Ancient DNA Reveals Genetic Stability Despite Demographic Decline: 3,000 Years of Population History in the Endemic Hawaiian Petrel

Andreanna J. Welch,*[,†,1,2] Anne E. Wiley,[3,4] Helen F. James,[4,2] Peggy H. Ostrom,[3] Thomas W. Stafford Jr,[5,6] and Robert C. Fleischer[1,2]

[1]Smithsonian Conservation Biology Institute, Center for Conservation and Evolutionary Genetics, National Zoological Park, Washington, DC

[2]Behavior, Ecology, Evolution and Systematics Program, University of Maryland

[3]Department of Zoology, Michigan State University

[4]Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC

[5]Stafford Research, Inc., Lafayette, CO

[6]Centre for GeoGenetics, University of Copenhagen, Copenhagen, Denmark

[†]Present address: Department of Biological Sciences, 109 Cooke Hall, University at Buffalo, Buffalo, NY 14260, USA

***Corresponding author:** E-mail: andreann@buffalo.edu.

Associate editor: Beth Shapiro

Abstract

In the Hawaiian Islands, human colonization, which began approximately 1,200 to 800 years ago, marks the beginning of a period in which nearly 75% of the endemic avifauna became extinct and the population size and range of many additional species declined. It remains unclear why some species persisted whereas others did not. The endemic Hawaiian petrel (*Pterodroma sandwichensis*) has escaped extinction, but colonies on two islands have been extirpated and populations on remaining islands have contracted. We obtained mitochondrial DNA sequences from 100 subfossil bones, 28 museum specimens, and 289 modern samples to investigate patterns of gene flow and temporal changes in the genetic diversity of this endangered species over the last 3,000 years, as Polynesians and then Europeans colonized the Hawaiian Islands. Genetic differentiation was found to be high between both modern and ancient petrel populations. However, gene flow was substantial between the extirpated colonies on Oahu and Molokai and modern birds from the island of Lanai. No significant reductions in genetic diversity occurred over this period, despite fears in the mid-1900s that this species may have been extinct. Simulations show that even a decline to a stable effective population size of 100 individuals would result in the loss of only 5% of the expected heterozygosity. Simulations also show that high levels of genetic diversity may be retained due to the long generation time of this species. Such decoupling between population size and genetic diversity in long-lived species can have important conservation implications. It appears that a pattern of dispersal from declining colonies, in addition to long generation time, may have allowed the Hawaiian petrel to escape a severe genetic bottleneck, and the associated extinction vortex, and persist despite a large population decline after human colonization.

Key words: ancient DNA, population bottleneck, gene flow, generation time, Hawaiian petrel, *Pterodroma*.

Introduction

There is no doubt that humans have had a great impact on global biodiversity, and this impact is particularly well documented for the islands of the Pacific Ocean (Steadman 2006). The Hawaiian Islands were one of the last habitable areas of the Pacific to be colonized by humans, with Polynesian people first arriving between 1200 and 800 years ago (Kirch 2000; Wilmshurst et al. 2011). Immediately prior to the first European contact in 1778 AD human population sizes in Hawaii likely exceeded 200,000 individuals (Kirch 1985). The fossil record indicates that the prehuman background extinction rate of birds in Hawaii was very low, but that it increased precipitously after the arrival of humans, due to factors such as direct exploitation, introduction of exotic mammalian predators, and habitat destruction (Olson and James 1982a; James 1987; Athens et al. 1991; Blackburn et al. 2004). Species ranging from raptors to giant flightless geese became extinct, including one seabird species, *Pterodroma jugabilis* (James and Olson 1991; Olson and James 1991). Many of the endemic Hawaiian species that were able to persist have experienced range contractions and declines in population size (James and Olson 1991; Olson and James 1991) and remain threatened by extinction.

Severe population declines can have large impacts on the genetic diversity of a species (Frankham et al. 2002). Declines in population size can lead to inbreeding, decreased survival, reduced reproductive success, and can limit evolutionary potential. All of these factors may, in turn, increase the risk of

Published by Oxford University Press on behalf of the Society for Molecular Biology and Evolution 2012.

extinction (Frankham 2005). Changes in past population size can be especially difficult to detect but ancient DNA techniques, which enable utilization of temporally spaced samples, increase power and can help elucidate the evolutionary history of a species (Ramakrishnan et al. 2005). Ancient DNA has been used, for example, to investigate whether temporal population dynamics of large mammals have been associated with the presence of humans or global climatic fluctuations (Shapiro et al. 2004; Campos et al. 2010). Ancient DNA has also revealed temporal changes in genetic diversity of Hawaiian species. The endangered Hawaiian goose, or nene, was found to have undergone a severe prehistoric genetic bottleneck about 500–800 years ago, after the arrival of humans in the islands (Paxinos et al. 2002). It remains unclear how genetic diversity has changed over time for other avian species that have survived through this period, and why some species have been able to persist despite population declines while others became extinct.

The endemic Hawaiian petrel (*Pterodroma sandwichensis*) is a long-lived pelagic seabird. Its lifespan, perhaps reaching nearly 40 years, is among the longest for endemic Hawaiian species, and is primarily rivaled only by that of other seabirds and perhaps the larger extinct flightless birds that formerly inhabited the Islands (Simons 1984; Kaufman 2011). Subfossil bones of the Hawaiian petrel have been found on all of the high islands (Hawaii, Maui, Lanai, Molokai, Oahu, and Kauai) but the petrel's range is considerably smaller today than it has been in the past (fig. 2). The lowlands of Oahu were previously the home of a large petrel colony, but historical records for it are lacking, indicating that petrels may have been extirpated from this island prior to the arrival of Europeans. Historical accounts indicate the presence of a large colony on Molokai with birds so plentiful that they "darkened the sky" (Munro 1955), however recent survey trips have failed to locate a substantial colony on this island (Simons and Hodges 1998). In the early and mid-1900s ornithologists feared that the species was extinct (Baldwin and Hubbard 1948; Banko 1980) due to an apparent lack of sightings combined with large-scale habitat destruction, the introduction of exotic mammalian predators, as well as archeological evidence and historical accounts of direct human exploitation in the past (Athens et al. 1991). Since that time the Hawaiian petrel has been rediscovered on four islands, (Hawaii, Maui, Lanai and Kauai; fig. 2), but colonies remain only in high elevation sites with rugged terrain, whereas they were formerly more widely distributed from the coast up to the volcanic peaks (Olson and James 1982b). Recent census population estimates range from a total of 11,000 to 34,000 individuals with potentially 3,750 to 4,500 breeding pairs (Spear et al. 1995). Census population sizes on each island are not precisely known, but it is likely that currently there are several thousand birds each on Maui, Lanai, and Kauai. On Hawaii, populations appear to be strongly declining and there may be fewer than 500 birds remaining (BirdLife International 2011).

Here we investigate the temporal population dynamics of the endangered Hawaiian petrel. We obtained mitochondrial *Cytochrome b* sequences from 100 ancient, 28 historic, and 289 modern Hawaiian petrel samples collected from all islands where this bird has been known to breed, including the prehistorically extirpated colony on Oahu and a presumably historically extirpated colony on Molokai. We examined patterns of gene flow and divergence, as well as changes in effective population size and genetic diversity over the last 3,000 years beginning before, and extending through, the human era in the Hawaiian Islands.

Materials and Methods

Samples and Dating

A total of 512 Hawaiian petrel samples were obtained for population genetic analyses (supplementary tables 1 and 2, Supplementary Material online). Of these, 289 samples were obtained opportunistically from modern petrels on Hawaii, Maui, Lanai, and Kauai. Bone, feather, and tissue samples were collected from carcasses of birds that had been depredated in breeding colonies or that died as a result of grounding (e.g., due to attraction of fledglings to artificial light sources). Blood samples collected from chicks in Haleakala National Park, Maui, were also obtained (Browne et al. 1997). Considering that nonbreeders appear to depart early in the season (Simons and Hodges 1998), we assume that birds found on a given island represent breeders or their offspring. We also obtained samples from 28 museum specimens that were collected from the potentially extirpated population on Molokai, in 1907 and 1914 and subsequently deposited at the Bernice P. Bishop Museum and at the Natural History Museum of Los Angeles County. Finally, 195 ancient Hawaiian petrel bone samples from Hawaii, Maui, Lanai, Molokai, and Oahu, were acquired from the Smithsonian's National Museum on Natural History, the Bernice P. Bishop Museum, or collected in the field at archeological and paleontological sites. No ancient bones from Kauai were available for destructive analyses. In sites where skeletal remains were disarticulated, the same skeletal element was sampled throughout (e.g., only right humeri were sampled from that site) to prevent duplicate sampling of the same individual.

Radiocarbon dates of bones were obtained through accelerator mass spectrometry using a protocol modified from Stafford et al. (1991). Briefly, XAD-treated hydrolyzates of gelatinized bone collagen were combusted to CO_2, graphitized, and dated at the W. M. Keck Carbon Cycle Accelerator Mass Spectrometry Lab, at University of California, Irvine. Since Hawaiian petrels forage at sea, they obtain all of their carbon from the ocean. Due to a delay in mixing of carbon between the ocean and the atmosphere, as well as to mixing of ocean layers at upwelling zones (where some water has traveled thousands of kilometers over a period of several hundred years), carbon from the ocean is older and depleted in ^{14}C relative to carbon in the atmosphere. Therefore radiocarbon dates from marine organisms can be biased by 200–800 years or more. We applied the global marine reservoir correction (Hughen et al. 2004) plus a regional correction of 54 years developed specifically for the Hawaiian petrel (data not shown). Radiocarbon dates were calibrated using the program Calib v. 6.0 (Stuvier and Reimer 1993). The reported ages represent the median of the probability distribution and

are presented as calendar years before 1950 AD (supplementary table 3, Supplementary Material online). Radiocarbon dates were obtained for all bones found in paleontological contexts. For bones found in archeological sites, we estimated the period of accumulation using a series of radiocarbon dates. Since these bones were deposited over a short period of time (≤100 years), the remaining samples were either assigned to the same time bin or given the average of the median ages of the dated bones from the site, depending on the analysis.

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from blood and tissue samples using the DNEasy tissue kit (Qiagen) and from bone, feather, and museum samples via phenol/chloroform extraction and centrifugal dialysis (Fleischer et al. 2000). Stringent protocols were maintained to prevent contamination of ancient samples. All extractions for ancient and historical samples were performed in a dedicated ancient DNA laboratory that was physically isolated from all polymerase chain reaction (PCR) products. Multiple extraction and negative reagent controls were also used to detect contamination. Additionally, for nearly 50% of the ancient and 100% of the historical samples, a sample from various different species was extracted in between Hawaiian petrel samples. These nonpetrel samples were amplified with petrel-specific primers to detect cross-contamination. For the remaining ancient samples Hawaiian petrel bones from different islands were alternated whenever possible. Initial mitochondrial DNA sequencing of ten ancient DNA extracts indicated the presence of cytosine deamination artifacts in three samples. Therefore, samples were treated with uracil-DNA glycosylase to eliminate these miscoding lesions (Hofreiter et al. 2001). For all ancient DNA samples from bone, 50 μl aliquots were incubated with 1X uracil-DNA glycosylase buffer and 1 unit uracil-DNA glycosylase at 37°C for 10 min, followed by incubation with 1 unit uracil glycosylase inhibitor (New England Biolabs) at 37°C for 10 min, and a final incubation at 95°C for 10 min.

Since there is a duplication of the mitochondrial control region in procellariiform seabirds (Abbott et al. 2005), we amplified a 524 bp portion of the 5′ variable region of the mitochondrial *Cytochrome b* gene, which has been widely used for seabird studies and has high levels of variation in the Hawaiian petrel (Nunn and Stanley 1998; Welch et al. 2012). For ancient, historical, and degraded modern samples we amplified seven short (<150 bp) overlapping fragments (supplementary table 4, Supplementary Material online), and for ancient and historical samples each of these was amplified at least twice. PCR and sequencing were carried out as in Welch et al. (2012). Briefly, PCR was conducted with 15 μl total reaction volumes, 1 unit AmpliTaq Gold DNA polymerase (Applied Biosystems), 1–2 μl DNA template, with 35 cycles for modern samples, and 25 μl total reaction volumes, 1 unit Amplitaq Gold DNA polymerase, 2–4 μl DNA template, and 45 cycles for ancient and historical samples. All fragments were electrophoresed in an ABI 3130 xL Genetic Analyzer (Applied Biosystems) and sequences were aligned and visually inspected in Sequencher v 4.9 (GeneCodes). Sequences were deposited in the GenBank database under accession numbers JN015536-JN015862 and HQ420351-HQ420378. We also attempted to amplify nuclear intron and microsatellite loci in ancient and historical samples, but success was low. Consequently, these data were not utilized in further analyses.

Data Analysis

Sequences were characterized in MacClade v. 4.08 (Maddison and Maddison 2008) and translated in Dambe v. 1.5.2 (Xia and Xie 2001) to examine the potential presence of nuclear copies in the mitochondrial data set (Sorenson and Fleischer 1996). Of the 51 variable sites found, 21% occurred in the first codon position, 4% occurred in the second, and 75% occurred in the third. Ninety-one percent exhibited transitions. There were no gaps in the alignment and after translation zero nonsense or stop codons were found. This evidence indicates that a mitochondrial, and not nuclear, origin of the sequences is likely. The program jModelTest v. 0.1 (Posada 2008) and the Bayesian information criterion (BIC) were utilized to select the best fitting substitution model: the Hasegawa–Kishino–Yano (HKY) model with rate heterogeneity among sites modeled by a gamma distribution. To depict the relationship between haplotypes a statistical parsimony network was created in TCS v. 1.21 with a 95% connection limit.

Levels of differentiation were investigated between birds sampled on each island, including between modern samples and those from the extirpated colonies on Oahu and Molokai. We estimated pairwise F_{ST} values in Arlequin v. 3.1 (Excoffier et al. 2005) from a Kimura two-parameter distance matrix. Depaulis et al. (2009) demonstrated that there could be bias in summary statistics, such as F_{ST}, calculated from heterochronous (i.e., ancient DNA) data sets. However, bias should be small if sampling occurs over a relatively short time as compared with evolutionary time. Here, corrected and uncorrected estimates of F_{ST} differed by at most 0.002, and so uncorrected estimates are shown. Significance of *P*-values was determined after sequential Bonferroni correction for multiple tests (Rice 1989).

Several methods were used to investigate temporal changes in effective population size and genetic diversity. First, Bayesian skyride analyses were conducted for samples collected on Hawaii and Maui utilizing BEAST v.1.6.1 (Bayesian Evolutionary Analysis by Sampling Trees; Drummond and Rambaut 2007; Ho and Shapiro 2011). The analyses were performed using a starting tree generated in Garli v. 0.96b (Zwickl 2006) and the HKY + G substitution model. We used a log-normal distribution representing the 95% confidence interval of the radiocarbon date as a prior on the sampling time for ancient samples and an uninformative prior on effective population size (i.e., bounded between 0 and infinity). Analyses were also performed with and without a model of DNA damage (Rambaut et al. 2009) as well as under the coalescent constant population size model, for comparison. Analyses were performed for 1.5×10^8 generations sampling every 2,000 generations. Convergence was

assessed through multiple independent runs, and effective sample sizes were examined in Tracer v. 1.5 (Drummond and Rambaut 2007). Support for various models was compared using Bayes factors, as estimated in Tracer (Kass and Raftery 1995; Suchard et al. 2001).

In addition to coalescent-based estimation of changes in effective population size, we utilized the "temporal alleles" approach to estimate the variance effective population size. This method estimates the harmonic mean of N_e in the time period between the sampling points by assuming that changes in allele frequencies are due to genetic drift (Luikart et al. 2010). Samples were binned by age (e.g., supplementary tables 9–11, Supplementary Material online) and any sequences with greater than 15% missing data were excluded from the analysis. We also excluded samples older than 1,000 years because of the comparatively low sample size. Estimates were obtained using the program TM3.1 (Berthier et al. 2002) assuming a maximum N_e of 100,000 with 20,000 iterations. The maximum N_e value of 100,000 was selected because reviews of the literature (Frankham 1995) have suggested that, on average, the ratio of effective to census size for wildlife populations may be approximately 0.10, and because subfossil evidence indicates that prior to human colonization of the Hawaiian Islands the Hawaiian petrel was likely very abundant. This species may have been the most abundant seabird in the main islands, quite possibly reaching population levels of one million or more individuals. Similar levels are seen in some modern seabird species, like the Laysan albatross (*Phoebastria immutabilis*), which breeds primarily in the Northwestern Hawaiian Islands (Naughton et al. 2007). For comparison with temporal estimates of changes in N_e, nucleotide diversity and gene diversity were calculated for the same samples using Arlequin.

To further examine Hawaiian petrel population dynamics over the past century, when it was feared that this species was extinct but then later rediscovered, we modeled the census population growth rate under various scenarios. This allowed us to investigate whether it is biologically possible for petrels to have been near extinction in the recent past and then have undergone a demographic recovery to their current census population sizes. The last major record of the Hawaiian petrel from the early 20th century is from W. A. Bryan in 1914, who was collecting on Molokai and noted that population sizes were decreasing, likely due to predation (Bryan 1908; Banko 1980). After this, to our knowledge, there were no documented Hawaiian petrel sightings and no known breeding colonies until 1964 when between 150 and 300 birds were discovered in Haleakala National Park, Maui (Banko 1980). By 1995, Spear *et al.* estimated that there were 11,000–34,000 individuals, based on at-sea sightings. Since Hawaiian petrels have a long lifespan, delayed sexual maturity, and low annual fecundity, they have a very low population growth rate. We used the exponential growth equation $N_t = N_0 e^{rt}$ to model census population growth rates and test the hypothesis that petrels were near extinction around 1915. Here N represents census population size, r represents growth rate, and t represents time (in years). We tested various values for each parameter of the model (supplementary table 5, Supplementary Material online). For example, we held time (t) constant at 80 years (the difference between 1915 and 1995), held current census population size (N_t) constant at 11,000 (the lower 95% confidence interval of census population size in 1995) then varied the initial census population size (N_0) and estimated the necessary growth rate. This should result in an estimate of the absolute minimum required growth rate since it allows the longest period of recovery, the smallest amount of growth necessary, and assumes that resources are unlimited and that there is no predation. Logically, if growth rates estimated for Hawaiian petrels based on field observations are much lower than the absolute minimum required, then it would be unlikely that petrels had ever reached very small census population sizes (e.g., 250 individuals) in the past.

Finally, we conducted simulations to investigate the effect of generation time on the retention of genetic diversity. All simulations were conducted using the program BottleSim (Kuo and Janzen 2003) and assumed the absence of gene flow and selection. Initial genetic diversity for all species was set to match the haplotype frequencies observed in the ancient Hawaiian petrels from the island of Hawaii (supplementary table 6, Supplementary Material online). This data set was selected because it represented the most extensive sampling. We simulated a decline in effective population size 150 years ago to a stable N_e of 250 individuals and examined the effect of generation time on expected heterozygosity and allelic richness. In this way we could test how genetic diversity would change for multiple species with differing life history strategies if they had started with the exact same levels of genetic diversity and undergone the exact same population decline. We also performed simulations using a set of arbitrarily chosen allele frequencies (supplementary table 6, Supplementary Material online). We tested life history strategies that included that of the Hawaiian petrel as well of other endemic species that live in Hawaii or for which ancient DNA has been used to investigate changes in genetic diversity over time (supplementary table 7, Supplementary Material online). Life history information was obtained from the literature (Laursen and Bekoff 1978; Meagher 1986; Lent 1988; Banko et al. 1999; Banko et al. 2002; Debruyne et al. 2008). We also used simulations to investigate the severity of a decline needed to result in a decrease in genetic diversity of the Hawaiian petrel, given its life history characteristics. We simulated a decline 150 years ago to various effective population sizes ranging from 10 to 1000, while holding generation time constant. One hundred iterations were performed for each simulation.

Results and Discussion

Mitochondrial DNA sequences were obtained from a total of 417 Hawaiian petrel samples, representing a period of approximately 3500 years (supplementary tables 1–3, Supplementary Material online). Success rates for ancient DNA recovery ranged from 18% to 76% per island for ancient bone samples and 100% for historical museum specimens collected on Molokai in 1907 and 1914 (supplementary fig. 1, Supplementary Material online). The sequence length

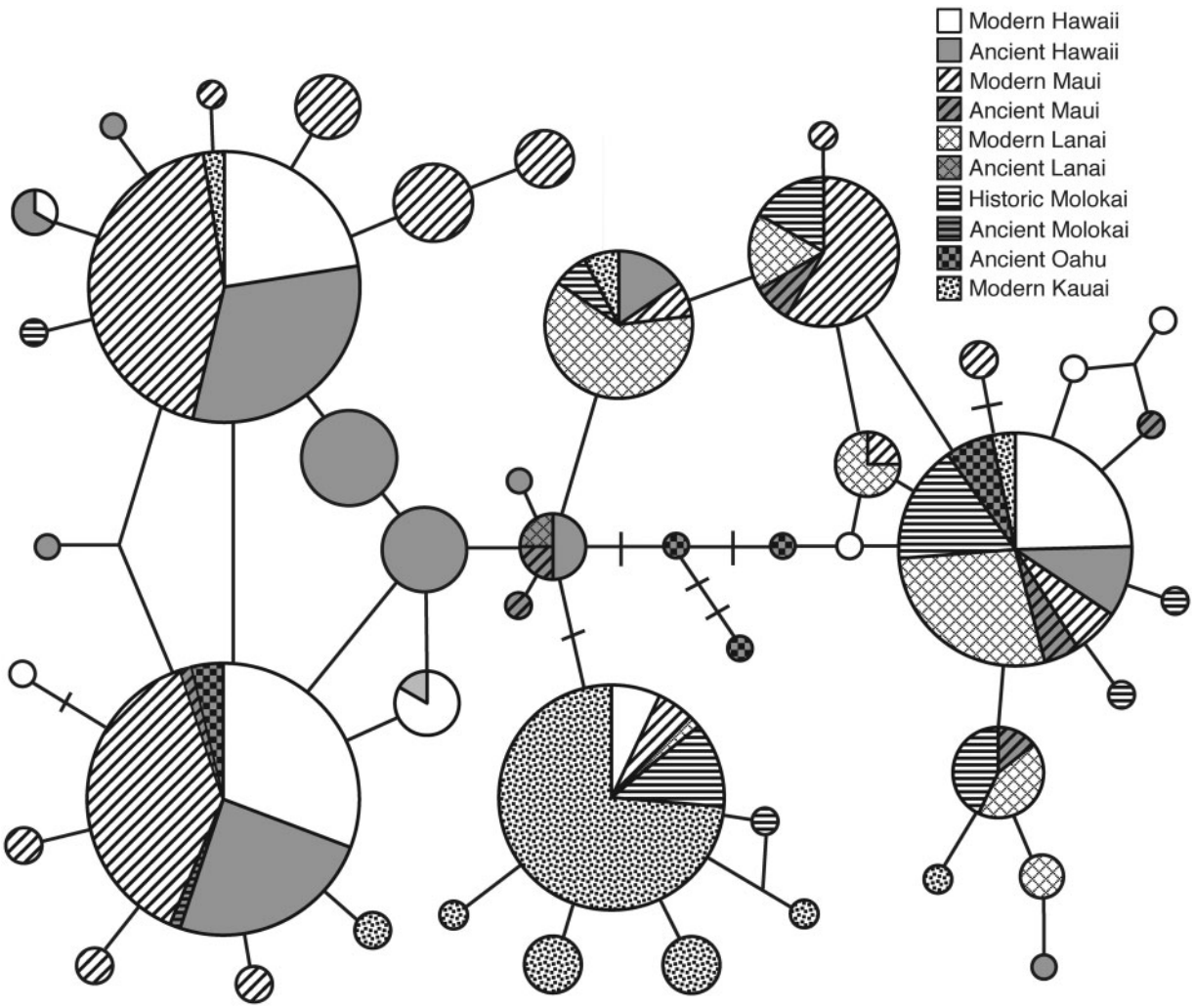

Fig. 1. Haplotype network depicting relationships between *Cytochrome b* sequences obtained for 417 ancient, historical, and modern Hawaiian petrels. Samples were obtained from all islands where the birds are currently known, or have been known in the past, to breed. Subfossil bones from Kauai were not available for destructive sampling, and the population on Oahu has been extirpated so no modern samples were available to include in the study. The size of the circle is proportional to the haplotype frequency, and all haplotypes differ by a single substitution unless otherwise indicated by dashes, which represent the number of additional substitutions.

was 524 bp for modern samples, and the average combined sequence length was approximately 480 and 400 bp for historic and ancient samples, respectively. No instances of Hawaiian petrel contamination were found in extraction, blank, or alternate species control samples. Additionally, Bayes factors indicate no support for BEAST analyses that included an age-dependent model of DNA damage over analyses without (2 ln Bayes factor = −4.70). Fifty-one variable sites were present in the data set and yielded a total of 46 haplotypes (fig. 1). Of these, 34 haplotypes were found in modern and historical samples, and 20 were found in ancient samples with 8 shared between time periods.

Gene Flow and Population Isolation

Investigation of gene flow and population isolation demonstrated that, in general, differentiation among Hawaiian petrels breeding on each island has been, and is currently, high (fig. 2*A*–*C*, supplementary tables 8 and 9, Supplementary Material online). In the past, F_{ST} ranged between 0.229 and 0.437 between individuals from Hawaii, Maui, and Oahu, with the highest level found between Hawaii and Oahu (fig. 2*A*). In the analysis with modern Hawaiian petrels from Hawaii, Maui, Lanai, and Kauai, F_{ST} ranged between 0.068 and 0.633 and was significantly greater than zero in all cases (fig. 2*B*). This indicates that although the Hawaiian petrel often makes foraging trips of greater than 10,000 km to the Gulf of Alaska, it appears to disperse less than 300 km to breed. In contrast to this general pattern, recent work (Welch et al. 2012) has shown high levels of gene flow between the extirpated population on Molokai and the modern population on Lanai. Here, both the extirpated populations on Oahu and Molokai show low, non-significant levels of divergence from one another, as well as from the modern population on Lanai (fig. 2*D*). Therefore it appears that population declines in the colony may lead Hawaiian petrels to deviate from their generally philopatric tendencies, perhaps because declining colonies do not offer sufficient social stimulus to attract young birds prospecting for new nesting sites (Danchin et al. 1998; Welch et al. 2012). Based on the age of the most recent

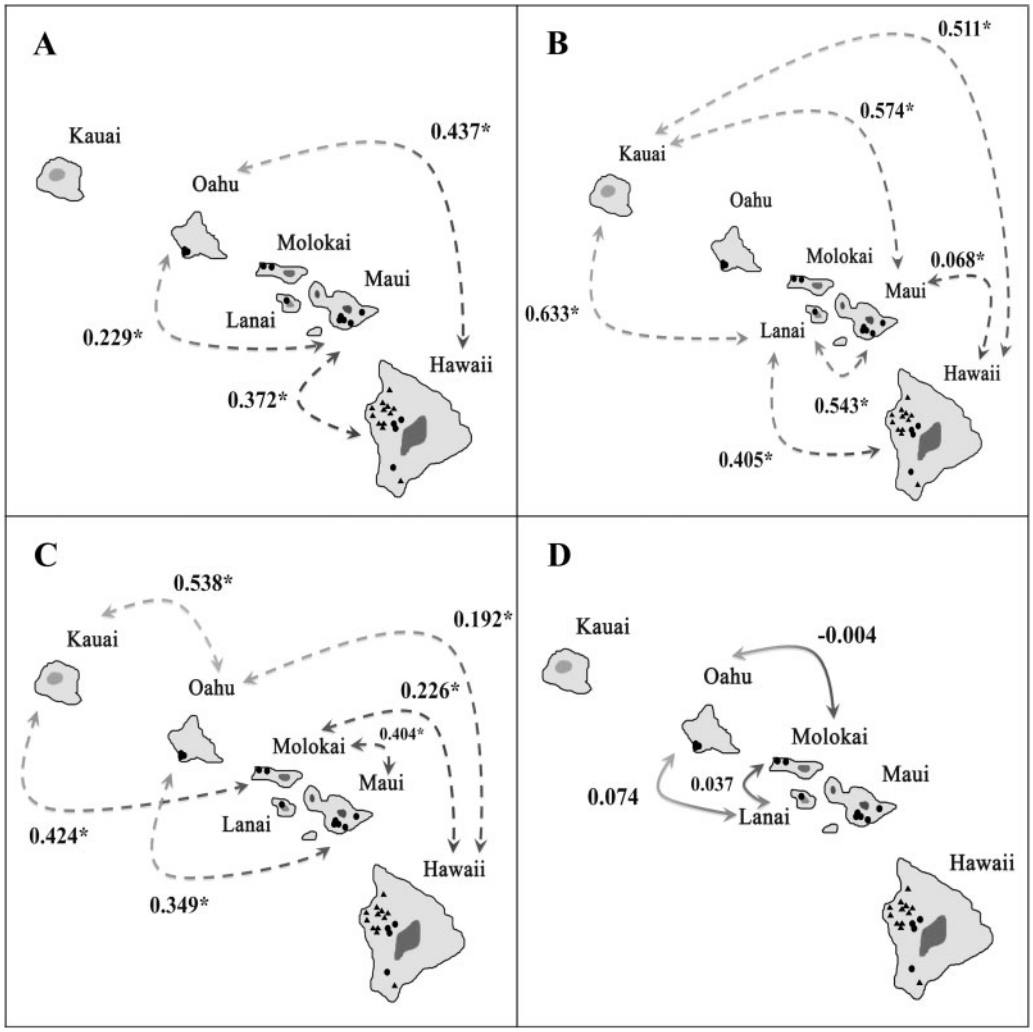

Fig. 2. Patterns of population isolation and gene flow through time. Map of the main Hawaiian Islands with approximate locations of modern (Hawaii, Maui, Lanai, and Kauai) and historically known (Molokai) breeding colonies shaded in dark gray. Approximate collection localities of subfossil bones are designated by triangles for archeological sites and circles for paleontological sites. Significant F_{ST} (*) was found between ancient populations (*A*), modern populations (*B*), and between two extirpated populations on Oahu and Molokai and most modern populations (*C*). However, estimates of F_{ST} between extirpated populations and modern birds on Lanai (*D*) were not significant.

radiocarbon-dated bones, the population on Oahu may have been extirpated around 550–660 years ago. Individuals from Oahu may have dispersed to either Lanai or Molokai as numbers in their own colony dwindled. Then later, as the population on Molokai declined, those birds may have dispersed to Lanai. Although mitochondrial DNA sequences only reflect the movements of females, differentiation has also been found between modern birds from each island using data sets consisting of multilocus microsatellite genotypes and nuclear intron sequences (Welch et al. 2012). Archeological and paleontological work on Lanai and Molokai has been relatively limited, and discovery of additional bones from these islands would be helpful in gaining a better understanding of dispersal as a potential response of the Hawaiian petrel to human-mediated disturbance.

Temporal Changes in Effective Population Size

Despite a documented contraction in the range of the Hawaiian petrel, genetic analyses indicate that there has not been a significant decline in the effective population size or genetic diversity of petrels on Hawaii and Maui. For individuals from the island of Hawaii, which had the largest ancient sample size ($N = 77$), the Bayesian skyride analysis shows that effective population size has been relatively stable since humans colonized the island (fig. 3*A*). The median estimate of effective population size shows there may have been a 40% decrease over the last approximately 800 years, but the 95% confidence interval indicates no significant change. In addition, Bayes factors indicate support for the constant population size model over the skyride model (2 ln Bayes factor = 27.37). Neither haplotype diversity or nucleotide diversity were significantly different between time bins, and estimates of effective size using the temporal alleles approach demonstrated no significant change (supplementary tables 10 and 11, Supplementary Material online). A similar pattern was found on Maui (fig. 3*B*). The Bayesian skyride analysis showed an increase in effective population size over time until about 1,100 years ago, after which it stabilized and remained

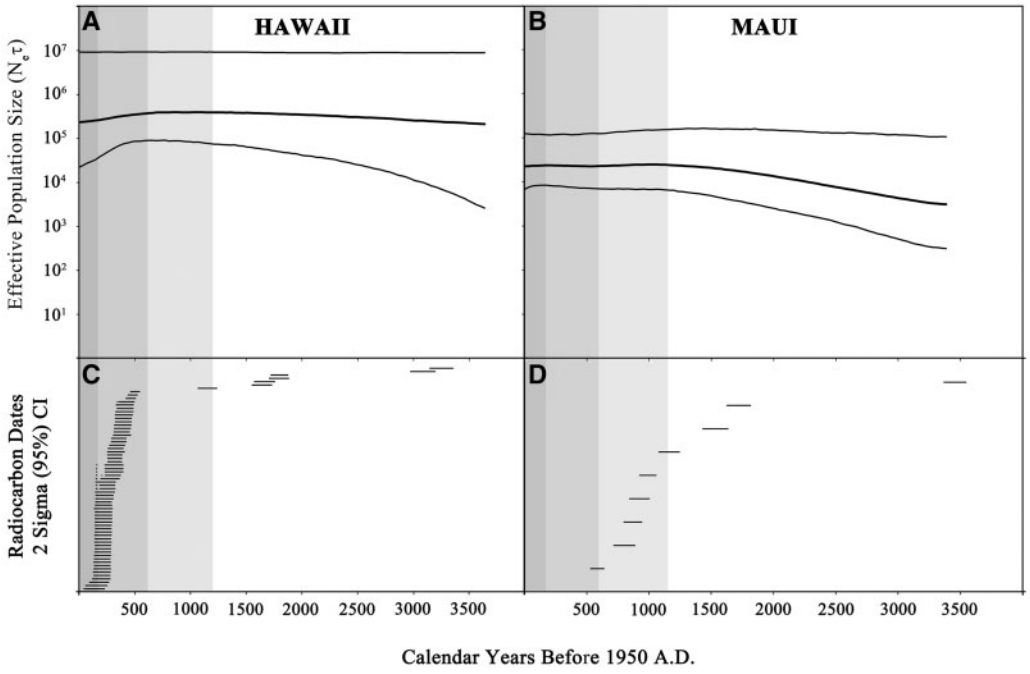

Fig. 3. Temporal trends in the effective population size of Hawaiian petrels. Upper plots show the Bayesian skyride of effective population sizes (thick line is the median posterior probability, thin lines are the upper and lower 95% confidence intervals) for Hawaii (*A*) and Maui (*B*). Lower plots show ^{14}C date ranges (2 sigma or 95% confidence intervals) in calendar years before 1950 AD for 77 samples from Hawaii (*C*) and nine samples from Maui (*D*). Gray shaded areas coincide with periods of colonization and expansion by humans. White: prior to human arrival, Light gray: Polynesian colonization and early population growth, Medium gray: Polynesian population expansion, and Dark gray: European arrival and expansion.

constant. This could be due to a relatively low ancient sample size ($N = 9$) for this island, which would result in few coalescent events in the past. Bayes factors indicate no support for the skyride model over the constant size model (2 ln Bayes factor = −8.80). No significant changes in haplotype or nucleotide diversity were found (supplementary table 12, Supplementary Material online). Although these findings are based only on mitochondrial DNA, it is unlikely that a comparable nuclear data set would show a different pattern because nuclear markers have an effective population size that is approximately four times higher than that of the mitochondrial genome, and therefore would take longer to acquire the signal of a decline.

The observed pattern of stasis in effective population size, especially on the island of Hawaii, is somewhat surprising. At some sites on this island approximately 70% of bird bones found in an archeological context belonged to the Hawaiian petrel (Athens et al. 1991), and historical accounts indicate that Hawaiian petrel chicks were considered a delicacy for the social elite (Henshaw 1902). Similarly, in a modern breeding colony on Mauna Loa, 19 of 41 burrows discovered during a survey occurred in pits that were apparently modified by humans in prehistoric or early historic times (Hu et al. 2001). In addition to direct human exploitation, exotic mammalian predators such as rats, which were first introduced by the Polynesians, and cats and mongooses, which were introduced later by Europeans, have been and continue to be significant sources of mortality for the Hawaiian petrel (Simons and Hodges 1998). Viability analyses indicate that the contemporary petrel population on Mauna Loa may even become extirpated in the near future (Hu et al. 2001). This information, in addition to subfossil evidence from around the island, indicates that the Hawaiian petrel has undergone a substantial range contraction on the island of Hawaii.

There are several potential explanations for the observed pattern of genetic stability. First, population genetics theory predicts that it can actually be quite difficult in some cases to lose genetic diversity. The proportion of heterozygosity retained (relative to the initial heterozygosity) is given by the equation $(1 - 1/(2N_e))^t$ (Frankham et al. 2002). Therefore, even in cases where a population is reduced to a single pair of individuals for one generation, 75% of the heterozygosity will be retained. Similarly, the effect of a sustained population reduction may take a long time to observe. For example, if a population was reduced to a N_e of 100, it would require approximately 57 generations before 25% of the heterozygosity would be lost. If the generation time of a species is long then this could lead to retention, or buffering, of genetic diversity over a relatively longer time (in years) as compared with a species with a shorter generation time.

Second, gene flow between populations can also help retain genetic diversity. Dispersal may have prevented the loss of Hawaiian petrel haplotypes formerly found in extirpated colonies on Oahu and Molokai, and this could be one mechanism preventing losses of genetic diversity in the species overall. However, the population on Hawaii appears to be isolated, as F_{ST} was found to be high between individuals from

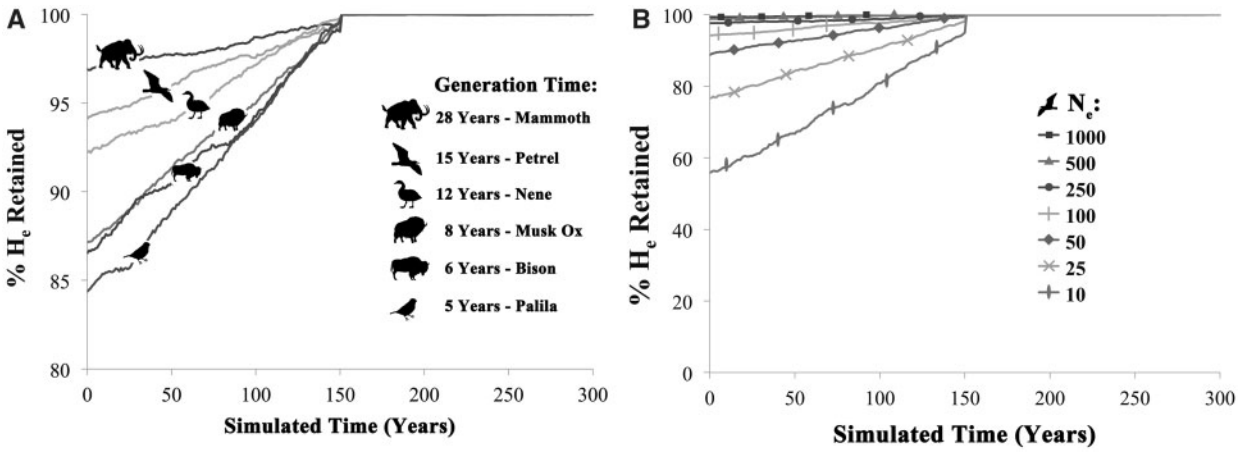

Fig. 4. Simulations of the effect of generation time and severity of decline on expected heterozygosity (H_e). (*A*) Comparison of the effect of generation time for mammoth (*Mammuthus* sp.), Hawaiian petrel (*Pterodroma sandwichensis*), nene (*Branta sandvicensis*), musk ox (*Ovibos moschatus*), bison (*Bison bison*), and the palila (*Loxioides bailleui*). (*B*) Effects of a decline in effective population size from 50,000 to less than 1,000 individuals at 150 years in the past.

Hawaii, Maui, and Oahu in the past and between modern individuals from all islands where this species is currently known to breed.

Third, high levels of genetic diversity would be maintained if perhaps the decline in census population size of the Hawaiian petrel was not as severe as previously thought. Burrow nesting seabirds can be difficult to study. Many species are morphologically similar, making taxonomic identification at sea, and hence estimation of population sizes, challenging (Spear et al. 1995). Additionally, colonies can be difficult to locate, particularly for nocturnal species. Identification of colony locations has only recently become more successful through implementation of several technological advances, such as radiotelemetry and marine surveillance radar surveys (Lawrence et al. 2008). Indeed, it is understandable that the Hawaiian petrel may have been overlooked. This species spends the majority of its life at sea, only coming to land for short periods to incubate or feed their chicks. Even then it returns to breeding colonies nocturnally, nesting in burrows that can be greater than 2 m long. In addition, extant colonies occur in rugged terrain and can be extremely challenging to access (Simons and Hodges 1998).

Finally, this pattern of stability could be caused by low power in the data set. Here, mitochondrial DNA sequences contained 51 variable sites, from which 46 haplotypes were discovered, suggesting relatively high levels of variability. Also, on Hawaii, ancient samples ($N = 77$) outnumbered the modern samples ($N = 71$). Additionally, other studies have been able to detect changes in effective population size over time using mitochondrial sequences of similar length (Shapiro et al. 2004; Campos et al. 2010). Although this indicates that this approach should have sufficient power to detect changes, at least in some circumstances, it can be particularly difficult to detect bottlenecks that do not reduce the population size by less than approximately 50%. Large nuclear data sets, perhaps consisting of data from tens or hundreds of thousands of SNPs, would likely give sufficient power to detect such fine scale changes in genetic diversity (Mourier et al. forthcoming). Next-generation sequencing approaches have the ability to generate these types of data sets, and genomic scale studies are becoming increasingly feasible in non-model organisms (Ekblom and Galindo 2011), but obtaining genome sequences from ancient samples is still challenging (Orlando et al. 2011). Future work may provide these resources for the Hawaiian petrel.

Therefore two explanations remain for the observed pattern of genetic stability: (1) the decline in census population size of the Hawaiian petrel was not as severe as previously thought, and/or (2) perhaps the long generation time of this species is buffering it against loss of genetic diversity over the short term.

Demographic Modeling

We used demographic models to investigate if the Hawaiian petrel, as a species, could have been on the brink of extinction in the mid-1900s, as previously feared, given their life history and recent estimates of census population size. We utilized the exponential growth model, since this model should give the absolute minimum growth rate required for recovery during this period, and then we compared these minimum necessary growth rates with rates obtained from observations in the field. If the Hawaiian petrel could not achieve even the minimum modeled growth rate, then it is highly unlikely they could have achieved rates necessary for demographic recovery to current census population size estimates, especially when taking into account predation from introduced mammals.

Assuming that the Hawaiian petrel was near extinction (i.e., 200 birds) in 1915, the year after the last major museum collection, a breeding frequency of 89%, annual adult survival of 93%, juvenile survival of 80%, and the maximum reproductive success of 72% observed during a three-year field study of this species (Simons 1984), a growth rate of 0.050 would be required to reach a census size of 11,000 individuals, the lower 95% confidence limit of the estimated census population size in 1995 (supplementary table 5, Supplementary Material online; Spear et al. 1995).

This is an order of magnitude larger than highest growth rate of 0.005 observed for a population of petrels studied on the island of Maui, where predator control programs were already in effect. This is also almost an order of magnitude larger than the growth rate of 0.008 resulting from the best possible projected scenario of 75% reproductive success proposed by Simons (1984). Therefore, it appears unlikely that the Hawaiian petrel was near extinction in the beginning of the last century. Probably more, and perhaps many more, individuals remained during the mid-1900s.

Simulated Declines in Effective Population Size

Although Hawaiian petrel populations may not have declined to the brink of extinction, the distribution of subfossil bones suggest that they were much more abundant in the past and that colonies were more widely distributed than they are today (Olson and James 1982b; Athens et al. 1991). Therefore it is possible that the long generation time of the Hawaiian petrel is buffering it against an immediate, substantial loss of genetic diversity (Amos and Balmford 2001). The Hawaiian petrel is thought to have an average lifespan of 36 years, and begins breeding in its fifth or sixth year, producing just a single offspring per year after that (Simons 1984). We performed simulations to explore the effect of generation time (i.e., the midpoint, or half the difference, between longevity and age at first reproduction) on the retention of genetic diversity when N_e was held at 100 for 150 years.

We compared the life history characteristics of the Hawaiian petrel with those of other species for which changes in genetic diversity have been investigated using ancient DNA (Paxinos et al. 2002; Shapiro et al. 2004; Debruyne et al. 2008; Campos et al. 2010), or which have also survived human arrival in Hawaii. These included continental species such as woolly mammoths, musk ox, and bison, and endemic Hawaiian species, such as the nene (the Hawaiian goose), and the endangered Hawaiian honeycreeper, the palila. Life history characteristics for the mammoth were inferred from modern proboscideans (elephants). Similar to other reports and consistent with population genetics theory (Amos and Balmford 2001; Hailer et al. 2006), we found that longer generation times resulted in the retention of higher levels of expected heterozygosity as well as allelic richness (fig. 4*A*, supplementary fig. 2*A*, Supplementary Material online). This is because a period of 150 years represents fewer generations during which genetic diversity can be lost for long-lived compared with short-lived organisms. Overall, species with generation times greater than 15 years lost only 5% or less of their expected heterozygosity, whereas when generation times were less than 10 years losses were nearly double. The palila, one example of an endangered endemic Hawaiian honeycreeper that has survived human colonization of the Hawaiian Islands, has the shortest generation time (5 years) of the species modeled here and demonstrates the strongest decline (16%). Mammoths and Hawaiian petrels, on the other hand, have the longest generation times (28 and 15 years, respectively), and show the lowest losses of genetic diversity, just 3–6%. Bison and musk ox, with generation times of 6 and 8 years, respectively, show intermediate losses (about 13%). Simulations using arbitrarily chosen allele frequencies demonstrated the same patterns (supplementary fig. 3, Supplementary Material online).

If all else were equal it might be expected that studies of long-lived species like Hawaiian petrels and mammoths would show fewer or less substantial changes in effective population size over time than either bison or musk ox. This is consistent with empirical findings (Shapiro et al. 2004; Debruyne et al. 2008; Campos et al. 2010). Nene should be intermediate between these groups, and changes in genetic diversity have been found (Paxinos et al. 2002). Of course, in reality, many assumptions of these simulations may be violated. For example, even if generation time is very short, gene flow may prevent significant losses of genetic diversity. Also, life history is more complex than that modeled here, including biased sex ratios, nonrandom mating, and, for some species, multiple offspring per reproductive event. Additionally, levels of initial genetic diversity and severity of population declines were not the same in each of the cases mentioned above. However, despite these violations, simulations suggest that the generation time of an organism may temporarily mask temporal changes in genetic diversity, for example, in response to climate change or anthropogenic impacts, and therefore careful interpretation of observed patterns is required.

Finally, we conducted simulations in order to determine the severity of a population decline that would be required to reduce genetic diversity in the Hawaiian petrel. Simulations were conducted of a decline in effective population size from 50,000 to 1,000 individuals or fewer. Results indicate that a very severe decline would be required to lose substantial levels of genetic diversity, even over long periods of time. Effective population size could decrease to 50 individuals and remain there for 150 years and only 10% of the expected heterozygosity would be lost (fig. 4*B*, supplementary fig. 2*B*, Supplementary Material online). More severe declines, to a N_e of 25 or 10, resulted in a loss of 23% and 44% of the expected heterozygosity, respectively.

Extinction versus Persistence

There has been increasing interest in determining which characteristics of species may be related to extinction versus persistence. Lorenzen et al. (2011) investigated extinctions of continental mammalian megafauna during the Late Quaternary period. For both the woolly mammoth and the woolly rhinoceros genetic diversity appears to have been stable over time, similar to the trend observed in the Hawaiian petrel. The buffering effect of long generation time on genetic diversity could, in the short term, prevent the marked effects of inbreeding and may enable species to avoid the extinction vortex (Gilpin and Soulé 1986; Saccheri et al. 1998). However, long-lived species, such as the Hawaiian petrel, may still be vulnerable to stochastic events when populations decline to very small sizes.

Lorenzen et al. (2011) also found evidence of population isolation in the mammoth, rhinoceros, and Eurasian musk ox

prior to their extinction. Populations of the Hawaiian petrel demonstrated a general pattern of isolation as well; however, there was substantial gene flow between extirpated colonies and the contemporary colony on Lanai. Gene flow can help increase and/or maintain genetic diversity, prevent inbreeding, and aid in demographic recovery (Johnson et al. 2010), and therefore such dispersal may be one mechanism contributing toward the persistence of this species.

Lorenzen et al. (2011) also investigated the role of human encroachment on the extinction of the woolly rhinoceros and the mammoth. Humans did not appear to be associated with extinction of the rhinoceros, but may have played a role in the extinction of the mammoth due to direct exploitation. Humans certainly have had direct impacts on Hawaiian petrel abundance, as indicated by archeological evidence and historical accounts (Henshaw 1902; Athens et al. 1991). However, the introduction of exotic mammals has also had large impacts on the Hawaiian petrel, through greatly increased predation rates and severe habitat degradation (Athens et al. 2002; Carlile et al. 2003). Although direct human exploitation of this species has substantially decreased in recent times, the legacy of indirect human impacts, such as the introduction of exotic mammalian species, may always haunt the Hawaiian petrel.

Overall, it appears that a combination of long generation time and the ability to disperse may have allowed genetic diversity to remain relatively stable in the Hawaiian petrel despite a demographic decline. Given that many seabird species demonstrate similar life history strategies, this could also account for the observation that although populations of many seabirds have been extirpated, relatively few species have gone extinct (Steadman 1995). However, these life history strategies may represent a double-edged sword. If a certain level of social stimulus is required for dispersal to occur, then Hawaiian petrels may be unlikely to colonize new sites. Consequently, this species may become increasingly vulnerable to extinction in the future, especially if the remaining contemporary breeding colonies become extirpated or decline substantially. Additionally, the buffering effect of generation time on levels of genetic diversity in the Hawaiian petrel is likely to only be temporary, particularly if census population sizes continue to decline or if they become very small for many generations. Unfortunately, the long generation time of the Hawaiian petrel will also prevent quick population growth. Following a severe decline, slow demographic recovery could result in increased extinction risk due to stochastic events, an extended population bottleneck, and a longer period of time during which genetic diversity would be vulnerable to loss (Allendorf and Luikart 2007). Therefore, it may be best for conservation management actions to focus on increasing the population growth rate on each island before it is too late.

Even in the absence of extinction or decreases in genetic diversity, the documented decrease in the census population size of the Hawaiian petrel may have broad impacts. Seabirds are important marine predators and may influence food webs over large areas because they forage thousands of kilometers from their nesting sites (Hobson et al. 1994). Seabirds can also transfer marine nutrients to terrestrial ecosystems in the form of guano, food remnants, and both chick and adult mortality in the nest (Polis and Hurd 1996). Declines in the number of breeding seabirds due to predation by alien mammals has been shown to alter the productivity and composition of plant communities on islands (Croll et al. 2005; Fukami et al. 2006). Therefore, even though the genetic diversity of the Hawaiian petrel has been stable over the past 3,000 years, there could still be ecosystem-wide consequences from its population decline.

Supplementary Material

Supplementary figures 1–3 and Supplementary tables 1–12 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

We would like to thank Josh Adams, David Anderson, David Duffy, Fern Duvall, Nick Holmes, Darcy Hu, Seth Judge, Jay Penniman, and Keith Swindle for samples and helpful discussions of petrel biology. We also thank Hawaii Volcanoes National Park, the Hawaii Experimental Tropical Forest, and the Hawaii Department of Land and Natural Resources for access to samples. For permission and assistance sampling museum specimens we thank: James Dean, Megan Spitzer, and Christina Gebhard at the Smithsonian Institution's National Museum of Natural History; Carla Kishinami and Lydia Garetano at the Bernice P. Bishop Museum; and Kimball Garrett at the Natural History Museum of Los Angeles County. Art Medeiros provided invaluable support for fieldwork. We are especially grateful to John Southon for assistance in obtaining radiocarbon dates. We also wish to thank Frank Hailer, Amy Wilson, Heather Lerner, Nancy Rotzel, Jon Beadell, and the members of the Center for Conservation and Evolutionary Genetics, for discussions on data analysis or technical support. Greg McDonald and three anonymous reviewers provided helpful comments on an earlier version of this article. This work was supported by the Behavior, Ecology, and Evolution Program at the University of Maryland, the Smithsonian Office of Fellowships, the Smithsonian's Walcott Fund, the Center for Conservation and Evolutionary Genetics, and the National Science Foundation (grant # DEB-0745604 to P.H.O., R.C.F., and H.F.J.).

References

Abbott CL, Double MC, Trueman JW, Robinson A, Cockburn A. 2005. An unusual source of apparent mitochondrial heteroplasmy: duplicate mitochondrial control regions in *Thalassarche* albatrosses. *Mol Ecol.* 14:3605–3613.

Allendorf FW, Luikart G. 2007. Conservation and the genetics of populations. Malden (MA): Blackwell Publishing.

Amos W, Balmford A. 2001. When does conservation genetics matter? *Heredity* 87:257–265.

Athens JS, Kaschko M, James H. 1991. Prehistoric bird hunters: high altitude resource exploitation on Hawai'i Island. *Bishop Museum Occasional Papers* 31:63–84.

Athens JS, Tuggle DH, Ward JV, Welch DJ. 2002. Avifaunal extinctions, vegetation change, and Polynesian impacts in prehistoric Hawaii. *Archaeol Oceania*. 37:57–78.

Baldwin PH, Hubbard DH. 1948. The Hawaiian dark-rumped petrel reappears on Hawaii. *Condor* 51:231–232.

Banko PC, Black JM, Banko WE. 1999. Hawaiian goose (*Branta sandvicensis*). In: Poole A, editor. The birds of North America online. Ithaca (NY): Cornell Lab of Ornithology.

Banko PC, Johnson L, Lindsey GD, Fancy SG, Pratt TK, Jacobi JD, Banko WE. 2002. Palila (*Loxioides bailleui*). In: Poole V, editor. The birds of North America online. Ithaca (NY): Cornell Lab of Ornithology.

Banko WE. 1980. Part I. Population histories—species accounts. Sea birds: Hawaiian dark-rumped petrel ('Ua'u). Honolulu (HI): Cooperative National Park Resources Studies Unit, University of Hawaii at Manoa, Department of Botany.

Berthier P, Beaumont MA, Cornuet J-M, Luikart G. 2002. Likelihood-based estimation of the effective population size using temporal changes in allele frequenceis: a genealogical approach. *Genetics* 160:741–751.

BirdLife International. 2011. Species factsheet: *Pterodroma sandwichensis*. Available from: http://www.birdlife.org, last accessed December 8, 2011.

Blackburn TM, Cassey P, Duncan RP, Evans KL, Gaston KJ. 2004. Avian extinction and mammalian introductions on oceanic islands. *Science* 305:1955–1958.

Browne RA, Anderson DJ, Houser JN, Cruz F, Glasgow KJ, Hodges CN, Massey G. 1997. Genetic diversity and divergence of endangered Galapagos and Hawaiian petrel populations. *Condor* 99:812–815.

Bryan WA. 1908. Some birds of Moloka'i. *Bernice P Bishop Museum Occasional Papers* 4:43–86.

Campos PF, Willerslev E, Sher A, et al. (20 co-authors). 2010. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. *Proc Natl Acad Sci U S A*. 107:5675–5680.

Carlile N, Priddle D, Zino F, Natividad C, Wingate DB. 2003. A review of four successful recovery programmes for threatened sub-tropical petrels. *Marine Ornithol*. 31:185–192.

Croll DA, Maron JL, Estes JA, Danner EM, Byrd GV. 2005. Introduced predators transform subarctic islands from grassland to tundra. *Science* 307:1959–1961.

Danchin E, Boulinier T, Massot M. 1998. Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology* 79:2415–2428.

Debruyne R, Chu G, King CE, et al. (21 co-authors). 2008. Out of America: ancient DNA evidence for a new world origin of late quaternary woolly mammoths. *Curr Biol*. 18:1320–1326.

Depaulis F, Orlando L, Hänni C. 2009. Using classical population genetics tools with heterochronous data: time matters! *PLoS One* 4: e5541.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol*. 7:214.

Ekblom R, Galindo J. 2011. Applications of next-generation sequencing in molecular ecology of non-model organisms. *Heredity* 107:1–15.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinformatics Online*. 1:47–50.

Fleischer RC, Olson SL, James HF, Cooper AC. 2000. Identification of the extinct Hawaiian eagle (*Haliaeetus*) by mtDNA sequence analysis. *Auk* 117:1051–1056.

Frankham R. 1995. Effective population size/adult population size ratios in wildlife: a review. *Genet Res*. 66:95–107.

Frankham R. 2005. Genetics and extinction. *Biological Conservation* 126: 131–140.

Frankham R, Ballou JD, Briscoe DA. 2002. Introduction to conservation genetics. Cambridge, UK: Cambridge University Press.

Fukami T, Wardle DA, Bellingham PJ, Mulder CPH, Towns DR, Yeates GW, Bonner KI, Durrett MS, Grant-Hoffman MN, Williamson WM. 2006. Above- and below-ground impacts of introduced predators in seabird-dominated island ecosystems. *Ecol Lett*. 9:1299–1307.

Gilpin ME, Soulé ME. 1986. Minimum viable populations: processes of extinction. In: Soulé ME, editor. Conservation biology: the science of scarcity and diversity. Sunderland (MA): Sinauer Associates. p. 19–34.

Hailer F, Helander B, Folkestad AO, et al. (11 co-authors). 2006. Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from a population decline. *Biol Lett*. 2:316–319.

Henshaw HW. 1902. Birds of the Hawaiian Islands: a complete list of the birds of the Hawaiian possessions. Honolulu (HI): Thos. G. Thrum.

Ho SYW, Shapiro B. 2011. Skyline-plot methods for estimating demographic history from nucleotide sequences. *Mol Ecol Resour*. 11: 423–434.

Hobson KA, Piatt JF, Pitocchelli J. 1994. Using stable isotopes to determine seabird trophic relationships. *J Anim Ecol*. 63:786–798.

Hofreiter M, Jaenicke V, Serre D, von Haeseler A, Pääbo S. 2001. DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Res*. 29: 4793–4799.

Hu D, Glidden C, Lippert JS, Schnell L, MacIvor JS, Meisler J. 2001. Habitat use and limiting factors in a population of Hawaiian dark-rumped petrels on Mauna Loa, Hawaii. In: Scott JM, Conant S, Van Riper CI, editors. Evolution, ecology, conservation and management of Hawaiian birds: a vanishing avifauna. Studies in avian biology. Camarillo (CA): Cooper Ornithological Society. p. 234–242.

Hughen KA, Maillie MGL, Bard E, et al. (27 co-authors). 2004. Marine04 marine radiocarbon age calibration, 0-26 cal kyr BP. *Radiocarbon* 46: 1059–1086.

James HF. 1987. A late Pleistocene avifauna from the island of Oahu, Hawaiian Islands. In: Mourer-Chauviré C, editor. L'Évolution des oiseaux d'après le témoignage des fossiles; Table Ronde Internationale de CNRS; 1985, Sept. 18–21. Lyon-Villeurbanne: Documents des Laboratoires de Géologie de Lyon. p. 121–128.

James HF, Olson SL. 1991. Descriptions of thirty-two new species of birds from the Hawaiian Islands: Part 2. Passeriformes. *Ornithol Monogr*. 46:1–88.

Johnson WE, Onorato DP, Roelke ME, et al. (16 co-authors). 2010. Genetic restoration of the Florida panther. *Science* 329:1641–1645.

Kass RE, Raftery AE. 1995. Bayes factors. *J Am Stat Assoc*. 90:773–795.

Kaufman L. 2011. Albatross is a mother at 60. New York: New York Times; Available from: http://green.blogs.nytimes.com/2011/03/08/albatross-is-a-mother-at-60.html, last accessed March 8, 2011.

Kirch PV. 1985. Feathered gods and fishhooks. Honolulu (HI): University of Hawaii Press.

Kirch PV. 2000. On the road of the winds. Los Angeles (CA): University of California.

Kuo C-H, Janzen J. 2003. BOTTLESIM: a bottleneck simulation program for long-lived species with overlapping generations. *Mol Ecol Notes*. 3:669–673.

Laursen L, Bekoff M. 1978. *Loxodonta africana*. In: Anderson S, editor. Mammalian species. Lawrence (KS): American Society of Mammalogists. p. 1–8.

Lawrence HA, Taylor GA, Crockett DE, Millar CD, Lambert DM. 2008. New genetic approach to detecting individuals of rare and endangered species. *Conserv Biol.* 22:1267–1276.

Lent PC. 1988. *Ovibos moschatus*. In: Verts BJ, Anderson S, Phillips CJ, editors. Mammalian species. Lawrence (KS): American Society of Mammalogists. p. 1–9.

Lorenzen ED, Nogues-Bravo D, Orlando L, et al. (55 co-authors). 2011. Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature* 479:359–364.

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conserv Genet.* 11: 355–373.

Maddison DR, Maddison WP. 2008. MacClade v 4.08. Sunderland (MA): Sinauer Associates.

Meagher M. 1986. *Bison bison*. In: Verts BJ, Anderson S, Lawlor TE, editors. Mammalian species. Lawrence (KS): American Society of Mammalogists. p. 1–8.

Minin VN, Bloomquist EW, Suchard MA. 2008. Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. *Mol Biol Evol.* 25:1459–1471.

Mourier T, Ho SYW, Gilbert MTP, Willerslev E, Orlando L. Forthcoming 2012. Statistical guidelines for detecting past population shifts using ancient DNA. *Mol Biol Evol.* doi: 10.1093/molbev/mss094.

Munro GC. 1955. Hawaii's birds and their homes: how to save them from extinction. Part XI—Seabirds of the main group. *Elepaio* 16:46–47.

Naughton MB, Romano MD, Zimmerman TS. 2007. A conservation action plan for black-footed albatross (*Phoebastria nigripes*) and laysan albatross (*Phoebastria immutabilis*). Version 1.0. Portland (OR): US Fish and Wildlife Service, p. 1–37.

Nunn GB, Stanley SE. 1998. Body size effects and rates of cytochrome b evolution in tube-nosed seabirds. *Mol Biol Evol.* 15:1360–1371.

Olson S, James H. 1982a. Fossil birds from the Hawaiian Islands: evidence for wholesale extinction by man before western contact. *Science* 217: 633–635.

Olson SL, James H. 1982b. Prodromus of the fossil avifauna of the Hawaiian Islands. *Smithson Contrib Zool.* 365:1–59.

Olson SL, James HF. 1991. Descriptions of thirty-two new species of birds from the Hawaiian Islands: Part 1. Non-Passeriformes. *Ornithol Monogr.* 45:1–88.

Orlando L, Ginolhac A, Raghavan M, et al. (17 co-authors). 2011. True single-molecule DNA sequencing of a Pleistocene horse bone. *Genome Res.* 21:1705–1719.

Paxinos EE, James HF, Olson SL, Ballou JD, Leonard JA, Fleischer RC. 2002. Prehistoric decline of genetic diversity in the nene. *Science* 296: 1827–1827.

Polis GA, Hurd SD. 1996. Linking marine and terrestrial food webs: allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. *Am Nat.* 147:396–423.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol Biol Evol.* 25:1253–1256.

Ramakrishnan U, Hadly EA, Mountain JL. 2005. Detecting past population bottlenecks using temporal genetic data. *Molecular Ecol.* 14: 2915–2922.

Rambaut A, Ho SYW, Drummond AJ, Shapiro B. 2009. Accommodating the effect of ancient DNA damage on inferences of demographic histories. *Mol Biol Evol.* 26:245–248.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392:491–494.

Shapiro B, Drummond AJ, Rambaut A, et al. (27 co-authors). 2004. Rise and fall of the Beringian steppe bison. *Science* 306:1561–1565.

Simons TR. 1984. A population model of the endangered Hawaiian dark-rumped petrel. *J Wildl Manage.* 48:1065–1076.

Simons T, Hodges C. 1998. Dark-rumped petrel (*Pterodroma phaeopygia*). In: Poole A, Gill F, editors. The birds of North America. Philadelphia: Birds of North America, Inc.

Sorenson MD, Fleischer RC. 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proc Natl Acad Sci U S A.* 93:15239–15243.

Spear LB, Ainley DG, Nur N, Howell SNG. 1995. Population size and factors affecting at-sea distribution of four endangered Procellariids. *Codor* 97:613–638.

Stafford TWJ, Hare PE, Curie LA, Jull AJT, Donahue D. 1991. Accelerator radiocarbon dating at the molecular level. *J Archaeol Sci.* 18:35–72.

Steadman DW. 1995. Island birds: biodiversity meets zooarchaeology. *Science* 267:1123–1131.

Steadman DW. 2006. Extinction and biogeography of tropical Pacific birds. Chicago: University of Chicago Press.

Stuvier M, Reimer PJ. 1993. Extended ^{14}C data base and revised CALIB 3.0 ^{14}C age calibration program. *Radiocarbon* 35:215–230.

Suchard MA, Weiss RE, Sinsheimer JS. 2001. Bayesian selection of continuous-time Markov chain evolutionary models. *Mol Biol Evol.* 18:1001–1013.

Welch AJ, Fleischer RC, James HF, et al. (11 co-authors). 2012. Population divergence and gene flow in an endangered and highly mobile seabird. *Heredity* 109:19–28.

Wilmshurst JM, Hunt TL, Lipo CP, Anderson AJ. 2011. High-precision radiocarbon dating shows recent and rapid initial human colonization of East Polynesia. *Proc Natl Acad Sci U S A.* 108: 1815–1820.

Xia X, Xie Z. 2001. DAMBE: software package for data analysis in molecular biology and evolution. *J Hered.* 92:371–373.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. [dissertation]. [Austin (TX)]: University of Texas.