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Inferring colonization history and dispersal patterns of a long-lived seabird by combining genetic and empirical data

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Abstract

Identifying biological trends and threats to organisms that make long distance migrations are often the limiting factors in their conservation. Indeed, Laysan albatross *Phoebastria immutabilis* are highly vagile seabirds, foraging throughout the North Pacific Ocean. Despite mark–recapture data indicating natal philopatry, Laysan albatross recently re-colonized several anthropogenically extirpated breeding locations. At the same time, a breeding population in the north-western Hawaiian Islands was lost to erosion and it was hypothesized that the colonization events were due to displacement rather than dispersal. Nuclear and mitochondrial markers were used in a range wide survey to test whether natal philopatry corresponded to population structure in Laysan albatross, and to determine whether recent colonization events were a result of displacement from vanishing breeding habitat. Five microsatellite loci found little population structure ($F_{ST} = 0.01$, $P = 0.001$), and sequences from the mitochondrial control region revealed low population structure ($\pi_{ST} = 0.05$, $P < 0.001$). The results were consistent with male-mediated dispersal and strong, but not absolute, philopatry by females. Mixed stock analyses and banding records from the newly colonized sites indicated contributions from multiple source populations, which contradicted the displacement hypothesis of a single source population and instead supported species-wide dispersal from all source colonies. High genetic diversity ($\pi = 0.045$, $h = 0.989$), rapid colonization, and great dispersal potential bode well for the conservation of Laysan albatross. However, it may be necessary to protect high-island nesting sites, preserve genetic diversity and maintain breeding populations in the face of projected sea level rises and persistent bycatch.

Introduction

Identifying biological trends and potential threats to organisms that make long distance migrations are often the limiting factor in the conservation of marine animals (Avise, 2000). For example, Laysan albatross *Phoebastria immutabilis* bycatch in North Pacific longlining fisheries impacts breeding colonies thousands of miles away from foraging grounds (Gilman & Freifeld, 2002). The high vagility and remote breeding sites of seabirds has made it difficult to draw the links between anthropogenic threats, oceanographic changes and population trends. In many other taxa, dispersal capability and geographic isolation are often used to form *a priori* hypotheses concerning genetic diversity and variation among and within populations (Bohonak, 1999). However, seabirds are not typically limited by barriers to dispersal, as demonstrated through mark–recapture studies, and satellite telemetry (Fisher, 1976; Jouventin & Weimerskirch, 1990). Laysan albatross fall on the extreme end of this spectrum; they are highly vagile seabirds characterized by an almost entirely pelagic existence (Hyrenbach, Fernandez & Anderson, 2002; Young *et al.,* 2009*a*,*b*). However, despite an inherently high dispersal potential, and an almost complete lack of dispersal barriers, mark–recapture studies of Laysan albatross have shown them to be philopatric to their natal nest site (Fisher, 1976). This observation has led to predictions of restricted gene flow that have been confirmed in other species of albatross (Abbott & Double, 2003*a*; Bried *et al.,* 2007; Walsh & Edwards, 2005).

Despite reports of high natal philopatry, in the 1970's Laysan albatross began colonizing islands from Japan to Mexico and expanding their range beyond the north-western Hawaiian Islands (NWHI) where 97% of the species currently nest (Naughton, Romano & Zimmerman, 2007; Arata, Sievert & Naughton, 2009). Many of these colonization events were re-colonizations of anthropogenically extirpated breeding colonies such as those on Mukojima, Japan (Kurara, 1978), Wake Island in the Western Pacific, and Lehua, Oahu and Kauai in the main Hawaiian Islands (Zeillemaker & Ralph, 1977; VanderWerf *et al.,* 2007; Young *et al.*, 2009*a*,*b*). Factors contributing to their historical declines included human consumption, feather collecting, egg collecting, predation by introduced mammals and military activities (Arata *et al.,* 2009; Awkerman,

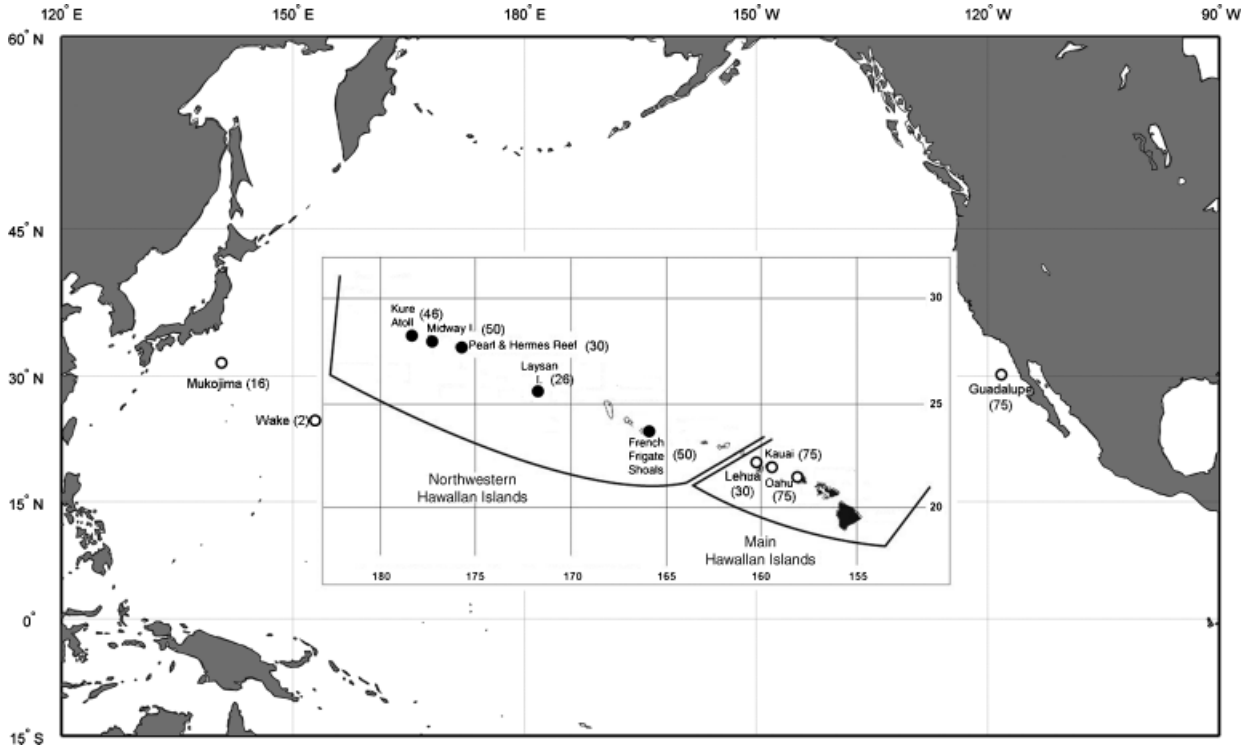

Figure 1 Sampling locations and number of samples (in parentheses) collected from each Laysan albatross *Phoebastria immutabilis* colony. Closed circles denote source colonies, and open circles denote new colonies.

Anderson & Whittow, 2009). In addition to the re-colonization of historical nesting sites, Laysan albatross also recently expanded their breeding range to Isla Guadalupe, Clarion and San Benedicto off of Mexico in the East Pacific (Fig. 1; Gallo-Reynoso & Figueroa-Carranza, 1996). These colonization events all occurred within the past 35 years (<2 albatross generations) and include <400 breeding pairs at each site. The few birds breeding in these new colonies that were banded elsewhere as chicks (<20 individuals colony^{-1}) have been primarily from French Frigate Shoals in the NWHI (Young *et al.,* 2009*a*,*b*). This site lost an important nesting area, Whale-Skate Island, in the extreme El-Nino event of 1997 after more than 30 years of erosion due to natural atoll processes and displaced hundreds breeding pairs of Laysan albatross (B. Flint, pers. comm., Arata *et al*., 2009). As a result of the loss of breeding habitat that coincided with the range expansion and re-colonization events, it has been hypothesized that climate-driven displacement was in fact responsible for the appearance of these new Laysan albatross colonies, rather than a low level of natural dispersal that went undetected in mark–recapture studies.

Here both nuclear DNA microsatellites and mitochondrial DNA sequences were employed in a range-wide survey of Laysan albatross rookeries to test whether the natal philopatry documented in mark–recapture studies is reflected in population structure of this species. If population structure is present, and provides sufficient power, the utility of mixed stock analyses in assigning colonizers back to their colony of origin will be tested and compared with banding records at these newly colonized sites. If recent colonization events can be attributed to a single colony's displacement, then the new nesting sites should have a relatively uniform genetic composition in mixed stock analyses. Alternatively, if the new nesting sites have multiple origins, this should be apparent in mixed stock analyses as indicated by contributions from multiple source colonies as well as banding records available from several of the new colonies. The Laysan albatross is listed as vulnerable by the International Union for the Conservation of Nature (IUCN, 2007) as a result of recent population fluctuations and the high rates of at-sea mortality from bycatch in North Pacific Fisheries. By resolving the range-wide population structure and the source(s) of immigrants that have colonized sites throughout the Pacific, this study will provide a stronger scientific foundation for identification of management units and conservation priorities for this species.

Methods

Sample collection

Blood samples were collected from chicks representing the entire extant range of Laysan albatross. Feathers were collected from fledglings in Japan, and tissue biopsies were collected from dead chicks from Pearl and Hermes Reef and

Laysan Island in lieu of blood. Sampling locations, number of samples, and classification of each colony (source, or NWHI vs. new) are documented in Fig. 1. French Frigate Shoals, the atoll where a breeding colony disappeared, is represented by Tern Island in this study, which is the next closest islet in the atoll.

Laboratory techniques

DNA was extracted from tissue and feathers using Qiagen® DNeasy™ extraction kits (Qiagen, Valencia, CA, USA) following the manufacturer's protocols and modified during the final two steps for a final elution volume of 175 μL in H_2O. DNA from blood was isolated using ID Labs IDetect™ (ID Labs Biotechnology Inc., London, ON, Canada) DNA purification kit for whole animal blood following manufacturer's protocols and eluted in 50 μL of H_2O.

Thirty-six microsatellite loci were screened from libraries developed for various albatross species by Burg (1999), Abbott & Double (2003*a*) and Dubois, Jarne & Jouventin (2005). Of these 36 loci, only six successfully amplified *P. immutabilis*, one of which was monomorphic and was excluded, leaving five suitable loci (Table 1). Thermal profiles and PCR conditions can be found in Young, Zaun & VanderWerf (2008). Loci were multiplexed on an ABI 3730 XL (Applied Biosystems, Foster City, CA, USA) automated sequencer with one primer of each primer pair fluorescently labeled. Each set of PCR reactions were performed with one positive and one negative control to check for amplification and contamination problems, respectively.

Oligonucleotide primers Spec 1 (5′-CAGCTTATGTAT AAATGCG-3′) and Glu 7 (5′-CGGGTTGCTGATTT CTCG-3′) initially developed for the vertebrate mtDNA control region of *Thalassarche* albatross species (Abbott & Double, 2003*b*) were used to amplify 189 bp of the control region of *P. immutabilis* following reaction conditions and PCR profiles of Abbott & Double (2003*b*). Five microliters of the PCR product was then added to 1 U of ExoSAP-IT® kit (USB Corporation, Cleveland, OH, USA) and incubated for 15 min at 37 °C followed by 15 min at 80 °C in order to purify PCR products. Products were sequenced at the Greenwood Core Facility at the University of Hawaii at Manoa in both forward and reverse directions.

Table 1 Characteristics of dinucleotide microsatellite loci

Name	Number of alleles	Size	Het (obs)	Het (exp)	Null
11H7	11	189	0.859	0.822	−0.0277
12H8	4	164	0.364	0.403	0.0602
D9	4	81	0.188	0.181	−0.0253
D21	4	162	0.521	0.530	0.0032
Tc65	8	137	0.533	0.503	−0.0545

Size is the size in base pairs of smallest allele, Het (obs) is the observed proportion of heterozygotes, Het (exp) is the expected heterozygosity, and Null is the per locus possible frequency of null alleles.

Data analysis

All microsatellite loci were scored independently at least twice with GENEMAPPER (Applied Biosystems, 850 Lincoln Centre Dr Foster City, CA, USA) and were tested for departures from Hardy–Weinberg equilibrium (HWE), linkage disequilibrium, and null alleles in GENEPOP 3.1b (Raymond & Rousset, 1997) and CERVUS 2.0 (Marshall *et al.,* 1998); no locus showed significant deviations from the above assumptions. A *P*-level of 0.05 was used in conjunction with a molecular analysis of variance (AMOVA) to determine population structure (F statistics) for microsatellites using ARLEQUIN version 3.0 (Excoffier, Laval & Schneider, 2005). A Bayesian maximum likelihood analysis using STRUCTURE version 2.0 (Pritchard & Wen, 2004) was performed to determine the number of effective populations. The results generated were based on running a series of independent simulations from one to five inferred populations (only source populations were included in this analysis). Each run used an admixture model with correlated allele frequencies, no prior population information, and 3×10^6 iterations after a burn-in of 2×10^5 iterations. The program was run independently 10 times for each number of populations (K) between one and five to test for convergence of the Markov chain (MCMC).

Mitochondrial DNA sequences were manually edited using SEQUENCHER 4.7 (Gene Codes Corporation, 775 Technology Dr Suite 100A, Ann Arbor, MI, USA). The problem of including inadvertently sequenced nuclear copies of mitochondrial genes (NUMTs; Sorenson & Quinn, 1998) or of nuclear heteroplasmy (Abbott *et al.,* 2005) was reduced in this study by using primers developed to minimize amplification of nuclear material (Abbott *et al.,* 2005), comparing both forward and reverse sequences, and checking for the presence of double peaks characteristic of NUMTs. No duplication or heteroplasmy were detected. Within each population, genetic diversity was estimated in DNAsp version 4.20 (Rozas *et al.,* 2003) using the nucleotide diversity index π as well as Tajimas D and Fu's F. Genetic differentiation between populations was quantified using the Φ_{ST} statistic computed using the program ARLEQUIN with 1000 MCMC simulations to determine statistical significance and an AMOVA was also done to estimate the extent of genetic differentiation between populations.

Program MIGRATE version 1.7.6 (Beerli, 1997) was used to estimate maximum-likelihood migration rates from the five source colonies into all other colonies with mtDNA sequences. This approach, based on coalescence using MCMC searches, is advantageous over conventional F_{ST}-based approaches in that it accounts for unequal effective population sizes and asymmetrical gene flow (Whitlock & McCauley, 1999; Beerli & Felsenstein, 2001), both of which are likely occurring in newly colonized sites in this dataset. The default settings of MIGRATE were used with the exception of changes made to the number of short and long Markov chains and the number of trees sampled which were increased (20 short chains sampling 10 000 trees and five long

Table 2 Population genetic statistics for Laysan albatross *Phoebastria immutabilis* populations[a] throughout the Pacific

	Kure	Midway	Pearl & Hermes	Laysan	Tern	Japan	Lehua	Kauai	Oahu	Mexico	Overall
# sequences obtained	45	41	23	24	52	15	23	37	42	54	358
Total # haplotypes	38	24	21	23	43	12	19	29	32	38	188
# unique haplotypes	21	17	7	11	22	5	7	9	13	19	131
Haplotype diversity (h)	0.986	0.851	0.992	0.996	0.988	0.971	0.984	0.986	0.986	0.983	0.989
Nucleotide diversity (π)	0.043	0.030	0.041	0.044	0.043	0.037	0.059	0.043	0.050	0.043	0.045
Theta	8.048	5.634	7.613	8.395	8.159	6.971	11.028	8.098	9.382	8.177	8.349
Tajimas D[b]	0.120	0.015	−0.118	0.603	−0.100	−0.388	0.274	0.316	0.316	0.054	−0.732
Fu's F[b]	−0.496	0.651	−12.580	0.494	−0.794	−3.308	−5.574	−0.124	0.100	−1.548	−264.852
# individuals amplified with MS	46	49	29	26	49	16	27	45	75	53	417
# microsatellite alleles	27	24	25	27	28	18	26	29	30	29	31

[a]Wake Island was not included in this table as with only two samples these statistics could not be calculated.
[b]No Tajima's D and Fu's F statistics were significant at $P \leq 0.05$.

chains sampling 100 000 trees, following an initial 'burn-in' period of 10 000 trees). A Mantel tests comparing gene flow estimates based on coalescent and F_{ST}-based approaches were run using MANTEL ver.32 (http://life.bio.sunysb.edu/morph/) and a two-sample t-test was done in Minitab to compare the average number of migrants for both source and newly established colonies.

To estimate the proportional contribution of each source colony in the NWHI to each new colony, a MCMC mixed stock analysis using the program BAYES was performed (ftp://ftp.afsc.noaa.gov/sida/mixture-analysis/Bayes/; Pella & Masuda, 2001). Estimated source colony contributions to new colonies were calculated based on 469–6640 resamplings (as determined by BAYES for each population) of one stock mixture expected to recruit individuals from source colonies along with their standard deviations. Bayesian mixed stock analyses used equal contributions from source populations as an initial (prior) distribution. A linear regression was used to test whether Bayesian estimated contributions from each source colony to each new colony depended on source colony population size or distance from the colonization site.

Results

Genetic diversity and neutrality

The control region of the mitochondria produced 188 haplotypes in 358 individuals, with several ancestral shared haplotypes that differed in frequency between source populations, but many that were also unique to a single population (Table 2, Appendix). Sequences can be accessed in GenBank under accession numbers GU395302–GU395489. Both haplotype and nucleotide diversity were high for all populations (Table 2). However, because the presence of new colonies confirms gene flow it should be noted that new colonies are by definition not in HWE due to migration (despite software indicating neutrality) and could be experiencing genetic drift due to small founding population sizes.

Population structure

The overall genetic partitioning with microsatellite data was $F_{ST} = 0.01$ ($P < 0.01$), and the overall value for mitochondrial data was $\Phi_{ST} = 0.05$ ($P < 0.0001$). Pairwise population F_{ST} and Φ_{ST} values were calculated for all populations with microsatellites and mtDNA, respectively (Table 3) and adjusted for multiple comparisons using a Bonferroni correction. The pairwise comparisons of new colonies were included for information purposes, but should be interpreted with caution because these sites violate assumptions of HWE and have structure that likely reflects the source of founding individuals rather than population separation.

Pairwise comparisons using microsatellite data produced only one significant comparison between source colonies (Tern and Laysan), but the low F_{ST} value (0.002) suggests high current gene flow (Hedrick, 1999). STRUCTURE analysis of genotypes from source populations identified only one inferred population ($K = 1$), indicating little population subdivision in microsatellite markers for this species.

In contrast, pairwise comparisons in mitochondrial data produced statistically significant population structure between source populations, particularly between Midway Atoll and all other colonies ($\Phi_{ST} = 0.06$–0.18). There was also significant structure between Tern and Laysan Island among source populations ($\Phi_{ST} = 0.04$).

Migration and source populations

Estimates of gene flow from a Φ_{ST} approach were not significantly correlated with those derived from a coalescent based approach in MIGRATE (Mantel $r^2 = 0.220$; $P = 0.73$). Moreover, 12 of 15 pairwise comparisons that showed significant population structure in mtDNA Φ_{ST} had >4 migrants per generation (Tables 3 and 4), indicative of high levels of gene flow using the coalescent approach. New populations did not have significantly more migrants per generation (486 ± 58) than source populations (394 ± 122, $P = 0.526$). Japan was the only population to show low levels of gene flow (<1 migrant generation^{-1} from 4/5 source populations) which is consistent with lack of

Table 3 Pairwise population[a] Φ_{ST} values for mtDNA control region (above the diagonal) and F_{ST} values for microsatellites (below diagonal) of Laysan albatross *Phoebastria immutabilis*

	Kure	Midway	Pearl & Hermes	Laysan	Tern	Japan	Lehua	Kauai	Oahu	Mexico
Kure	–	0.1084**	0.0327	0.0107	0.0188	0.0456	0.0537*	0.0248	0.022	0.0404**
Midway	0.0028	–	0.1540**	0.178**	0.0557**	0.1121**	0.1892**	0.1714**	0.1399**	0.1441**
Pearl & Hermes	0.0058	0.009	–	0.0154	0.0086	−0.1262	0.0106	0.0064	−0.0023	−0.0099
Laysan	−0.0004	−0.01	0.0113	–	0.035*	0.0359	−0.0019	0.0011	−0.0037	0.0096
Tern	−0.0016	0.0076	0.0175	0.002*	–	0.0045	0.0467**	0.018	0.0137	0.02*
Japan	0.0205	0.0246	0.0078*	0.0138**	0.0343	–	0.0407	0.04	0.0158	0.0016
Lehua	0.0242**	0.0262*	0.0064**	0.021*	0.0161	0.0005	–	0.0061	0.0044	0.019
Kauai	0.0141**	0.0125*	−0.0015*	0.0173**	0.0188	0.0104	0.0084	–	−0.0037	0.0044
Oahu	0.0109*	0.0133*	−0.0025**	0.0158**	0.0276	0.0016	0.0113*	−0.0018	–	0.00009
Mexico	0.0166**	0.0036	−0.0007	0.0045	0.0083	0.011	−0.0021	0.0035	0.0072	–

Source populations are contained in the upper left box.

*$P<0.05$; **$P<0.01$ denote statistically significant pairwise comparisons.

[a]Wake Island was not included in this table as with only two samples these statistics could not be calculated.

population growth that has been observed in the colony since its inception in 1976 (T. Deguchi, pers. comm.).

Bayesian mixed stock analyses indicated that new colonies were genetically diverse assemblages comprised of individuals from multiple source colonies in the NWHI. This was also confirmed in the coalescent analysis with high number of migrants coming from each source colony into each new colony and by banding records examined by Young *et al.* (2009*a*,*b*). Young *et al.* (2009*a*,*b*) found that the newly formed Oahu colony included migrants that came from French Frigate Shoals, Midway and Kauai. Primary contributions differed to some degree in each colony, with the exception of Midway Atoll which showed low contributions to all new colonies (3–15%; Table 5) despite hosting about 72% of the world's breeding population of Laysan albatross (Naughton *et al.,* 2007). These results are consistent with the high genetic structure that was found in pairwise comparisons between Midway Atoll and all other colonies. Contributions from all other colonies (5–51%; Table 5) had overlapping standard errors, but indicate that most source colonies contribute to most new colonies. Estimated contributions from each source colony to each new colony were not related to distance between colonies or source colony size (Table 6). Based on the mixed stock analysis, all colonies have low rates of emigration rather than a single colony having a high rate of emigration.

Discussion

The goals of this study were to test hypotheses about the effects of natal philopatry on population structure and to determine whether colonization events could be attributed to dispersal or displacement in Laysan albatross. The results indicate that despite an apparent lack of barriers to dispersal and high vagility, that high natal philopatry and population unique haplotypes appear to lead to the low population structure in Laysan albatross. Contributions from multiple source colonies to newly colonized sites in both empirical observation from Young *et al.* (2009*a*,*b*) and the mixed stock analysis from this study were contrary to the expectation of a displacement hypothesis from a single source colony. Instead, these data indicate low levels of dispersal from all colonies as the source of colonization events.

Genetic diversity

The high haplotype and nucleotide diversities observed in this study indicate that the control region may be useful as a genetic monitoring tool in documenting changes in Laysan albatross populations and corresponding genetic diversity. The levels of both haplotype and nucleotide diversity in *P. immutabilis* populations were higher than in many other avian taxa (Qu *et al.,* 2005). The high number of haplotypes was also consistent with population expansion, which often enhances the retention of novel mutations and creates an excess of haplotypes differing by one or a few mutations (Slatkin & Hudson, 1991; Rogers & Harpending, 1992).

Population structure and dispersal

The pattern of genetic structuring among Laysan albatross colonies likely reflects both historical and contemporary gene flow. The mtDNA analysis demonstrated low but significant population structure ($\Phi_{ST} = 0.05$). Midway Atoll proved to be distinct in every pairwise combination, and the high Φ_{ST} values from these comparisons (0.06–0.24) would also serve to influence the overall mitochondrial structure despite weak Φ_{ST} values in most other pairwise comparisons. The observed structure is likely a result of historical population subdivision, since contemporary gene flow is obviously occurring.

In contrast, there was virtually no differentiation between populations when examined with microsatellites. This discrepancy between the markers is likely a result of their ability to detect structure on different time scales as well as differences in migration rates between the sexes. The greater mtDNA structure found in this study is indicative of female natal philopatry and male mediated dispersal. Sex-biased

Table 4 Migration estimates (migrants/generation) from source populations (rows) into recipient populations[a] (columns) based on a coalescent approach using the computer program MIGRATE for mtDNA

	Kure	Midway	Pearl & Hermes	Laysan	Tern	Japan	Lehua	Kauai	Oahu	Mexico
Kure	–	4.6 (4.0–7.5)	7.7 (4.5–11.5)	0.6 (0–6.2)	27.7 (17.8–47.1)	1.0 (0.4–1.7)	0.3 (0–4.5)	0.4 (0–2.2)	13.0 (7.3–22.2)	2.6 (2.0–3.8)
Midway	0.5 (0–1.1)	–	5.0 (2.1–11.7)	4.5 (0.8–9.8)	15.0 (10.6–18.5)	0.1 (0–0.4)	17.5 (10.0–24.8)	33 (22.3–41.1)	14.1 (6.0–18.4)	7.5 (6.4–9.5)
Pearl & Hermes	0.2 (0–1.2)	3.3 (2.1–4.6)	–	50.1 (34.9–63.1)	0.4 (0–1.1)	1.6 (1.3–2.1)	13.2 (8.9–18.6)	4.5 (2.5–9.4)	0.4 (0–1.0)	0.04 (0–0.1)
Laysan	6.2 (4.0–8.7)	0.2 (0–0.4)	0.1 (0–0.5)	–	0.9 (0–1.6)	0.4 (0.1–0.7)	3.7 (1.1–8.2)	1.4 (0.6–3.2)	2.6 (1.0–4.5)	2.7 (2.2–3.4)
Tern	17.4 (11.2–24.5)	3.5 (2.0–5.9)	4.5 (2.7–7.3)	22.8 (10.7–45.2)	–	0.1 (0–0.3)	3.7 (1.8–6.6)	6.8 (3.6–11.6)	0.9 (0.1–3.8)	26.0 (10.9–29.5)
Japan	19.5 (7.4–45.5)	1.3 (0.8–2.4)	7.6 (4.7–11.9)	3.7 (0–12.3)	4.2 (2.7–6.8)	–	14.0 (3.1–21.2)	5.5 (2.6–10.4)	2.6 (1.1–4.1)	1.5 (0–2.4)
Lehua	7.9 (7.2–14.6)	0.5 (0.2–1.1)	0.2 (0–0.9)	22.9 (10.7–42.5)	32.4 (23.2–46.2)	0.02 (0–0.3)	–	1.0 (0.1–3.6)	13.7 (9.5–18.2)	0.7 (0.4–1.1)
Kauai	0.4 (0–1.18)	2.9 (1.4–4.2)	8.5 (3.1–12.5)	15.3 (7.4–25.2)	11.9 (3.9–15.7)	0.2 (0.1–0.5)	23.7 (9.5–31.8)	–	8.1 (4.1–13.7)	0.5 (0.2–0.7)
Oahu	8.2 (5.3–10.5)	2.7 (1.3–3.9)	6.5 (3.5–10.0)	10.2 (4.5–25.7)	1.5 (0.8–2.3)	0.1 (0–0.4)	3.8 (1.6–7.1)	4.8 (2.2–8.0)	–	0.4 (0.2–0.6)
Mexico	19.0 (8.0–23.1)	3.9 (2.5–6.2)	9.9 (6.6–17.2)	24.6 (11.2–49.3)	13.2 (10.1–18.0)	0.1 (0–0.6)	35.7 (24.6–39.4)	13.4 (9.4–20.5)	3.0 (1.2–6.6)	–
Total	79.3	22.9	50	154.7	107.2	3.62	115.6	70.8	58.4	41.94

The first value is the best estimate given by migrate, the second set of values in parentheses are the range representing the 95% confidence interval.
[a]Wake Island was not included in this Table because these statistics could not be calculated from the small sample ($n = 2$) at this site.

dispersal is a common pattern observed in many other avian species (Clarke Saether & Roskaft, 1997). Notably, the adult sex ratio in the new colony on Oahu, Hawaii is significantly female biased as a result of greater female immigration (Young *et al.,* 2008). While these results may initially appear to be contradictory, several females from Kauai have been observed to mate with males on Oahu, and then return to Kauai (L. C. Y. pers. obs.). Hence the male mediated gene flow does not require males to disperse, if females are highly vagile yet philopatric (see Bowen *et al.,* 2005).

With respect to contemporary gene flow, which is represented better by microsatellites in this study, there are multiple examples on Kauai and Oahu of fledglings that relocate from their natal colony and subsequently breed at the new colonies (Young *et al.,* 2009*a*,*b*). On Oahu *c*. 10% of nesters are from Kauai (Young *et al.,* 2009*a*,*b*) which is contrary to previous studies finding virtually no inter-island natal dispersal in this species (Fisher, 1976). There is also evidence of movements by adults between colonies. For example, *c*. 15% of all adults resighted on Oahu each year were banded elsewhere; the majority (66%) from the next closest colony on Kauai (137 km), but some from as far away as Midway Atoll (2052 km). Contemporary observations of the rapid increase in population size following the founding in these two colonies suggest a large contribution by immigrants, rather than growth via self-recruitment.

Migration and colonization patterns

The low population structure observed in Laysan albatross is indicative of some level gene flow as demonstrated in the coalescent migration analysis; the colonization events that occurred across the Pacific empirically confirm this. However, the presence of population structure between source colonies in mtDNA did allow for a mixed stock analysis to test the displacement hypothesis. The hypothesis that new colonies were comprised entirely of birds displaced from French Frigate Shoals was not supported by the mixed stock, migration analyses or banding records which instead revealed that new colonies were comprised of varying mixtures of birds from all source populations in the NWHI. While a certain proportion of the recruits in new colonies are likely displaced individuals from French Frigate Shoals, there are probably displaced birds that colonized closer sites in the NWHI as a result of conspecific attraction provided by existing breeding colonies which is a common phenomenon in colonial nesting birds (Wagner & Danchin, 2003). The lack of correlation between source colony population size, or distance to the colonization site indicates that some other factor, perhaps nest site limitation or distance to foraging grounds, is responsible for the differences in source colony contribution to newly colonized sites.

Recent counts of Laysan albatross at Midway, Laysan, and Tern Island in the NWHI, which account for ~93% of the global population, have reported stable or growing numbers of breeding birds (Arata *et al.,* 2009) and the appearance of new colonies may be related to this growth.

Table 5 Proportional contribution of source populations to newly colonized populations of Laysan albatross *Phoebastria immutabilis* spanning the Pacific based on results obtained in BAYES

	New colony											
	Japan		Lehua		Kauai		Oahu		Mexico		Wake	
Source population		SE		SE		SE		SE		SE		SE
Kure	0.41	0.36	0.05	0.10	0.07	0.12	0.20	0.23	0.03	0.06	0.14	0.23
Midway	0.07	0.15	0.04	0.10	0.03	0.07	0.03	0.06	0.02	0.05	0.18	0.28
Pearl & Hermes	0.19	0.28	0.49	0.30	0.51	0.31	0.07	0.12	0.03	0.05	0.36	0.34
Laysan	0.24	0.30	0.31	0.26	0.28	0.28	0.30	0.32	0.68	0.26	0.19	0.28
Tern	0.09	0.15	0.10	0.10	0.12	0.15	0.40	0.30	0.25	0.25	0.14	0.22

SE, standard error.

Table 6 Regression of proportional contribution of source populations to newly colonized populations of Laysan albatross *Phoebastria immutabilis* using source colony size and source colony distance as predictive variables

New colony	R^2	P
Japan	0.59	0.41
Lehua	0.18	0.83
Kauai	0.29	0.71
Oahu	0.80	0.20
Guadalupe	0.31	0.69
Wake	0.16	0.84

In the wandering albatross *Diomedea exulans*, juvenile dispersal is density dependent, leading to higher juvenile dispersal when the local population density is high (Inchausti & Weimerskirch, 2002). If a similar mechanism were functioning in Laysan albatross, then increases at new colonies may be driven by juveniles dispersing from high density nesting colonies in the NWHI.

Conclusions and conservation implications

High genetic diversity, ability to rapidly colonize new locations and great dispersal potential bode well for the future of Laysan albatross and could explain this species success relative to other albatross species. However, there are still a myriad of threats to Laysan albatross, from fisheries bycatch to global warming, that will likely increase in magnitude in the future. Consequently, it is important to prioritize management actions aimed at preserving the genetic diversity and maintaining existing colonies. Because of its size and genetic isolation, Midway Atoll should be maintained as a distinct management unit. With the remaining colonies of Laysan albatross being genetically similar, efforts should be made to preserve colonies at the extreme edges of the range (Japan and Mexico) as well as colonies on high islands (Lehua, Kauai and Oahu) that could provide refugia in the event of future sea level rises (Baker, Littman & Johnston, 2006). The research conducted here, in conjunction with mark–recapture studies, indicate that displaced birds and natural colonizers will find and use these refuges and that providing predator-free refuges on high islands in the Hawaiian archipelago could help mitigate potential habitat loss in the future.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Geographic distribution of mtDNA control region haplotypes in Laysan Albatross.

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