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ORIGINAL ARTICLE

Could specialization to cold-water upwelling systems influence gene flow and population differentiation in marine organisms? A case study using the blue-footed booby, *Sula nebouxii*

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ABSTRACT

Aim We assessed population differentiation and gene flow across the range of the blue-footed booby (*Sula nebouxii*) (1) to test the generality of the hypothesis that tropical seabirds exhibit higher levels of population genetic differentiation than their northern temperate counterparts, and (2) to determine if specialization to cold-water upwelling systems increases dispersal, and thus gene flow, in blue-footed boobies compared with other tropical sulids.

Location Work was carried out on islands in the eastern tropical Pacific Ocean from Mexico to northern Peru.

Methods We collected samples from 173 juvenile blue-footed boobies from nine colonies spanning their breeding distribution and used molecular markers (540 base pairs of the mitochondrial control region and seven microsatellite loci) to estimate population genetic differentiation and gene flow. Our analyses included classic population genetic estimation of pairwise population differentiation, population growth, isolation by distance, associations between haplotypes and geographic locations, and analysis of molecular variance, as well as Bayesian analyses of gene flow and population differentiation. We compared our results with those for other tropical seabirds that are not specialized to cold-water upwellings, including brown (*Sula leucogaster*), red-footed (*S. sula*) and masked (*S. dactylatra*) boobies.

Results Blue-footed boobies exhibited weak global population differentiation at both mitochondrial and nuclear loci compared with all other tropical sulids. We found evidence of high levels of gene flow between colonies within Mexico and between colonies within the southern portion of the range, but reduced gene flow between these regions. We also found evidence for population growth, isolation by distance and weak phylogeographic structure.

Main conclusions Tropical seabirds can exhibit weak genetic differentiation across large geographic distances, and blue-footed boobies exhibit the weakest population differentiation of any tropical sulid studied thus far. The weak population genetic structure that we detected in blue-footed boobies may be caused by increased dispersal, and subsequently increased gene flow, compared with other sulids. Increased dispersal by blue-footed boobies may be the result of the selective pressures associated with cold-water upwelling systems, to which blue-footed boobies appear specialized. Consideration of foraging environment may be particularly important in future studies of marine biogeography.

Keywords

Foraging ecology, genetic differentiation, marine biogeography, seabird, *Sula nebouxii*, Sulidae, tropical Pacific Ocean, upwelling.

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INTRODUCTION

Marine ecosystems support an enormous proportion of the Earth's biodiversity, but knowledge of how these systems function has only recently started to increase (Ruckelshaus *et al.*, 2008; Palumbi *et al.*, 2009; Nichols *et al.*, 2010). More specifically, the factors that influence the distribution and extent of population differentiation in marine organisms are poorly understood (Hellberg, 2009). The complex interplay between ocean currents and dispersal influences both species distributions throughout the world's oceans and levels of genetic differentiation between populations (Palumbi, 1994; Palumbi *et al.*, 1997; Riginos & Nachman, 2001). Gaining a better understanding of these processes is an important aspect of successful management.

Seabirds, although potentially less influenced by ocean currents than are more sedentary organisms, exhibit a range of patterns of population differentiation (reviewed in Friesen *et al.*, 2007a). In recent years, interest in seabird population genetic structure and mechanisms of speciation has increased as various populations and species are threatened by climate change, fisheries, and pollution (Blight & Burger, 1997; Thompson & Ollason, 2001; Karpouzi *et al.*, 2007; Watkins *et al.*, 2008). Population genetic structure and speciation in seabirds may be influenced by many factors, including both physical and non-physical barriers to dispersal (Steeves *et al.*, 2005a,b; Friesen *et al.*, 2007b), foraging ecology (Burg & Croxall, 2001), habitat preference and mate choice (Liebers *et al.*, 2001), non-breeding distribution, and philopatry (Friesen *et al.*, 2007a). The relative importance of the various factors is unclear; however, at least two patterns have emerged: (1) northern temperate species tend to exhibit only weak, if any, population genetic structure, while tropical seabirds generally have strongly structured metapopulations, as do some southern temperate species, and (2) foraging ecology can have a significant influence on gene flow in seabirds.

The extent of population differentiation in northern temperate seabirds has often been attributed to glaciation events during the Pleistocene (Moum & Bakke, 2001). Many temperate seabirds appear to have been restricted to refugia during these glaciations, and the weak population genetic structure they currently exhibit may reflect expansion and recolonization from glacial refugia (Friesen *et al.*, 2007a; Morris-Pocock *et al.*, 2008). Although tropical environments changed during glaciations, the extent of these changes appears to have been much less than that in temperate environments (Hewitt, 2000). Results from recent studies of tropical members of the Sulidae (Aves: Pelecaniformes; boobies and gannets) revealed high levels of population genetic differentiation both between and within ocean basins for masked (*Sula dactylatra*), red-footed (*S. sula*) and brown (*S. leucogaster*) boobies (Steeves *et al.*, 2003, 2005a; Morris-Pocock *et al.*, 2010). Patterns in sulids are similar to those in other tropical seabird species: all 11 tropical species reviewed in Friesen *et al.* (2007a) exhibited strong population differentiation. Thus, the tropical/temperate distinction appears to be a robust pattern, even across very different groups of seabirds.

Population genetic data from tropical boobies all come from pelagically feeding species (red-footed and masked boobies) or inshore feeding species (brown boobies) that do not rely on cold-water upwelling systems for foraging (Nelson, 1978; Weimerskirch *et al.*, 2006, 2008, 2009). Cold-water upwelling systems can be unpredictable, especially during El Niño–Southern Oscillation (ENSO) events, which are characterized by an influx of warm surface water throughout a large area of the eastern tropical Pacific and subsequent depression of primary production (Pennington *et al.*, 2006). Cold-water epipelagic fish (i.e. sardines and anchovies) are intolerant of these conditions and are often unavailable to foraging seabirds during ENSO events (Jordan, 1971; Anderson, 1989). Reliance on an unpredictable foraging environment during breeding may influence intercolony dispersal.

Blue-footed boobies (*Sula nebouxii* Milne Edwards, 1882) are distributed throughout the eastern tropical Pacific Ocean, and two subspecies are currently recognized: *S. n. excisa* (Todd), which is endemic to the Galapagos Archipelago; and *S. n. nebouxii*, which breeds along the coast from Mexico to northern Peru (Nelson, 1978). Unlike their tropical relatives, blue-footed boobies breed exclusively in close proximity to areas of cold-water upwelling. Known breeding areas coincide with areas of high chlorophyll *a* and low sea surface temperature, oceanographic conditions that are also associated with the major prey species of blue-footed boobies: sardines (Clupeidae, *Sardinops* spp.; Weimerskirch *et al.*, 2009) and anchovies (Engraulidae, *Engraulis* spp.; Zavalaga *et al.*, 2007; see Appendix S1 in the Supporting Information). Records of blue-footed boobies breeding outside areas of cold-water upwelling are poorly supported [e.g. Revillagigedos (Jehl & Parkes, 1982; Howell & Webb, 1990) and the Gulf of Panama (Loftin, 1991; M. Miller, Smithsonian Tropical Research Institute, pers. comm.)], and ENSO events in the eastern Pacific are known to cause blue-footed booby chick mortality, breeding failure, and colony abandonment (Ricklefs *et al.*, 1984; Anderson, 1989). Blue-footed boobies disperse widely during ENSO events (Simeone *et al.*, 2002), and increased dispersal should increase gene flow between colonies relative to other sulids. No association between breeding colonies and regions of cold-water upwelling exists for brown, red-footed or masked boobies, which can breed at significant distances from upwelling systems, and which forage predominantly in warm tropical waters on flying fish (Exocoetidae) and flying squid (Ommastrephidae) (Nelson, 1978; Weimerskirch *et al.*, 2006, 2008, 2009).

Given the blue-footed booby's range (Fig. 1) and age (see Discussion), the species has probably been restricted to the eastern Pacific for its entire evolutionary history and has experienced the selective pressures of a variable foraging environment throughout. We analysed variation in a 540-bp fragment of the mitochondrial control region and seven microsatellite loci across the range of the blue-footed booby. Our aim was to evaluate global population genetic structure to determine (1) the universality of the hypothesis that tropical

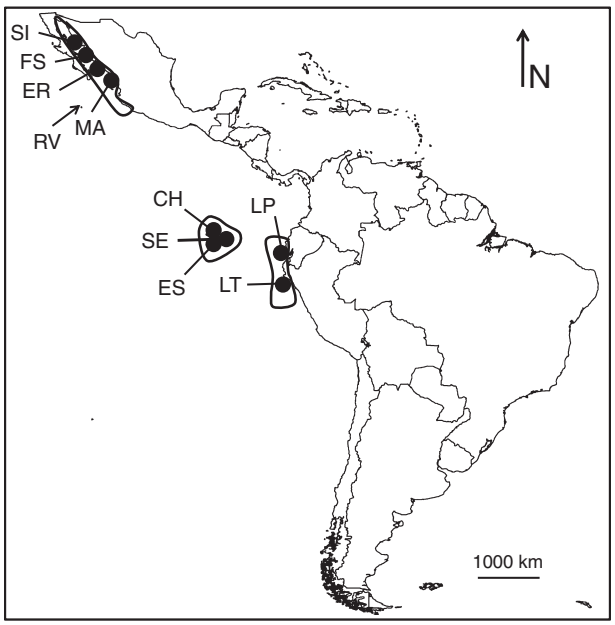

Figure 1 Distribution of blue-footed booby (*Sula nebouxii*) sampling sites. Breeding distributions are outlined in black; sampling locations are indicated by black circles. Colony codes are in parentheses after colony names, and the numbers in parentheses indicate the number of individuals sampled per colony. Isla San Ildefonso, Mexico (SI; 10), Farallon de San Ignacio, Mexico (FS; 15), El Rancho, Mexico (ER; 15), Islas Marietas, Mexico (MA; 3), Revillagigedos Islands (RV; not sampled), La Plata, Ecuador (LP; 50), Lobos de Tierra, Peru (LT; 55), Champion Island, Galapagos (CH; 10), Seymour Island, Galapagos (SE; 11), Espanola Island, Galapagos (ES; 10).

seabirds exhibit higher levels of population genetic structure than their northern temperate counterparts, and (2) whether specialization to cold-water upwelling systems increases gene flow in blue-footed boobies compared with that in other tropical sulids.

MATERIALS AND METHODS

Blood samples were obtained from 174 blue-footed booby nestlings from breeding colonies throughout the species' range (Fig. 1). Because the sample size from Islas Marietas was small, and no significant genetic differences were found among Mexican colonies (see Results), Islas Marietas samples were combined with those from the next closest colony, El Rancho, for all analyses. Additional sample treatment details are given in Appendix S2.

A 540-bp fragment of the mitochondrial control region was amplified from 154 individuals, and seven microsatellite loci were amplified from 172 individuals. Both marker types were used because they differ in effective population size, mutation rate and inheritance pattern, and their combined analysis can provide a more comprehensive view of within-species population genetic differentiation. Owing to degradation, clean control region sequence could not be amplified for 20 individuals, and microsatellites could not be amplified from two of these individuals. Methods for DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing of the mitochondrial DNA (mtDNA) control region and microsatellite data, including GenBank accession numbers, are given in Appendix S2.

Population genetic analyses of the mtDNA control region

To determine if control region variation deviated from neutrality or mutation-drift equilibrium (the assumptions of most subsequent analyses), Ewens–Watterson (Ewens, 1972; Watterson, 1978) and Chakraborty's (Chakraborty, 1990) tests of selective neutrality were conducted for each colony using Arlequin 3.11 (Excoffier *et al.*, 2005). In addition, fluctuate 1.4 (Kuhner *et al.*, 1998) was used to address the null hypothesis that each population's growth rate was zero, recognizing that growing populations may not be in mutation-drift equilibrium. fluctuate was run using an initial population growth rate of 1 and a Watterson estimate of θ (where θ is the product of the effective population size and the per site neutral rate of mutation; Kuhner *et al.*, 1998), and population size was allowed to vary. Each run consisted of 30 short chains of 1000 steps and four long chains of 100,000 steps, and chains were sampled every 20 steps. Runs were performed three times with different random seeds, and statistical significance was determined by testing whether *g* (the growth parameter) was significantly different from zero, both for each region and for the total sample, using log-likelihood ratio tests (Kuhner *et al.*, 1998).

Arlequin was used to index population genetic structure by calculating pairwise population differentiation (Φ_{ST}) and net sequence divergence (δ) from mitochondrial control region sequences, as well as to evaluate the significance of geographic subdivisions among colonies using a hierarchical analysis of molecular variation (AMOVA). Five colony groupings were created in an attempt to maximize global between-group population structure (Φ_{CT}), under the assumption that the most likely geographic subdivisions of colonies were those that maximized global Φ_{CT} (Stanley *et al.*, 1996). Colony groupings were made on the basis of subspecies designations, results from pairwise Φ_{ST} calculations, or the geographic separation of colonies. All analyses were conducted using Kimura's (1980) two-parameter substitution model with a rate parameter (α) of 0.45, and significance was determined by comparing the results with 10,000 random permutations of the data at a significance level of 0.05 using the Benjamini–Yekutieli (B-Y) correction for multiple tests (Narum, 2006).

To test for a positive correlation between genetic differentiation and geographic distance between colonies, a Mantel test was performed in Arlequin using Wright's linearized F_{ST} and log-transformed distances (Mantel, 1967). Between-colony distances were calculated using an online resource (http://www.movable-type.co.uk/scripts/latlong.html) that calculated the great-circle distance between two latitude/longitude points using the Haversine formula (Sinnott, 1984).

Relationships between control region haplotypes were inferred by construction of a statistical parsimony network in TCS 1.21 (Clement *et al.*, 2000). Ambiguous connections in the statistical parsimony network (loops) were resolved using a hierarchical set of guidelines based on coalescent criteria (Crandall & Templeton, 1993; Steeves *et al.*, 2005b). Remaining ambiguities were broken both conservatively (with least geographic structure) and non-conservatively (with most geographic structure). Parsimony trees were subsequently nested according to Templeton *et al.* (1987), and the existence of phylogeographic structure was assessed with GEODIS 2.5 (Posada *et al.*, 2000). GEODIS used contingency tests and 10,000 permutations of the data to examine the correlation of nesting pattern (the distribution of a clade relative to others within the nesting category) and inter-clade geographic distance (the geographical spread of a clade).

Based on results from both mitochondrial and nuclear data suggesting that colonies in Mexico were genetically isolated (see Results), Hey & Nielsen's (2004) Isolation with Migration, IM, was used to test if migration between Mexico and the other sampling locations was different from zero using mitochondrial control region data. The model assumed that the populations being examined were each other's closest relatives, that no genetic structure existed within the two populations being examined, and that no other populations exchanged genes with the delineated populations. It then modelled a situation in which the two populations descended from a common ancestor at some time in the past, *t*, and diverged either with or without gene flow. Asymmetrical gene flow was allowed, and priors for theta, immigration rate and divergence time were assigned based on results from five preliminary trial runs; wide uninformative priors were set originally but were adjusted so that the posterior probability curves reached convergence. Single-chain runs without heating were conducted, using a burn-in of at least 200,000 steps, and results were recorded every 30 min. To ensure that the program was running well it was run three times using different random number seeds, but identical parameters, and was allowed to run for at least 10,000,000 steps.

Population genetic analyses of the microsatellites

ARLEQUIN was used to test for deviations of microsatellite genotype frequencies from Hardy–Weinberg equilibrium (HWE), and to test for deviations from linkage equilibrium using ln likelihoods. ARLEQUIN was also used to estimate pairwise population differentiation (F_{ST}) between all colony pairs, to perform AMOVA, and to perform a Mantel test as above.

STRUCTURE 2.3.1 (Pritchard *et al.*, 2000; Falush *et al.*, 2003) was used to test for population genetic structure in microsatellite variation. STRUCTURE analyses were performed using an admixture model, correlated allele frequencies, a burn-in period of 50,000 cycles, and 500,000 additional cycles (determined from test runs to be sufficient for parameter stabilization). Although models without admixture may be more sensitive to detecting small amounts of population genetic structure than admixture models (Falush *et al.*, 2003), using the no-admixture model did not produce significantly different results (data not shown). Analyses were repeated 20 times for $K = 1$–8, where K is the number of genetic populations, and posterior probability, $\ln[P(D)]$, was used to infer the most likely number of genetic populations as described in Pritchard & Wen (2004). In addition, the method of Evanno *et al.* (2005) was used to infer the most likely value of K using the second-order rate of change of the likelihood function with respect to K, divided by the standard deviation (ΔK). DISTRUCT 1.1 (Rosenberg, 2004) was used to redraw the output from STRUCTURE with the highest likelihood.

BAYESASS 1.1 (Wilson & Rannala, 2003) was used to estimate migration (gene flow) among regional populations using nuclear loci. BAYESASS was chosen for this purpose because the model does not assume migration drift equilibrium, unlike other programs that estimate migration (e.g. Kuhner, 2006). BAYESASS was run using 3,000,000 Markov chain Monte Carlo (MCMC) iterations, 1,000,000 burn-in iterations, and sampling every 2000 iterations. Initial deltas for allele frequencies, migration rates, and inbreeding were set at 0.15, the default value.

RESULTS

Mitochondrial control region

Among 154 blue-footed boobies from nine colonies there were 104 haplotypes defined by 39 variable sites (listed in Appendices S3 and S4). Haplotype diversity ranged from 0.95 ($\pm$ 0.40) at Farallon de San Ignacio to 1.00 ($\pm$ 0.1) at Seymour, Champion and Española, and was fairly evenly distributed at *c.* 0.97 in the remaining colonies (Table 1). Haplotype diversity is a measure of the uniqueness of a haplotype within a population; a value of one indicates that all haplotypes within a colony are unique. Nucleotide diversity was highest at Lobos de Tierra and La Plata (Table 1). Significant overall population growth was detected using FLUCTUATE (growth parameter $g = 460$, $\chi^2 = 80.51$, $\chi^2_1 = 3.84$, $P < 0.005$); however, no neutrality test statistics were significantly different from expected values (all $P > 0.05$; Table 1).

Six estimates each of pairwise population differentiation (Φ_{ST}) and net sequence divergence (δ) were significantly greater than zero after B-Y correction for the non-conservative data set (Table 2); however, only the pairwise estimate between Farallon de San Ignacio and La Plata was significant for the conservative data set (see Appendix S2 for description of data sets). AMOVA detected weak but significant global population structure ($\Phi_{ST} = 0.05$; $P < 0.0001$), and a maximum estimate of between-group global population structure (Φ_{CT}) was obtained by grouping (1) Mexican colonies, (2) colonies from Galapagos and (3) Lobos de Tierra with La Plata ($\Phi_{CT} = 0.07$, $P < 0.05$; $\Phi_{SC} = 0.004$, $P > 0.05$). Grouping all colonies together produced the next highest estimate ($\Phi_{ST} = 0.05$, $P < 0.05$). Grouping colonies by subspecies produced a small

Table 1 Haplotype diversities, nucleotide diversities and neutrality test (Ewens–Watterson and Chakraborty's) results for sampled colonies of blue-footed boobies (*Sula nebouxii*) based on mitochondrial control region sequences. No significant deviations from neutral expectations were detected (all $P > 0.05$). Obs. is the observed test statistic; Exp. is the test statistic expected under neutrality. Colony abbreviations are as in Fig. 1. N/A indicates that it was impossible to conduct the test because all gene copies were different.

Colony	*N*	Haplotype diversity (*h*)	Nucleotide diversity (π) (%)	Ewens–Watterson		Chakraborty's	
				Obs.	Exp.	Obs.	Exp.
SI	10	0.96 ± 0.059	1.4 ± 0.0078	0.14	0.15	8.0	8.3
FS	15	0.95 ± 0.040	1.3 ± 0.0072	0.11	0.11	11.0	11.2
ER	13	0.95 ± 0.051	1.3 ± 0.0071	0.12	0.12	10.0	9.90
LP	44	0.98 ± 0.11	1.5 ± 0.0080	0.036	0.034	39.0	37.3
LT	55	0.99 ± 0.083	1.8 ± 0.0092	0.032	0.029	38.0	36.7
SE	6	1.00 ± 0.096	1.2 ± 0.0080	N/A	N/A	N/A	N/A
CH	6	1.00 ± 0.096	1.3 ± 0.0080	N/A	N/A	N/A	N/A
ES	3	1.00 ± 0.27	1.2 ± 0.010	N/A	N/A	N/A	N/A

Table 2 Population pairwise differentiation (Φ_{ST}) and net sequence divergence (δ) estimates (lower matrix) calculated from non-conservative blue-footed booby (*Sula nebouxii*) mitochondrial control sequence data. Population pairwise F_{ST} estimates (upper matrix) calculated from microsatellite data. Φ_{ST}, δ and F_{ST} estimates that were significant after Benjamini–Yekutieli correction are denoted with an asterisk (*$P < 0.05$). Colony abbreviations are as in Fig. 1.

	Galapagos			Mexico			Coastal South America	
	SE	ES	CH	SI	ER	FS	LT	LP
SE	–	0.01	0.01	0.08*	0.14*	0.13*	0.03*	0.02
ES	−0.16/0.95	–	0.04	0.11*	0.19*	0.18*	0.06*	0.04*
CH	−0.02/−0.16	−0.03/−0.15	–	0.05*	0.14*	0.14*	0.04*	0.03*
SI	0.06/0.49	0.02/0.27	0.00/0.19	–	0.00	−0.01	0.04*	0.04*
ER	0.04/0.27	0.05/0.34	0.05/0.37	−0.01/−0.07	–	0.01	0.10*	0.10*
FS	0.10/0.85	0.13/1.18	0.14/1.22	0.11/0.90	−0.00/−0.00	–	0.07*	0.07*
LT	−0.02/0.56	−0.02/0.35	0.00/0.23	0.09*/1.05*	0.08*/0.85*	0.10*/1.05*	–	0.00
LP	0.23/−0.10	−0.01/0.20	0.01/0.18	0.10*/0.93*	0.08*/0.72*	0.10*/0.91*	0.01/0.06	–

and non-significant value of between-group differentiation (Φ_{CT}), and a significant value of within-group differentiation (Φ_{SC}) ($\Phi_{CT} = 0.00$, $P > 0.05$; $\Phi_{SC} = 0.05$, $P < 0.05$). A Mantel test provided no evidence for a correlation between genetic and geographic distance ($r = 0.23$, $P > 0.05$).

The mitochondrial haplotype tree generated in TCS showed some clustering of haplotypes by geographic location, and nested contingency analysis indicated significant phylogeographic structure at the highest clade level ($\chi^2 = 58.01$, $P < 0.001$) (Fig. 2). This significant phylogeographic structure was primarily the result of haplotype frequency differences between Mexican colonies and colonies further south (Fisher's exact test on marginal frequencies, $P < 0.0001$): haplotype frequencies at the highest clade levels were significantly different for colonies in Mexico versus Galapagos (Fisher's exact test, $P < 0.001$), and for colonies in Mexico versus coastal Ecuador and Peru (Fisher's exact test, $P < 0.0001$).

Results from IM revealed asymmetrical gene flow between Mexico and colonies to the south, which are separated by a distance of *c.* 3500 km. Migration into Mexico, *m1*, peaked near zero, and the confidence interval included the lowest bin of the probability distribution [90% highest posterior density interval (HPD): 0.0004–0.1732]: thus *m1* was not significantly different from zero (Fig. 3). In contrast, gene flow out of Mexico peaked at 0.0483, and the confidence interval did not include the lowest bin of the probability distribution (90% HPD: 0.0022–0.1353), suggesting that migration out of Mexico is significantly greater than zero, but low (Fig. 3). Highest posterior density intervals represent the minimum-length confidence intervals for a Bayesian posterior distribution (Hey & Nielsen, 2004).

Microsatellites

There were between 4 and 17 alleles per microsatellite locus, with an average of eight alleles per locus, and genotype frequencies showed no significant deviations from HWE either at a single locus across colonies, or at a single colony across loci (all $P > 0.001$; Appendix S5). Tests for linkage disequilibrium did not detect any deviations for any pair of loci within any colony (all $P > 0.05$).

Twenty-one pairwise population differentiation (F_{ST}) estimates were significantly greater than zero after B-Y correction, and all significant comparisons were between colonies from

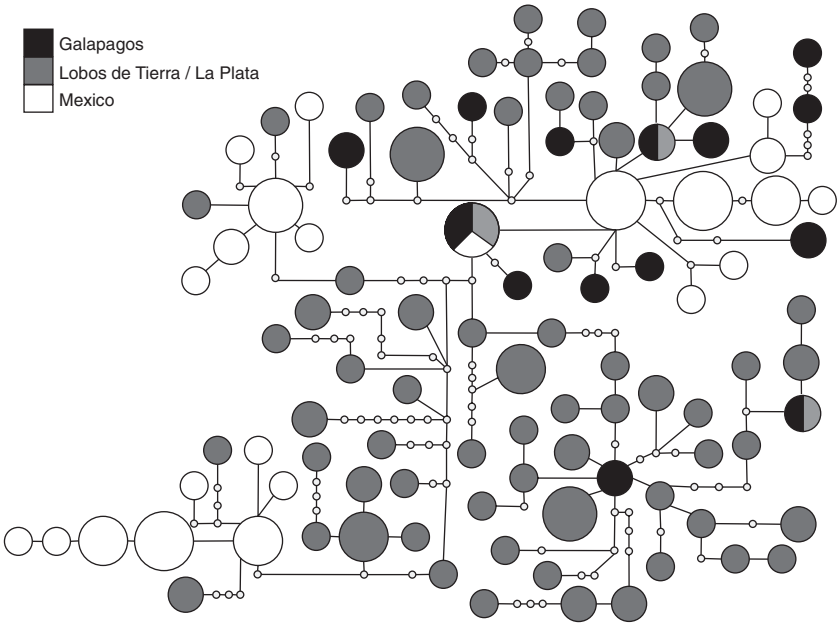

Figure 2 Most parsimonious tree of blue-footed booby (*Sula nebouxii*) control region haplotypes derived from TCS. Small grey circles represent haplotypes not represented in the current sample, black circles indicate haplotypes found in the Galapagos, large grey circles indicate haplotypes found in Lobos de Tierra and La Plata, and white circles indicate haplotypes found in Mexico. Circles are proportional to the number of individuals with the haplotype, and pie-slice sizes indicate the number of individuals with the shared haplotype. A random clustering of regional haplotypes would indicate the absence of phylogeographic structure.

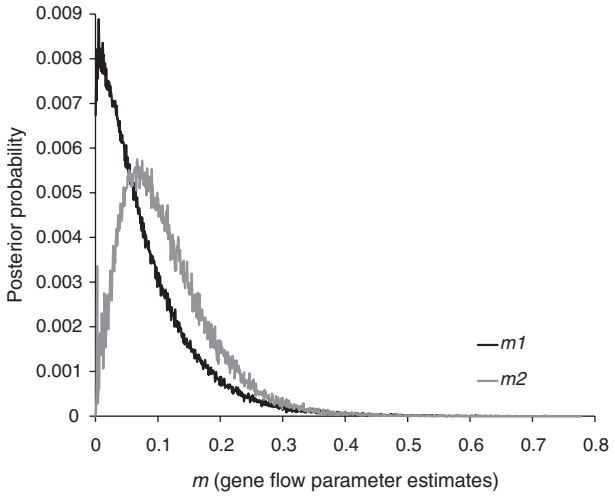

Figure 3 Posterior probability distributions from Hey & Nielsen's (2004) Isolation with Migration, IM, for blue-footed booby (*Sula nebouxii*) migration rates between Mexican colonies and colonies located south of Mexico. *m1* represents migration into Mexico, and *m2* represents migration out of Mexico going forwards in time.

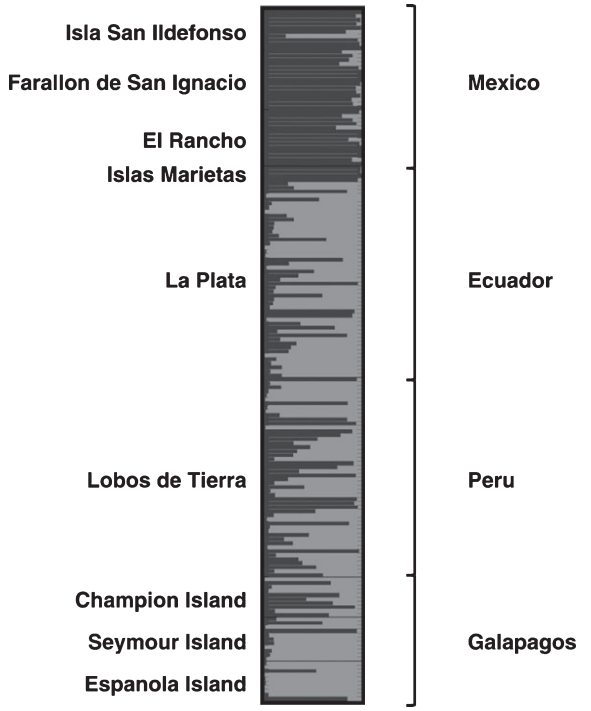

Figure 4 Bayesian assignment probabilities for individual blue-footed boobies (*Sula nebouxii*) at $K = 2$, the most probable number of genetic populations as determined using STRUCTURE 2.3.1 (Pritchard *et al.*, 2000). Each horizontal line represents an individual, and the shades of grey indicate the probability that an individual's genotype is assigned to a particular genetic population.

different geographic groups (Table 2). No pairwise estimates of population differentiation (F_{ST}) between colonies within geographic groups were significant. The global estimate of population differentiation (global F_{ST}) was 0.05 ($P < 0.001$), indicating weak but significant population genetic structure, and a maximum between-group differentiation estimate (F_{CT}) of 0.08 ($P < 0.02$) was obtained by comparing two geographic groups: Mexican colonies and colonies to the south of Mexico. When colonies were grouped according to the subspecies designation, we obtained a between-group differentiation estimate (F_{CT}) of 0.03 ($P = 0.03$) and a within-group differentiation estimate (F_{SC}) of 0.05 ($P < 0.001$): this grouping explains less between-group variation than the previous grouping.

A Mantel test showed a significant relationship between genetic differentiation and geographic distance ($R = 0.66$,

$P = 0.006$). The most probable number of genetic populations as determined using STRUCTURE and the method of either Pritchard & Wen (2004) or Evanno *et al.* (2005) was two [$\Pr(K = 2) = 1.00$; see Appendix S6]. Individuals from Mexico tended to assign with highest probability to one genetic population, while individuals from Galapagos and coastal Ecuador and Peru tended to assign to the other (Fig. 4).

For initial runs of BAYESASS, colonies were grouped by region. However, results indicated that non-migration (residency) rates were too low for a reliable estimation of migration. Low non-migration rates can result from a lack of information in the data, or from high migration rates. Given that the same level and distribution of population differentiation were found in preliminary analyses of 18 microsatellite loci (S. A. Taylor *et al.*, unpublished data) as from the present seven loci, it was inferred that migration rates between Galapagos and the coastal colonies of Ecuador and Peru were sufficient to prevent genetic differentiation. When colonies were subsequently pooled into Mexico versus southern regions, results from BAYESASS indicated that non-migration rates were high enough for the reliable estimation of migration rates. For this analysis, estimates of migration from Mexico into southern colonies (mean = 0.017, SD = 0.014) and from southern colonies into Mexico (mean = 0.016, SD = 0.015) were low, indicating essentially no migration between Mexico and colonies to the south of Mexico.

DISCUSSION

Contrary to the hypothesis that tropical seabirds exhibit high levels of population genetic structure, the neutral markers used here indicated that population structure in blue-footed boobies is an order of magnitude lower than that in brown, red-footed and masked boobies (Figs 2 & 4; Table 2; Steeves *et al.*, 2005a; Morris-Pocock *et al.*, 2010). With the exception of Mexican versus other colonies (Fig. 3), gene flow between most colonies is probably quite high. This represents the first evidence that tropical seabird colonies can exhibit little genetic differentiation across a wide geographic distance. Furthermore, the weak genetic differentiation between blue-footed booby colonies supports the hypothesis that blue-footed boobies should exhibit weaker population genetic structure than tropical species that are not associated with cold-water upwelling systems and are therefore less critically influenced by ENSO events.

Blue-footed booby population differentiation compared with other sulids

Unless the foraging environment utilized by blue-footed boobies is considered, the weak overall genetic structure they exhibit is surprising given the high levels of genetic structure across similar geographic scales in other tropical seabirds (reviewed in Friesen *et al.*, 2007a), especially in the closely related brown booby (Morris-Pocock *et al.*, 2010). Unlike blue-footed boobies, brown boobies are pantropical; however, they have a similar distribution to blue-footed boobies within the eastern tropical Pacific Ocean (Nelson, 1978). Estimates of population differentiation between eastern Pacific brown booby colonies from eight microsatellites and a fragment of the mitochondrial control region are high and significant ($F_{ST} = 0.11$, $P < 0.05$, J. A. Morris-Pocock *et al.*, Queen's University, pers. comm.; $\Phi_{ST} = 0.73$, $P < 0.05$, Morris-Pocock *et al.*, 2010). At a smaller geographic scale, brown booby colonies are still genetically differentiated: colonies within the Gulf of California at Farallon de San Ignacio and San Pedro Martir are genetically distinct from those outside the Gulf at Piedra Blanca, and these colonies are separated by only 540 km ($F_{ST} = 0.16$, $P < 0.05$, J. A. Morris-Pocock *et al.*, pers. comm.; $\Phi_{ST} = 0.69$, $P < 0.05$, Morris-Pocock *et al.*, 2010). Even at a larger geographic scale, blue-footed boobies exhibit less population genetic structure than brown boobies. Estimates of F_{ST} and Φ_{ST} between blue-footed booby colonies within the Gulf of California and those on the Galapagos, sites separated by *c.* 3500 km, are only 0.14 and 0.04, respectively, and Φ_{ST} is not significant after B-Y correction. Furthermore, within-basin population differentiations of red-footed ($\Phi_{ST} = 0.80$, J. A. Morris-Pocock *et al.*, pers. comm.) and masked ($\Phi_{ST} = 0.39$, T. E. Steeves *et al.*, University of Canterbury, pers. comm.) boobies are also higher than that of blue-footed boobies.

Potential explanations for the observed pattern

In the present paper we hypothesized that lower population differentiation should exist in blue-footed boobies than in other tropical sulids given their foraging environment and the potential for increased dispersal between colonies; however, the extent of genetic differentiation between colonies is determined by several factors, including time since separation, effective population size and gene flow (Wright, 1931; Whitlock & McCauley, 1999; Friesen *et al.*, 2007a). Recent separation between colonies could result in low population differentiation because opportunities for selection and/or drift to take place within a colony would be reduced compared with more historically diverged colonies. Blue-footed boobies are believed to have diverged from their common ancestor with the Peruvian booby (distributed from northern Peru to south-central Chile) 0.2–0.45 million years ago (Ma), while brown boobies (pantropical in distribution) are believed to have diverged from other boobies 2.0 to 3.86 Ma (Friesen & Anderson, 1997; Patterson *et al.*, 2010). Thus, blue-footed boobies may not have had sufficient time to establish population genetic structure. Although molecular data suggest that the blue-footed booby is a considerably younger species than the brown booby, brown boobies in the eastern tropical Pacific, which apparently became isolated from other brown booby colonies between 0.13 and 0.38 Ma, exhibit significantly more population genetic structure than equivalent groups of blue-footed booby colonies (Morris-Pocock *et al.*, 2010). As such, recent divergence does not appear to explain the lower levels of population differentiation in blue-footed boobies.

Small effective population size at a colony could lead to drift and potentially to the development of high population differentiation between colonies. For example, high population genetic structure between brown, red-footed and masked booby colonies could result from genetic bottlenecks during colony formation if each colony was founded by only a small number of individuals. Given the low probability that all tropical seabirds examined thus far have experienced severe bottleneck events, this scenario is unlikely. Furthermore, brown and red-footed booby populations do not exhibit signatures of bottlenecks in their control region variation (Morris-Pocock *et al.*, 2010). As such, low effective population sizes do not explain the high levels of population differentiation in tropical seabirds compared with that in blue-footed boobies.

Increased intercolony dispersal and gene flow (which can be influenced by several factors) would prevent populations from diverging, and this appears to be the best explanation for the weak population genetic structure in blue-footed boobies. Comprehensive studies examining the intercolony breeding dispersal of blue-footed boobies are absent from the literature; however, recent investigations of intracolony breeding and natal dispersal indicate that blue-footed boobies on Isla Isabel in the Gulf of California do not necessarily exhibit fidelity to their hatching site (Kim *et al.*, 2007a,b), in contrast to the case for most other seabirds (Greenwood & Harvey, 1982; Coulson, 2002), and that juveniles may undergo long-distance natal dispersal (13 chicks nested 476 km south of Isla Isabel during the study period, Kim *et al.*, 2007a,b). Furthermore, three chicks marked in Galapagos were later recovered off the coast of Ecuador, making it possible that birds breed in non-natal colonies (Nelson, 1978). Given that the movement of even one individual per generation between colonies is enough to homogenize genetic variation at neutral markers (Wright, 1931; Mills & Allendorf, 1996), natal dispersal may be at least partially responsible for the weak population differentiation exhibited by blue-footed booby colonies. Although comprehensive studies of between-colony breeding dispersal by blue-footed boobies are absent from the literature, juvenile blue-footed boobies appear more dispersive than other sulids. Dispersive behaviour may persist in a sufficient number of adults to reduce population differentiation, and we find the suggestion of a dispersive blue-footed booby phenotype by Kim *et al.* (2007b) intriguing.

The influence of foraging ecology on seabird population differentiation

We hypothesized that blue-footed boobies would exhibit less genetic structure than other tropical sulids given their reliance on unpredictable foraging environments because increased dispersal between colonies, potentially the result of an unpredictable foraging environment, should increase gene flow compared with other sulids. By comparing levels of population differentiation between closely related booby species that breed within the same geographic region (brown boobies) or across similar geographic scales (red-footed and masked boobies), but that differ in foraging ecology, we have found some support for our hypothesis. Although too few studies have been completed to test the generality of this pattern, studies to date seem to support the hypothesis. Low levels of population genetic structure have recently been detected in other marine organisms that rely on cold-water upwelling systems during foraging, including dusky dolphins (*Lagenorhynchus obscures*, Cassens *et al.*, 2005), Humboldt penguins (*Spheniscus humboldti*, Schlosser *et al.*, 2009), Peruvian boobies (*S. variegata*, Taylor *et al.*, in press) and Peruvian pelicans (*Pelecanus thagus*, S. A. Taylor *et al.*, unpublished data).

Our results represent the first example of a tropical seabird that exhibits extremely low levels of population genetic differentiation across a large geographic distance, refuting the hypothesis that tropical seabirds always exist in highly genetically differentiated metapopulations (Friesen *et al.*, 2007a). This is an important finding and one that should encourage other researchers to consider thoroughly the ecology of tropical seabirds when making assumptions about levels of population differentiation or gene flow between colonies. This may become especially important as climate change, competition with fisheries, and pollution threaten tropical seabird colonies, and potentially species, with extinction (Walsh & Edwards, 2005; Barbraud & Weimerskirch, 2006; Devney *et al.*, 2009). Even within a small group of seabirds, the Sulidae, we see a variety of patterns of population differentiation, which appear to be related, at least in part, to foraging ecology. Foraging ecology also appears to influence population differentiation in other seabirds (Friesen, 1997; Friesen *et al.*, 2007a). Population-specific differences in the foraging distribution of some albatross taxa, for example, are consistent with genetic differences between populations (Burg & Croxall, 2001, 2004), and inshore feeding seabird species tend to exhibit greater population genetic structure than offshore feeders (Morris-Pocock *et al.*, 2010). Given that foraging environment commonly influences population differentiation in seabirds, it may have the potential to influence gene flow and genetic differentiation in a variety of marine taxa. Thus, consideration of foraging environment may be particularly important to future investigations in marine biogeography and conservation.

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REFERENCES

Anderson, D.J. (1989) Differential responses of boobies and other seabirds in the Galápagos to the 1986–87 El Niño-Southern Oscillation event. *Marine Ecology Progress Series*, **52,** 209–216.

Barbraud, C. & Weimerskirch, H. (2006) Antarctic birds breed later in response to climate change. *Proceedings of the National Academy of Sciences USA*, **103,** 6248–6251.

Blight, L.K. & Burger, A.E. (1997) Occurrence of plastic particles in seabirds from the eastern North Pacific. *Marine Pollution Bulletin*, **34,** 323–325.

Burg, T.M. & Croxall, J.P. (2004) Global population structure and taxonomy of the wandering albatross species complex. *Molecular Ecology*, **13,** 2345–2355.

Burg, T.P. & Croxall, J.P. (2001) Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. *Molecular Ecology*, **10,** 2647–2660.

Cassens, I., Van Waerebeek, K., Best, P.B., Tzika, A., Van Helden, A.L., Crespo, E.A. & Milinkovitch, M.C. (2005) Evidence for male dispersal along the coasts but no migration in pelagic waters in dusky dolphins (*Lagenorhynchus obscurus*). *Molecular Ecology*, **14,** 107–121.

Chakraborty, R. (1990) Mitochondrial DNA polymorphism reveals hidden heterogeneity within some Asian populations. *American Journal of Human Genetics*, **47,** 87–94.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9,** 1657–1660.

Coulson, J.C. (2002) Colonial breeding in seabirds. *Biology of marine birds* (ed. by E.A. Schreiber and J. Burger), pp. 87–113. CRC Press, London.

Crandall, K.A. & Templeton, A.R. (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, **134,** 959–969.

Devney, C.A., Short, M. & Congdon, B.C. (2009) Sensitivity of tropical seabirds to El Niño precursors. *Ecology*, **90,** 1175–1183.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14,** 2611–2620.

Ewens, W.J. (1972) Sampling theory of selectively neutral alleles. *Theoretical Population Biology*, **3,** 87–112.

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

Falush, D., Stephens, M. & Pritchard, J.K. (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. *Genetics*, **164,** 1567–1587.

Friesen, V.L. (1997) Population genetics and the spatial scale of conservation of colonial waterbirds. *Colonial Waterbirds*, **20,** 353–368.

Friesen, V.L. & Anderson, D.J. (1997) Phylogeny and evolution of the Sulidae (Aves: Pelecaniformes): a test of alternative modes of speciation. *Molecular Phylogenetics and Evolution*, **7,** 252–260.

Friesen, V.L., Burg, T.M. & McCoy, K.D. (2007a) Mechanisms of population differentiation in seabirds. *Molecular Ecology*, **16,** 1765–1785.

Friesen, V.L., Smith, A.L., Gómez-Díaz, E., Bolton, M., Furness, R.W., González-Solís, J. & Monteiro, L.R. (2007b) Sympatric speciation by allochrony in a seabird. *Proceedings of the National Academy of Sciences USA*, **104,** 18589–18594.

Greenwood, P.J. & Harvey, P.H. (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics*, **13,** 1–21.

Hellberg, M.E. (2009) Gene flow and isolation among populations of marine animals. *Annual Review of Ecology, Evolution, and Systematics*, **40,** 291–310.

Hewitt, G. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405,** 907–913.

Hey, J. & Nielsen, R. (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pesudoobscura* and *D. persimilis*. *Genetics*, **167,** 747–760.

Howell, S.N.G. & Webb, S. (1990) The seabirds of las Islas Revillagigedo, México. *The Wilson Bulletin*, **102,** 140–146.

Jehl, J.R., Jr & Parkes, K.C. (1982) The status of the avifauna of the Revillagigedo Islands, México. *The Wilson Bulletin*, **94,** 1–19.

Jordan, R. (1971) Distribution of anchoveta (*Engraulis ringens* J.) in relation to the environment. *Investigacion Pesquera*, **35,** 113–126.

Karpouzi, V.S., Watson, R. & Pauly, D. (2007) Modeling and mapping resource overlap between seabirds and fisheries on a global scale: a preliminary assessment. *Marine Ecology Progress Series*, **343,** 87–99.

Kim, S.-Y., Torres, R., Rodriguez, C. & Drummond, H. (2007a) Effects of breeding success, mate fidelity and senescence on breeding dispersal of male and female blue-footed boobies. *Journal of Animal Ecology*, **76,** 471–479.

Kim, S.-Y., Torres, R., Dominguez, C.A. & Drummond, H. (2007b) Lifetime philopatry in the blue-footed booby: a longitudinal study. *Behavioral Ecology*, **18,** 1132–1138.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16,** 111–120.

Kuhner, M.K. (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics*, **22,** 768–770.

Kuhner, M.K., Yamato, J. & Felsenstein, J. (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics*, **149,** 429–434.

Liebers, D., Helbig, A.J. & de Knijff, P. (2001) Genetic differentiation and phylogeography of the gulls in the *Larus cachinnans–fuscus* group (Aves: Charadriiformes). *Molecular Ecology*, **10,** 2447–2462.

Loftin, H. (1991) An annual cycle of pelagic birds in the Gulf of Panama. *Ornitologia Neotropical*, **2,** 85–94.

Mantel, N. (1967) The detection of disease clustering and a generalised regression approach. *Cancer Research*, **27,** 209–220.

Mills, L.S. & Allendorf, F.W. (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10,** 1509–1518.

Morris-Pocock, J.A., Taylor, S.A., Birt, T.P., Damus, M., Piatt, F.F., Warheit, K.I. & Friesen, V.L. (2008) Population genetic structure in Atlantic and Pacific Ocean common murres: natural replicate tests of post-Pleistocene evolution. *Molecular Ecology*, **17,** 4859–4873.

Morris-Pocock, J.A., Steeves, T.E., Estela, F.A., Anderson, D.J. & Friesen, V.L. (2010) Comparative phylogeography of brown (*Sula leucogaster*) and red-footed boobies (*S. sula*): the influence of physical barriers and habitat preference on gene flow in pelagic seabirds. *Molecular Phylogenetics and Evolution*, **54,** 883–896.

Moum, T. & Bakke, I. (2001) Mitochondrial control region structure and single site heteroplasmy in the razorbill (*Alca torda*; Aves). *Current Genetics*, **39,** 198–203.

Narum, S.R. (2006) Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation Genetics*, **7,** 783–787.

Nelson, B.J. (1978) *The Sulidae: gannets and boobies*. Oxford University Press, Aberdeen University Series, Oxford.

Nichols, W.J., Seminhoff, J.A. & Etnoyer, P. (2010) Biodiversity, function and interconnectedness: a revolution in our understanding of marine ecosystems and ocean conservation. *Handbook of marine fisheries conservation and management* (ed. by R.Q. Grafton, R. Hilbron, D. Squires, M. Tait and M. Williams), pp. 43–59. Oxford University Press, Oxford.

Palumbi, S.R. (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, **25,** 547–572.

Palumbi, S.R., Grabowsky, G., Duda, T., Geyer, L. & Tachino, N. (1997) Speciation and population genetic structure in tropical Pacific sea urchins. *Evolution*, **51,** 1506–1517.

Palumbi, S.R., Sandifer, P.A., Allan, J.D., Beck, M.W., Fautin, D.G., Fogarty, M.J., Halpern, B.S., Incze, L.S., Leong, J.-A., Norse, E., Stachowicz, J.J. & Wall, D.H. (2009) Managing for ocean biodiversity to sustain ecosystem services. *Frontiers in Ecology and the Environment*, **7,** 204–211.

Patterson, S.A., Morris-Pocock, J.A. & Friesen, V.L. (2010) A multilocus phylogeny of the Sulidae (Aves: Pelecaniformes). *Molecular Phylogenetics and Evolution*, doi: 10.1016/j.ympev.2010.11.021.

Pennington, J.T., Mahoney, K.L., Kuwahara, V.S., Kolber, D.D., Calienes, R. & Chavez, F.P. (2006) Primary production in the eastern tropical Pacific: a review. *Progress in Oceanography*, **69,** 285–317.

Posada, D., Crandall, K.A. & Templeton, A.R. (2000) GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Molecular Ecology*, **9,** 487–488.

Pritchard, J.K. & Wen, W. (2004) *Documentation for structure software: version 2.2*. University of Chicago, Chicago, IL. Available at: http://pritch.bsd.uchicago.edu/structure.html.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **159,** 945–959.

Ricklefs, R.E., Duffy, D. & Coulter, M. (1984) Weight gain of blue-footed booby chicks: an indicator of marine resources. *Ornis Scandinavica*, **15,** 162–166.

Riginos, C. & Nachman, M.W. (2001) Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology*, **10,** 1439–1453.

Rosenberg, N.A. (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4,** 137–138.

Ruckelshaus, M., Klinger, T., Knowlton, N. & DeMaster, D.P. (2008) Marine ecosystem-based management in practice: scientific and governance challenges. *BioScience*, **58,** 53–63.

Schlosser, J.A., Dubach, J.M., Garner, T.W.J., Araya, B., Bernal, M., Simeone, A., Smith, K.A. & Wallace, R.S. (2009) Evidence for gene flow differs from observed dispersal patterns in the Humboldt penguin, *Spheniscus humboldti*. *Conservation Genetics*, **10,** 839–849.

Simeone, A., Garthe, S., Sepúlveda, F.G. & Luna-Jorquera, G. (2002) *Sula nebouxii* en Isla Pájaros, Región de Coquimbo. *Boletin Chileno de Ornitologia*, **9,** 48.

Sinnott, R.W. (1984) Virtues of the Haversine. *Sky and Telescope*, **68,** 158.

Stanley, H.F., Casey, S., Carnahan, J.M., Goodman, S., Harwood, J. & Wayne, R.K. (1996) Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). *Molecular Biology and Evolution*, **13,** 368–382.

Steeves, T.E., Anderson, D.J., McNally, H., Kim, M.H. & Friesen, V.L. (2003) Phylogeography of *Sula*: the role of physical barriers to gene flow in the diversification of tropical seabirds. *Journal of Avian Biology*, **34,** 217–223.

Steeves, T.E., Anderson, D.J. & Friesen, V.L. (2005a) The Isthmus of Panama: a major physical barrier to gene flow in a highly mobile pantropical seabird. *Journal of Evolutionary Biology*, **18,** 1000–1008.

Steeves, T.E., Anderson, D.J. & Friesen, V.L. (2005b) A role for nonphysical barriers to gene flow in the diversification of a highly vagile seabird, the masked booby (*Sula dactylatra*). *Molecular Ecology*, **14,** 3877–3887.

Taylor, S.A., Zavalaga, C.B., Luna-Jorquera, G., Simeone, A., Anderson, D.J. & Friesen, V.L. (in press) Panmixia and high genetic diversity in a Humboldt Current endemic, the Peruvian Booby (*Sula variegata*). *Journal of Ornithology*.

Templeton, A.R., Boerwinkle, E. & Sing, C.F. (1987) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping 1. Basic theory and an analysis of alcohol dehydrogenase activity in Drosophila. *Genetics*, **117,** 343–351.

Thompson, P.M. & Ollason, J.C. (2001) Lagged effects of ocean climate change on fulmar population dynamics. *Nature*, **413,** 417–420.

Walsh, H.E. & Edwards, S.V. (2005) Conservation genetics and Pacific fisheries bycatch: mitochondrial differentiation and population assignment in black-footed albatross (*Phoebastria nigripes*). *Conservation Genetics*, **6,** 289–295.

Watkins, B.P., Petersen, S.L. & Ryan, P.G. (2008) Interactions between seabirds and deepwater hake trawl gear: an assessment of impacts in South African waters. *Animal Conservation*, **11,** 247–254.

Watterson, G.A. (1978) Homozygosity test of neutrality. *Genetics*, **88,** 405–417.

Weimerskirch, H., Le Corre, M., Ropert-Coudert, Y., Kato, A. & Marsac, F. (2006) Sex-specific foraging behaviour in a seabird with reversed sexual dimorphism, the red-footed booby. *Oecologia*, **146,** 681–691.

Weimerskirch, H., Le Corre, M. & Bost, C.A. (2008) Foraging strategy of masked boobies from the largest colony in the world: relationship with environmental conditions and fisheries. *Marine Ecology Progress Series*, **362,** 291–302.

Weimerskirch, H., Shaffer, S.A., Tremblay, Y., Costa, D.P., Gadenne, H., Kato, A., Ropert-Coudert, Y., Sato, K. & Aurioles, D. (2009) Species- and sex-specific differences in foraging behaviour and foraging zones in blue-footed and brown boobies in the Gulf of California. *Marine Ecology Progress Series*, **391,** 267–278.

Whitlock, M.C. & McCauley, D.E. (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity*, **82,** 117–125.

Wilson, G.A. & Rannala, B. (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163,** 1177–1191.

Wright, S. (1931) Evolution in Mendelian populations. *Genetics*, **16,** 97–159.

Zavalaga, C.B., Benvenuti, S., Dall'Antonia, L. & Emslie, S. (2007) Foraging areas of breeding blue-footed boobies *Sula nebouxii* in northern Peru, as determined by direction recorders. *Journal of Avian Biology*, **39,** 405–412.

SUPPORTING INFORMATION

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

Appendix S1 Chlorophyll *a* concentrations (mg m^{-3}), average daily sea surface temperature (SST, °C), and distributions of Pacific sardine (*Sardinops sagax/caeruleus*) and Peruvian anchovy (*Engraulis ringens*) in the eastern tropical Pacific Ocean.

Appendix S2 Methods for laboratory protocols, including GenBank accession numbers.

Appendix S3 Consensus sequence of the most common control region haplotype.

Appendix S4 Mitochondrial control region haplotype frequencies.

Appendix S5 Microsatellite loci summary statistics, including sample sizes, allele frequencies, and observed and expected heterozygosities.

Appendix S6 Summary of results from STRUCTURE.

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BIOSKETCH

Scott A. Taylor is a PhD candidate at Queen's University, Canada. He is interested in mechanisms of speciation, particularly in the absence of physical barriers to gene flow where ecology is likely to play a central role in divergence, and in using molecular methods to gain a better understanding of such natural systems.

Author contributions: S.A.T. and V.L.F. conceived the ideas; S.A.T., L.M. and D.J.A. collected the data; S.A.T. and L.M. analysed the data; V.L.F. and D.J.A. funded the project; and S.A.T. led the writing with the help of all authors.

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