Lanai and Kauai (F_{ST}=0.633), but differentiation was also high between Maui and Lanai (F_{ST}=0.543). However, F_{ST} was not significantly different from zero between birds from Lanai and the historic birds from Molokai. Mantel tests indicated that there was no significant relationship between genetic and geographic distance (P=0.15). Results from MIGRATE concurred with estimates of F_{ST}, showing low migration between all pairs of populations. Posterior distributions for all analyses were unimodal and narrow, and all analyses showed that migration rates were very low. The highest migration estimate obtained was 0.004 migrants per generation, but the average migration rate was ~0.002 migrants per generation (Supplementary Table 3). For 13 out of 20 migration parameters, the 95% confidence interval included a migration rate of zero.

Nuclear intron sequences

Three nuclear introns were sequenced for each of 164 individuals, for a combined total of 741 bp (Table 1). Sequences were deposited in the GenBank database under accession numbers HQ420460–HQ420515, HQ420604–HQ420659, HQ420746–HQ420801 and JN015863–JN016231.

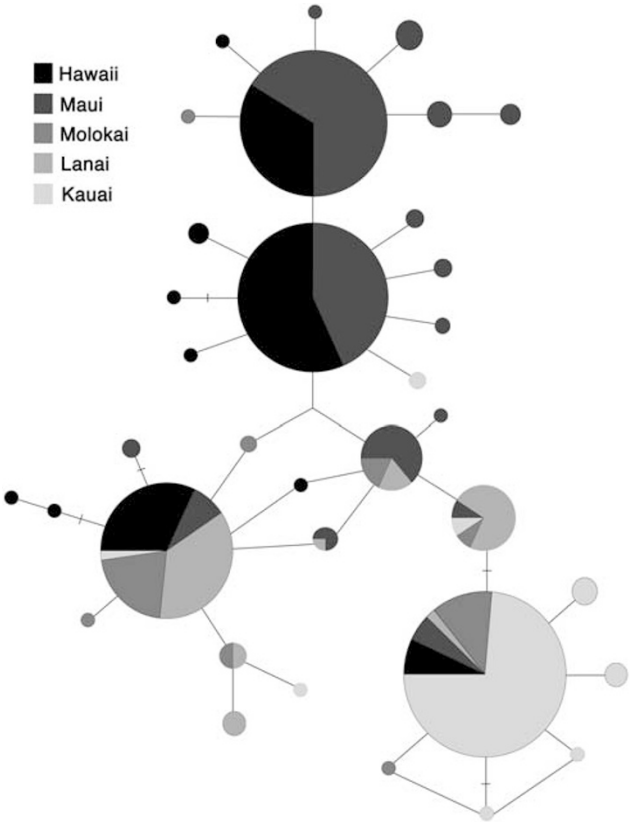

Figure 2 Haplotype network for modern and historic Hawaiian petrel mitochondrial *Cytochrome b* sequences. The sizes of the circles are proportional to the haplotype frequency. All haplotypes differ by a single substitution, unless otherwise indicated by a hash mark, which represents the number of additional substitutions.

Table 2 Population differentiation of historic and modern Hawaiian petrels based on mitochondrial and nuclear intron data sets

	Hawaii	*Maui*	*Lanai*	*Molokai*	*Kauai*
Hawaii	—	0.092[a]	0.060	NA	0.064[a]
Maui	0.068[a]	—	0.095[a]	NA	−0.030
Lanai	0.405[a]	0.543[a]	—	NA	0.145[a]
Molokai	0.226[a]	0.404[a]	0.037	—	NA
Kauai	0.511[a]	0.574[a]	0.633[a]	0.424[a]	—

Abbreviation: NA, not available.
Pairwise F_{ST} values for the *Cytochrome b* gene are below the diagonal, whereas those for a data set of sequences from three nuclear introns are above.
[a]Indicates the estimate is significantly different from zero after correction for multiple tests.

Sufficient nuclear data could not be obtained for historical Molokai samples, and so they were not included in further analyses. Sixteen variable sites were discovered in the remaining four populations, and of these fourteen were transitions and two were transversions. A total of 24 haplotypes were found: 3 for the Enol locus, 8 for Lam and 13 for RP40.

Nuclear sequences revealed a slightly different pattern of differentiation than that obtained from mitochondrial sequences. Overall, significant differentiation was found (global F_{ST}=0.066, $P<0.001$), with F_{ST} for pairwise comparisons ranging from −0.030 to 0.145 (Table 2). One of the highest pairwise F_{ST} values occurred between Maui and Lanai; however, there was no significant differentiation

between birds from Hawaii and Lanai or between Maui and Kauai. Mantel tests suggested that there was no relationship between genetic isolation and geographic distance (P=0.76). Results from MIGRATE indicated migration rates of 0.467 to over 10 migrants per generation (Supplementary Table 4). For seven of the twelve migration estimates, the 95% confidence intervals overlapped with zero, but all intervals also contained rates greater than 2 migrants per generation.

Microsatellite data set

A total of 232 individuals were genotyped for 18 microsatellite loci and there was an average of 6.7 alleles per locus (Supplementary Table 5). Expected heterozygosity ranged from 0.08 to 0.88, with an average expected heterozygosity for each population between 0.57 and 0.62. Results from MICROCHECKER indicated that no locus exhibited large-allele dropout, but two loci (*Ptero06* and *Ptero10*) did display evidence for the presence of null alleles. Examination of observed and expected heterozygosities also indicated an excess of homozygotes. Simultaneous estimation of the inbreeding coefficient and the null allele frequency from INEST indicated that inbreeding was low and that null allele frequencies ranged from 0.115 to 0.247 per population for *Ptero06*, and from 0.125 to 0.252 for *Ptero10*. The *Ptero10* locus was previously found to contain a 38-bp deletion in some individuals (Welch and Fleischer, 2011), and additional undetected insertions or deletions could explain the relatively high incidence of null alleles. Primers could not be redesigned for this locus because sufficient flanking region was lacking. The cause of the observed excess of homozygotes for the *Ptero06* locus remains unclear. Regardless, both loci were discarded from further analyses. No other loci deviated from Hardy–Weinberg equilibrium after correction for multiple tests. Two loci (*Parm01* and *RBG29*) were found to be in linkage disequilibrium after correction for multiple tests, therefore the *Parm01* locus was also discarded and a total of 15 loci were used in further analyses. These data are deposited in the Dryad repository at http://dx.doi.org/10.5061/dryad.1rk18128. Despite the removal of three loci, simulations demonstrated that the microsatellite data set still contained sufficient power to detect very weak population structure. Population structure was detected with 100% accuracy for simulated populations with an F_{ST} of 0.005. Even when F_{ST} was decreased to 0.0025, structure was correctly detected in 93% of simulations.

The microsatellite data set revealed patterns of differentiation similar to those of the mitochondrial data set. Overall, statistically significant differentiation was found (global F_{ST}=0.019, $P<0.001$). The highest values of F_{ST} occurred between Lanai and Maui (Table 3). Estimates of G'_{ST} ranged from 0.015 to 0.057, and estimates of D ranged from 0.022 to 0.060. Both were higher than F_{ST}, as expected (Table 3), and all were significant after correction for multiple tests. Again, mantel tests showed no significant relationship between genetic and geographic distance (P=0.94). Point estimates of migration rates from the program MIGRATE ranged from 0.001 to about 8 migrants per generation (Supplementary Table 6). Overall, 95% confidence intervals were broad and ranged from 0 to about 5 migrants per generation for most parameters.

Population structure was also investigated using a Bayesian clustering analysis. Initial runs with all individuals included in the analysis and the number of clusters (K) set to 2 grouped individuals from Hawaii and Lanai separately from individuals on Maui (Figure 3), similar to the pattern suggested by the nuclear intron data set. Kauai birds appeared to be an admixture of the two groups. With K=3, Hawaii and Lanai remained grouped together, but Kauai was separated from that group as well as from Maui. Finally, with K=4, birds from each island formed separate groups, but individuals from Hawaii still contained some admixture with the Lanai population. Under the criteria of Evanno *et al.* (2005), the grouping K=2 received the greatest support (Supplementary Figure 1) as ΔK decreases substantially and tends to level off for values beyond this. Even though it is not possible to assess the level of support for the situation where K=1 using the Evanno criteria, the log probability of the data under this model was much lower (109 units) compared with models where K=2 and above. Further, posterior probability was highest for K=2, and effectively zero for K=1. While K=4 also did not receive strong support under the Evanno criteria in this initial analysis, it had the second highest log probability, which was just 19 likelihood units smaller than K=2.

As the Evanno method has been found to underestimate the number of genetic clusters present when population structure is weak (Waples and Gaggiotti, 2006), we conducted further analyses by dividing the data set as suggested by the initial results for K=2 and analyzing these groups separately. In the analysis that included Hawaii, Lanai and Kauai, the Evanno method gave high support for K=3 (Supplementary Figure 2), and for the analysis including Maui and Kauai, it gave high support for K=2 (Supplementary Figure 3). Investigation of the posterior probabilities concurred. Therefore, overall the STRUCTURE analyses support the presence of a genetic cluster corresponding to each island, similar to the F_{ST} results.

Table 3 Population differentiation for the microsatellite data set in modern Hawaiian petrels

	Hawaii	*Maui*	*Lanai*	*Kauai*
Hawaii	—	0.028[a]	0.030[a]	0.021[a]
Maui	0.016[a]	—	0.057[a]	0.015[a]
Lanai	0.021[a]	0.033[a]	—	0.046[a]
Kauai	0.010[a]	0.012[a]	0.027[a]	—

Pairwise F_{ST} is shown below the diagonal and G'_{ST} is shown above.
[a]Indicates the estimate is significantly different from zero after correction for multiple tests.

DISCUSSION

We conducted a population genetic study of the endangered Hawaiian petrel using three data sets: sequences of the mitochondrial *Cytochrome b* gene, sequences of three nuclear intron loci and

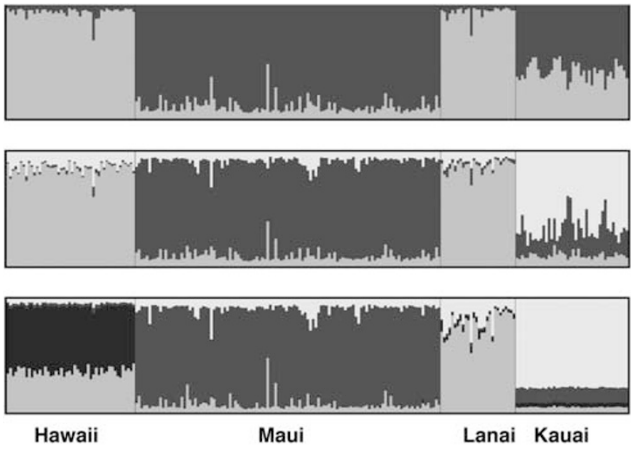

Figure 3 Genetic ancestry of Hawaiian petrels as estimated by the program STRUCTURE from the microsatellite data set using the admixed model with correlated allele frequencies and sampling location as a prior. Top K (the number of genetic clusters)=2, middle K=3 and bottom K=4.

genotypes from 15 microsatellite loci (data from three microsatellite loci were discarded). Analyses of these data revealed significant population genetic structure among all four modern populations. Contrary to expectations, differentiation was found even at very short distances between adjacent islands. In addition, significant differentiation was found between museum specimens collected from the potentially extirpated colony on Molokai and contemporary birds breeding on all islands except for those on Lanai.

Contemporary population differentiation

Consistent with the findings of Welch *et al.* (2011), the more extensive analysis conducted here on multiple data sets shows support for genetic isolation between modern populations of the Hawaiian petrel. Analyses of the mitochondrial DNA and microsatellite data sets showed significant differentiation between all contemporary population pairs. Similarly, nuclear intron results also showed significant isolation between most populations. Finally, a Bayesian clustering analyses performed using the program STRUCTURE indicated the presence of four genetic populations. Although sampling location was included as prior information in this analysis, which results in increased sensitivity (Hubisz *et al.*, 2009), this model has not been found to be biased in cases where population structure is absent (Hubisz *et al.*, 2009). Also, as mentioned above, the presence of population differentiation found in the STRUCTURE analysis is corroborated by other data sets and analyses. For the nuclear intron and microsatellite data sets, some of the highest pairwise F_{ST} values were found between birds sampled on the adjacent islands of Maui and Lanai, which are separated by just $\sim$75 km. The highest pairwise F_{ST} in the mitochondrial data set occurred between Lanai and Kauai, which are $\sim$300 km apart. This is a very small distance when taking into account the fact that Hawaiian petrels are capable of, and in fact often make, foraging trips $>$10 000 km (Adams and Flora, 2010).

Overall, the signal of differentiation among populations appears to be stronger in the mitochondrial data set than in the nuclear data set. From mitochondrial DNA sequences the average pairwise F_{ST} was 0.53. However, the average G'_{ST} calculated from the microsatellite data (which corrects for higher levels of variability and also ranges from 0 to 1; Hedrick, 2005) was 0.033. The mitochondrial levels of differentiation appear to be much closer to their theoretical maximum than do levels observed in the microsatellite data set. This is also demonstrated in the MIGRATE analyses, where the average migration rate was 0.002 for the mitochondrial data set, but was $>$0.780 for the nuclear intron and microsatellite data sets. This is not likely to be related to low power in the microsatellite data set, as simulations demonstrated that extremely weak population genetic structure (for example, an F_{ST} of 0.005) could be accurately detected in 100% of the tests. Instead, the pattern observed here could also be due to low levels of male-biased dispersal, which has been documented in some seabirds (Burg and Croxall, 2001). It could also be explained by recent divergence, which may be the case for the Hawaiian petrel (Welch *et al.*, 2011). Without information from banding studies, it will be difficult to disentangle the genetic signatures of low levels of male-biased gene flow and incomplete lineage sorting. However, even if the strength of the signal is somewhat different, both mitochondrial and microsatellite data sets support the presence of four genetic populations within the Hawaiian petrel.

Potential isolating mechanisms

Population genetic structure has been documented in other procellariid seabird species. In the closely related Galapagos petrel an endangered, endemic species that is only known to breed on five islands, microsatellite data indicated the presence of four distinct populations. The fifth population was suggested to be the result of a recent colonization (Friesen *et al.*, 2006). Strong natal philopatry was proposed as an important mechanism promoting isolation between populations. In band-rumped storm petrels (*Oceanodroma castro*), populations from the Atlantic and Pacific Oceans showed high levels of divergence, likely due to separation by continental landmasses, but even within the same ocean basin populations showed some evidence of differentiation (Smith *et al.*, 2007). However, population genetic structure has also been found to be lacking in many species, such as the gray-headed albatross (*Thalassarche chrysostoma*), and the white-capped albatross (*T. steadi*), even though differentiation was found in closely related species (Burg and Croxall, 2001; Abbott and Double, 2003). Thus, it is not easy to predict the presence of population genetic structure *a priori* in procellariiform seabirds, and further investigations of the mechanisms of isolation are necessary.

Geographic barriers are not likely factors in promoting divergence among Hawaiian petrel populations. Hawaiian petrel colonies are located in the same ocean basin, and are at most $\sim$500 km apart, so there are no obvious physical barriers to dispersal in this species. Patterns of differentiation could be linked to distance (Burg *et al.*, 2003), however, this does not appear to be the case for Hawaiian petrels either. Mantel tests failed to find a relationship between genetic and geographic distance. In addition, populations on the adjacent islands of Maui and Lanai, which are just $\sim$75 km apart, show evidence of significant genetic isolation. In the Hawaiian archipelago, island age has been found to be related to genetic isolation for some species (Fleischer *et al.*, 1998). However, Hawaiian petrels split from Galapagos petrels $\sim$550 000 years ago (Welch *et al.*, 2011), and by that time all of the high islands present today had been formed. Finally, wind patterns can be an important factor in the flight of seabirds (Spear and Ainley, 1997) and could affect dispersal. Given the prevailing easterly trade winds in Hawaii, there is no evidence that wind direction or speed differ substantially between islands in a manner consistent with the patterns observed here, although further study would be beneficial.

Habitat characteristics could also influence levels of migration. Hawaiian petrels at present use two general types of breeding habitat: colonies on Maui and Hawaii both occur at high elevation sites with little precipitation and scrubby vegetation (Brandt *et al.*, 1995; Hu *et al.*, 2001), whereas the habitat on the islands of Lanai, Kauai and Molokai consists of lower elevation wet forest (Ainley *et al.*, 1997). Birds may be reluctant to disperse between these different types of habitat. However, habitat type does not appear to be the only explanation for the observed patterns, because significant differentiation was found even between colonies occurring in similar habitat (for example, Lanai and Kauai). Habitat quality at the colony may also influence seabird dispersal, particularly if quality declines due to competition for nesting sites or other density-dependent factors (Inchausti and Weimerskirch, 2002; Kildaw, 2005). The Hawaiian petrel has been declining in abundance since the arrival of humans in the archipelago, so there may be little competition for nest sites and therefore reduced drive for contemporary dispersal (Schreiber and Burger, 2002; Pyle and Pyle, 2009).

Friesen *et al.* (2007a) suggested that population-specific foraging ranges, particularly in the nonbreeding season, may act to reduce gene flow. If birds spend the nonbreeding season in population-specific areas, then they may return together to the same breeding colony (Burg and Croxall, 2001). Pair formation and copulation are both thought to occur primarily on the breeding grounds for the Hawaiian petrel (Simons, 1985; Warham, 1990), and therefore

population-specific foraging or nonbreeding distributions could lead to population differentiation. Wiley *et al.* (2012, in preparation), examined the stable isotope composition of primary feathers, which are grown during the nonbreeding season, and found population-specific signatures. Adults from Hawaii and Lanai had a very similar stable carbon and nitrogen isotope composition (congruent with STRUCTURE results with K=2 and 3), whereas individuals from Maui and Kauai both differed significantly from them in nitrogen isotope values, likely due to differences in foraging location. This could be one mechanism leading to population isolation in the Hawaiian petrel.

Strong natal philopatry could also lead to population differentiation (Friesen *et al.*, 2007a). Banding studies suggest that, in general, procellariiform seabirds are highly philopatric (Warham, 1990). This mechanism is often invoked when population structure is found in species for which banding information is lacking and there are no other obvious factors preventing dispersal (Friesen *et al.*, 2007a). Natal philopatry followed by drift could explain the genetic differentiation found between populations of the Hawaiian petrel. It could also explain recently documented biological differences between populations. For example, vocalizations of Hawaiian petrels were found to be unique on each island (Judge, 2011). Little is known about how procellariiform seabirds acquire their calls, but in some petrel species it may be innate: a snow petrel chick (*Pagodroma nivea*) raised by another species developed snow petrel vocalizations (Bretagnolle, 1996). Differences in call may have developed due to drift in isolated populations. Judge (2011) also found that adults from Maui were significantly larger than adults from Hawaii and Kauai, in both wing chord and tarsus length, and that birds on Maui breed about 30 days earlier than birds on all of the other islands. Taken together, all of these factors may work together to minimize contemporary gene flow.

Population extirpation and gene flow

Investigation of a presumably extirpated population on the island of Molokai revealed significant differentiation from all of the modern populations except for the adjacent island of Lanai. By the early 1900s, the number of Hawaiian petrels breeding on Molokai had apparently already begun declining, likely due to the introduction of mongoose as well as habitat degradation by introduced feral ungulates (Bryan, 1908). During the same time period, habitat conditions for petrels on Lanai were probably also less than ideal due to grazing by ungulates, which led to severe habitat degradation as early as 1870. Continued foraging impeded natural recovery until 1911 when goats were removed from the island (Munro, 2007) and forest restoration began. While petrels may not have ever been completely extirpated from Lanai, the recent discovery of a large breeding colony there (Birdlife International, 2011), just a handful of generations later, is somewhat unexpected given that these long-lived petrels lay a single egg each year and exhibit delayed maturity. Findings of significant population structure among extant populations, and failure of biologists to relocate formerly large colonies on Molokai, coupled with no significant divergence between birds from Molokai and Lanai, may indicate that petrels from Molokai were displaced to Lanai as the Molokai colony dwindled due to anthropogenic influences. It is also possible that a recent colonization event occurred on these islands or that there was a substantial level of gene flow between them prior to the decline of the Molokai population.

Several lines of evidence indicate that displacement of Hawaiian petrels from Molokai to Lanai could be possible. The islands of Molokai and Lanai are separated by just $\sim$30 km, exhibit peaks of similar elevations and have similar habitats (Wagner *et al.*, 1999), which could have facilitated movement between colonies on these islands. In addition, social attraction has been found to be important for many seabirds (Parker *et al.*, 2007) and therefore individuals, particularly young birds prospecting for nest sites, may preferentially disperse away from dwindling colonies. There is also evidence that seabirds may disperse from colonies in response to declining conditions, and that dispersal can be substantial in some cases. Kildaw (2005) found that seabirds, including previously established breeders, dispersed in response to poor habitat quality and reduced reproductive success. Similarly, Oro and Ruxton (2001) demonstrated large-scale dispersal to a newly formed seabird colony. However, these examples arise from charadriiform seabirds and may not translate directly to procellariiform seabirds. Within this order, Black-browed albatrosses have recently been confirmed to be breeding on several islands in the south-west Pacific, despite their apparent absence there early in the twentieth century, providing evidence that procellariiform seabirds do indeed disperse from natal colonies (Moore *et al.*, 2001). Wandering albatrosses (*Diomedea exulans*) are known to disperse when population density at the colony is high (Inchausti and Weimerskirch, 2002), and accordingly no evidence for population structure has been found in this species (Burg and Croxall, 2004; Milot *et al.*, 2008). Unfortunately the densities of Hawaiian petrel populations on Lanai and Molokai prior to human contact remain unknown. Therefore, while levels of dispersal between the populations on Lanai and Molokai may have consistently been large in the past, it is also possible that petrels from Molokai were displaced to Lanai as that colony became extirpated due to predation and habitat disturbance.

If petrels are able to overcome isolating mechanisms and disperse prior to complete extirpation then this could explain why some species do not seem to exhibit population genetic differentiation, despite evidence of strong philopatry from mark-recapture studies. For example, the Laysan albatross is known to exhibit strong natal philopatry (Fisher, 1976), but very weak population structure was found in this species (Young, 2010). While density-dependent dispersal was suggested as one potential cause, displacement from several extirpated colonies could also lead to weak population structure. A pattern of dispersal due to displacement could also explain why seabird extinction is relatively rare in the Pacific despite the high prevalence of local population extirpation (Steadman, 1995).

Conservation implications

Resources for conservation management of endangered species are always limited, and therefore an understanding of population differentiation and connectivity can help identify conservation priorities and inform management decisions. For example, if populations were panmictic then it might be more beneficial to focus resources on large populations or those that are more easily managed instead of utilizing a disproportionately large amount of resources on conserving small, severely declining and remote populations. Here our results indicate that Hawaiian petrel populations on each island could be considered distinct management units (Fraser and Bernatchez, 2001) and targeted for conservation actions to prevent the loss of genetic diversity. Significant levels of differentiation indicate that each population contains some unique genetic variation and might be embarking on its own evolutionary trajectory. This is concordant with recent work indicating that some populations may also be ecologically distinct (Wiley *et al.*, 2012). Practically, the strategy of managing each population separately is also necessary because management concerns are unique on each island. In addition to differences among inter-island nesting habitats mentioned above, the number and types of predators differ (for example, cats, rats, mongooses, owls), as do the presence and magnitude of other anthropogenic threats, such as wind

turbines, attraction to artificial light sources and collisions with power lines (Ainley *et al*., 1997; Cooper and Day, 1998; Hodges and Nagata, 2001; Carlile *et al*., 2003).

The colony on Mauna Loa, Hawaii warrants particular attention. This population appears to be declining at a faster rate than the others, and population viability analyses indicate that it could become extirpated in the near future (Hu *et al*., 2001). At a minimum, more comprehensive measures to reduce predation within nesting areas are urgently needed if this population is to remain viable. This population may also benefit from translocations (Miskelly *et al*., 2009). One potential option is to move eggs or chicks from the relatively large colonies on Maui or Lanai to Hawaii. Populations from Lanai and Hawaii appear to be ecologically similar (Wiley *et al*., in preparation), but the demographic trend on Lanai remains unclear. The colony at Haleakala, Maui, is perhaps the largest colony of Hawaiian petrels and may be beginning to stabilize (Carlile *et al*., 2003). Although significant genetic differentiation was observed between all of these populations, those on Maui and Lanai also appear to be most genetically similar to the Hawaii population, and the levels of differentiation found are not to the extent where outbreeding depression would be a major issue (Frankham *et al*., 2011). Artificial gene flow would cause some genetic homogenization of the Hawaii population, but without it that population, and its unique genetic variation, may be lost completely from the species. In addition, admixture with another population, such as may have occurred between individuals from Molokai and Lanai, could be a natural response in these petrels. Such translocations would only be beneficial, however, if predation by introduced mammals could be effectively controlled or eliminated. If that is not possible, then translocating eggs or chicks away from the colony on Mauna Loa (and potentially also encouraging prospecting adults to move away as well) to another safer, more easily managed location could be a better option.

CONCLUSION

Here we demonstrate that populations of Hawaiian petrels, even those separated by as little as 75 km, are genetically isolated from one another, potentially due to strong natal philopatry or population-specific nonbreeding distributions. Many seabirds, especially endemic species, are increasingly threatened by extinction, and identifying isolated populations can be important for preserving genetic diversity and developing conservation management plans and priorities. This is true for enigmatic species, such as nocturnal, burrow-nesting seabirds like the Hawaiian petrel, but also for seabird species that are more well known, because distinct evolutionary lineages may go unnoticed (Hailer *et al*., 2011) and become extirpated without further investigation.

DATA ARCHIVING

Mitochondrial and nuclear intron sequence data have been deposited in the GenBank database under accession numbers HQ420351–HQ420378, JN015638–JN015862, HQ420460–HQ420515, HQ420604–HQ420659, HQ420746–HQ420801 and JN015863–JN016231. Microsatellite data have been deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.1rk18128.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Heredity website (http://www.nature.com/hdy)