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ORIGINAL ARTICLE

Panmixia and high genetic diversity in a Humboldt Current endemic, the Peruvian Booby (*Sula variegata*)

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Abstract Marine ecosystems and their inhabitants are increasingly under threat from climate change, competition with humans for resources, and pollution. Species that are endemic to particular currents or regions of the world's oceans have the potential to be at higher risk due to localized overfishing, pollution, or locally severe impacts of climate change such as more intense, or longer, El Niño Southern Oscillation events. Understanding patterns of population differentiation in endemic marine organisms may be particularly important for their conservation and persistence. Peruvian Boobies (*Sula variegata*) are endemic to the Humboldt Current upwelling system and have experienced population fluctuations throughout their evolutionary history due to both dramatic reduction of food supplies, and anthropogenic influence over the last ~150 years. Recent research on other members of the Sulidae indicates that populations of these primarily tropical seabirds show a high degree of genetic differentiation; however, the sister species of the Peruvian Booby, the Blue-footed Booby (*S. nebouxii*), exhibits only weak range-wide population genetic structure. We characterized population genetic differentiation and diversity in 153 Peruvian Boobies using sequence variation of 540 base pairs of the mitochondrial control region and seven microsatellite loci. Although we found evidence of panmixia, a signature of isolation by distance appears to exist between the five sampled colonies. We also found unexpectedly high genetic diversity given this species' recent population decline. Our results are similar to those for the Humboldt Penguin (*Spheniscus humboldti*), another endemic of the Humboldt Current upwelling system.

Keywords Humboldt current · Marine ecosystems · Dispersal · Sulid · Population genetics

Zusammenfassung Marine Ökosysteme und ihre Bewohner sind zunehmend bedroht durch den Klimawandel, den Wettbewerb mit Menschen um Ressourcen und durch Umweltverschmutzung. Arten, die endemisch in bestimmten Meeresströmungen oder Regionen vorkommen, sind hierbei potentiell stärker bedroht durch lokale Überfischung, Umweltverschmutzung oder lokal stark ausgeprägte Auswirkungen des Klimawandels wie z. B. intensivere oder länger andauernde El Niño Südliche Oszilllation-Ereignisse. Das Verständnis von Mustern der Populationsdifferenzierung endemischer mariner Organsimen kann von besonderer

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Bedeutung für ihren Schutz und ihr Weiterbestehen sein. Guanotölpel (*Sula variegata*) sind endemisch im Auftriebsgebiet der Humboldtströmung und haben Populationsschwankungen über ihre evolutionäre Vergangenheit auf Grund von dramatischen Reduktionen von verfügbarer Nahrung als auch durch anthropogene Einflüsse der letzten etwa 150 Jahre erfahren. Neuere Forschung an weiteren Arten der Sulidae weist auf eine hochgradige genetische Populationsdifferenzierung dieser primär tropischen Seevögel hin. Dem entgegen steht jedoch eine nur schwache genetische Populationsstruktur der Schwesterart des Guanotölpels, dem Blaufußtölpel (*S. nebouxii*), über dessen Verbreitungsgebiet. Wir beschreiben die genetische Populationsdifferenzierung und Diversität von 153 Guanotölpeln an Hand der Sequenzvariation einer 540 Basenpaaren langen Sequenz der mitochondrialen Kontrollregion und von sieben Mikrosatellitenmarkern. Obwohl wir Hinweise gefunden haben die auf Panmixie hinweisen, scheint ein Muster der Isolation durch Distanz zwischen den fünf beprobten Kolonien vorzuherrschen. Außerdem haben wir eine unerwartet hohe genetische Diversität gefunden, obwohl diese Art kürzlich einen Populationsrückgang erfahren hat. Unsere Ergebnisse ähneln denen die für den Humboldtpinguin (*Spheniscus humboldti*) gefunden wurden, einer weiteren endemischen Art des Auftriebsgebietes der Humboldtströmung.

Introduction

Determining the factors that influence population differentiation is crucial to gaining a better understanding of the generation and maintenance of biodiversity, particularly in ecosystems under threat from climate change, resource extraction, and pollution. Marine ecosystems face a number of these threats, and are more able to withstand perturbations when biodiversity is high (Palumbi et al. 2008). If the goal of marine ecosystem management is to maintain natural processes and biodiversity, investigations of population differentiation in marine systems should be an important aspect of marine conservation strategies (Allendorf and Luikart 2006; Mills 2007; Burton 2009; Polunin 2009). These investigations may be of particular importance when they concern endemic marine species, whose restricted distributions may increase their risk of decline due to localized overfishing, pollution, or more severe impacts of climate change.

Comparing patterns of population differentiation between species can shed light on factors generating diversity (Avise 2000). Patterns of population differentiation vary widely within the Sulidae (Steeves et al. 2003, 2005a, b; Morris-Pocock et al. 2010a), a group of tropical, sub-tropical, and temperate seabirds comprising the gannets and boobies (Nelson 1978). The boobies, in particular, exhibit a range of levels of population differentiation from very high colony-specific differentiation, as in Brown Boobies (*Sula leucogaster*) (Morris-Pocock et al. 2010a), to relatively low differentiation across large geographic distances, as in Blue-footed Boobies (*S. nebouxii*; Taylor et al. in press). The picture developing from these results involves a complex mix of drivers of genetic differentiation, including geographic and ecological obstacles to dispersal (Steeves et al. 2003, 2005a, b; Morris-Pocock et al. 2010a), degree of natal philopatry (Huyvaert and Anderson 2004), and disruptive effects of El Niño Southern Oscillation (ENSO) events (Taylor et al. in press). The wide variation in this phylogenetically small group presents a fertile opportunity to identify ecological and distributional factors underlying the diverse patterns of population differentiation in marine organisms. Understanding why these patterns vary is an important step toward a better understating of diversification and speciation in this group, and may aid sulid conservation. In this paper, we provide the first description of population differentiation in the Peruvian Booby (*S. variegata*), permitting a comparison to other sulids and especially to their sister taxon, the Blue-footed Booby (Friesen and Anderson 1997). Comparing patterns among closely related organisms adds power to inferences regarding the relative influences of ecology, behavior, and the environment on the process of population differentiation (Thompson 1999; Pauls et al. 2009).

Peruvian Boobies are endemic to the upwelling induced by the Humboldt Current, one of the most productive marine ecosystems in the world (Murphy 1923; Chavez and Messié 2009), and breed on coastal islands and protected headlands from Northern Perú to South-Central Chile (Murphy 1936; Nelson 1978). They are one of three seabird species identified as guano birds, producing guano in commercially significant amounts (Coker 1908, 1920). Population size has varied dramatically during the past ~150 years due to anthropogenic and natural factors, including habitat degradation and modification, introduced species (especially cats and rats), human disturbance (LaValle 1918), competition with fisheries (Duffy 1983a, c; Jahncke 1998; Cushman 2005), ENSO effects, and ectoparasitism (Coker 1920; Murphy 1925; Duffy 1983b, c). ENSO events can induce mass chick and juvenile mortality and dispersal of adult Peruvian Boobies as far north as the Gulf of Panama (Duffy 1983a, c; Aid et al. 1985), and ectoparasitism, particularly from blood-sucking argasid ticks (*Ornithodorus* spp.), can cause chick mortality and colony abandonment (Coker 1920; Duffy 1983b).

Given the unpredictable nature of the Humboldt Current upwelling system, and the tendency of Peruvian Boobies to disperse during ENSO events or due to high ectoparasite loads, we predict less range-wide population genetic

differentiation in the Peruvian Booby than in sulids living outside the Humboldt Current upwelling system. Further, given the recent population declines and potential for genetic bottleneck events we also predict low genetic diversity in Peruvian Boobies compared to other sulids. We used sequence data from the mitochondrial control region and seven microsatellite loci to test these predictions, examining population differentiation and genetic diversity throughout the range of this species.

Methods

Sampling and DNA extraction

Blood samples were obtained from 153 dependent young (one per nest) or breeding adult Peruvian Boobies from throughout their range (Fig. 1). Sampling in the southern part of the range was restricted to Isla Pajaros. Although additional colonies exist south of Isla Pajaros, ~95% of the population can be found between Isla Pajaros and Lobos de Tierra (Nelson 1978). Blood was collected as described in Zavalaga et al. (2009). Samples were preserved in 70% ethanol at ambient temperature in the field, and are archived at Queen's University at −80°C. DNA was extracted using a standard protease-K phenol/chloroform technique (Sambrook and Russell 2001).

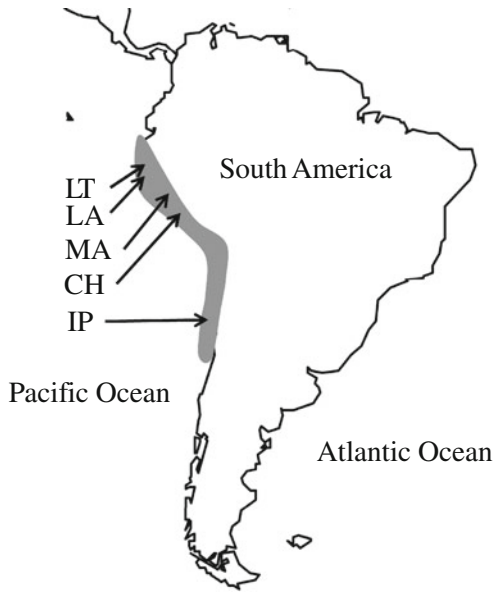

Fig. 1 Approximate breeding distribution (*shaded gray area*), and locations of sampled Peruvian Booby breeding colonies. *LT* Lobos de Tierra, Peru, 6°26′S, 80°51′W (32), *LA* Lobos de Afuera, Peru, 6°57′S, 80°41′W (30), *MA* Isla Mazorca, Peru, 11°23′S, 77°43′W (30), *CH* Isla Chincha Norte, Peru, 13°38′S, 76°22′W (32), *IP* Isla Pajaros, Chile, 29°37′S, 71°24′W (29); numbers in parentheses indicate number of individuals sampled per colony

Laboratory protocols: mitochondrial DNA

Preliminary analyses showed that Peruvian Boobies, like their sister taxon the Blue-footed Booby and other close relatives in the Sulidae, possess within their mitochondrial genome two fragments that are undergoing concerted evolution (Morris-Pocock et al. 2010b). These fragments contain copies of the control region; the genes for tRNA-Glu, ND6, tRNA-Pro and tRNA-Thr; and a partial copy of cytochrome b. Several lines of evidence suggest that both fragments are functional: e.g., proper inferred folding of tRNAs to cloverleaf shape, absence of stop codons in coding regions of cytochrome b and ND6, and the presence of conserved sequence blocks in the proper locations. Because the fragments are undergoing concerted evolution, both fragments are informative for population genetic studies. However, within a study, the same fragment should be used throughout (Morris-Pocock et al. 2010b). Generic primers amplify both copies of the control region, making unambiguous sequencing of the PCR product impossible (Morris-Pocock et al. 2010b). To solve this problem, the primers SdMCR-H750 and SlMCR-160A were used to amplify the "A" copy as outlined by Morris-Pocock et al. (2010b): a 540-base pair fragment of the mitochondrial control region containing all of Domains I and II and part of Domain III from 139 Peruvian Boobies (Supplementary Fig. 1).

After electrophoresis of 5 µl of amplified DNA through 2% agarose gels to confirm successful amplification, the remaining product was sequenced at Genome Quebec using a 3730xl DNA Analyzer system from Applied Biosystems® (McGill University, Quebec). Sequences were aligned using CLUSTALW (Thompson et al. 1994) as implemented in BioEdit Ver. 7.0.5.3 (Hall 1999) and each variable site was checked against the sequencing trace using FinchTV Ver. 1.4.0 (Geospiza Inc., http://www.geospiza.com/finchtv.html). As in Red-footed (*S. sula*), Brown (*S. leucogaster*), and Blue-footed Boobies, some samples (~15%) had two bases at some sites (Morris-Pocock et al. 2010a; Taylor et al. in press). Examination of similar ambiguous sites in Red-footed Boobies indicated that they represent true heteroplasmy and not amplification of either a nuclear copy of the mitochondrial control region or of both mitochondrial copies of the control region (Morris-Pocock et al. 2010a). To address this issue, two sequence files ("conservative" and "non-conservative") were created for the mitochondrial control region sequence data. In the conservative file, any individual with heteroplasmic bases was deleted prior to analysis. Fifteen individuals were deleted to create the conservative sequence file. In the non-conservative file, heteroplasmic bases were assigned as described in Steeves et al. (2005a). All subsequent analyses were carried out on both datasets. Results

presented throughout are for the non-conservative dataset unless stated otherwise.

Laboratory protocol: microsatellites

Seven microsatellite loci (Taylor et al. in press) were amplified from 153 individuals. Six loci were combined in two multiplexes (Sn2B-68, Sv2A-53, Ss1B-100; Ss1B-88, Sn2B-83, Sv2A-47), and locus Ss2B-138 was amplified alone in 5-μL volumes, each containing 2.5 μL Multiplex Mix (Qiagen), 0.15 μM forward and reverse primers, 1 μL of DNA, and 1 μM of M13F + D4 proprietary dye. Amplifications were performed using a BIOMETRA T-gradient Thermocycler (Biometra Analytik, Goettingen, Germany) with temperature profiles used in Taylor et al. (2010). The success of amplifications was confirmed by electrophoresis in 2% agarose gels in 1 mM Tris–acetate pH 8.0. DNA fragments were sized using a Beckman-Coulter CEQ^{TM} 8000 genetic analysis system at the Core Genotyping Facility (Department of Biology, Queen's University).

Data analyses: mitochondrial control region

Ewens–Watterson (Ewens 1972; Watterson 1978) and Chakraborty's (1990) tests of selective neutrality were conducted using ARLEQUIN Ver. 3.11 (Excoffier et al. 2005) to determine if control region variation deviates from mutation-drift equilibrium, and populations were examined for evidence of growth using the program FLUCTUATE (Kuhner et al. 1998). FLUCTUATE was run using an initial population growth rate of 1 and a Watterson estimate of θ. Population size was allowed to vary. Each run consisted of 30 short chains of 1,000 steps and four long chains of 100,000 steps. Each chain was sampled every 20 steps. Runs were performed three times with different random seeds. The parameter g was compared to zero using a log-likelihood ratio test to determine statistical significance for the entire dataset (Kuhner et al. 1998).

Substitutional relationships among control region haplotypes were inferred by constructing a statistical parsimony network in TCS Ver. 1.21 (Clement et al. 2000). The network was subsequently assessed for the presence of phylogeographic structure.

ARLEQUIN was used to index population genetic structure by calculating Φ_{ST} and δ (net sequence divergence; Wilson et al. 1985) between colony pairs, and to determine the statistical significance of genetic differences between colonies. Analyses of control region haplotypes were conducted using Kimura two-parameter distances (Kimura 1980) with a rate parameter (α) of 0.45. Significance was determined by comparison to 10,000 random permutations of the sequence data. ARLEQUIN was also used to test for range-wide isolation by distance using Wright's linearized Φ_{ST} and log-transformed geographic distances between colonies (Mantel 1967). Given failure to detect global population genetic structure (see "Results"), no hierarchical AMOVA or Bayesian analysis of population genetic structure or gene flow could be conducted.

Data analyses: microsatellites

Microsatellite data were analyzed with ARLEQUIN to test for deviations from Hardy–Weinberg equilibrium (HWE) and gametic equilibrium (LE), to estimate global F_{ST} (Wright 1931), to estimate pairwise F_{ST} values between colonies, and to perform a Mantel's test as above.

The program BOTTLENECK (Cornuet and Luikart 1996) was used to examine the data for the signature of a recent population bottleneck or recent growth. Because the mutation model for the microsatellite loci was unknown, the two-phase (TPM) mutation model with 30% variance and 70% step-wise mutation model (SMM) was used and the analysis included 1,000 iterations. Results of this analysis were robust to changes in the % variance and % SMM in the TPM mutation model. This analysis was performed on the total sample.

Results

Mitochondrial variation

Ninety-seven haplotypes were found, defined by 101 variable sites including 91 transitions, 10 transversions, and 3 insertions or deletions (Supplementary Table 1; GenBank accession numbers HQ592377–HQ592522). One site involved both a transition and a transversion. Haplotypes differed by 1–36 substitutions. The haplotype network generated by TCS was complex but did not reveal any phylogeographic structure: sampling locations appeared to be distributed randomly throughout the network. Due to the uninformative nature of the network, it is not presented here. Haplotype diversities ranged from 0.97 at Lobos de Afuera to 0.99 at Chincha Norte (Table 1). Nucleotide diversity ranged from 1.9 to 2.1 (Table 1). Few individuals shared haplotypes. No neutrality test statistics were significantly different from expected values; however, FLUCTUATE detected significant overall population growth ($g = 412.8$, $\chi^2 = 67.13$, $P = 0.0005$). AMOVA did not detect any global population genetic structure ($\Phi_{ST} = 0.005$; $P = 0.20$), no Φ_{ST} estimates were significantly different from zero between any pair of colonies (Table 2), and Mantels' test did not show a significant relationship between genetic differentiation and geographic distance ($r = 0.36$, $P = 0.14$).

Table 1 Number of individuals sequenced (*n*), *haplotype diversity (h)* ± SD, *nucleotide diversity* (π) ± SD and results of neutrality tests (*Ewens–Watterson* and *Chakraborty's*) for colonies of Peruvian Boobies (*Sula variegata*) based on mitochondrial control region sequences

Colony	*n* (mtDNA)	Haplotype diversity (h)	Nucleotide diversity (π) (%)	Ewens-Watterson		Chakraborty's	
				Obs.	Exp.	Obs.	Exp.
LT	32	0.98 ± 0.011	2.1 ± 0.002	0.041	0.040	28.0	27.8
LA	29	0.97 ± 0.020	1.9 ± 0.010	0.063	0.052	24.0	21.7
MA	27	0.98 ± 0.015	1.9 ± 0.010	0.053	0.054	22.0	22.2
CH	26	0.99 ± 0.015	2.1 ± 0.011	0.044	0.045	27.0	27.1
IP	25	0.98 ± 0.017	2.0 ± 0.010	0.053	0.051	22.0	21.5

Obs observed value, *Exp* value expected under neutrality. Abbreviations as in Fig. 1

Table 2 Pairwise Φ_{ST}/net sequence divergence (δ) estimates (from control region variation; lower matrix) and F_{ST} estimates (from microsatellite variation; upper matrix) between Peruvian Booby colonies

Colony	LT	LA	MZ	CN	PA
LT	–	−0.007	−0.002	−0.000	0.017
LA	0.001/0.014	–	−0.005	−0.001	0.011
MA	−0.009/−0.10	−0.008/−0.09	–	−0.001	0.006
CH	0.004/0.045	0.014/0.19	−0.002/−0.02	–	0.013
IP	0.030/0.030	0.005/0.084	−0.011/−0.11	0.020/0.23	–

No estimates were significantly different from zero after Benjamini–Yekutieli correction. Abbreviations as in Fig. 1

Nuclear allele variation

Between 4 and 17 alleles were found per microsatellite locus, with an average of 8 alleles per locus (Supplementary Table 2). Genotype frequencies showed no significant deviations from HWE either within a single locus across colonies or within a single colony across loci (Supplementary Table 2). No loci deviated from gametic equilibrium.

The global estimate of F_{ST} was low and not significantly different from zero ($F_{ST} = 0.003$, $P = 0.99$) and no pairwise F_{ST} estimates were significantly different from zero after Benjamini–Yekutieli correction for multiple tests (Narum 2006) (Table 2). A Mantel's test showed a significant relationship between genetic differentiation and geographic distance, suggesting isolation by distance ($r = 0.82$, $P = 0.01$). A strong relationship between genetic differentiation and geographic distance remained when the southernmost colony sampled, Isla Pajaros, was removed from the analysis ($r = 0.73$, $P = 0.04$).

Results from the sign and Wilcoxon tests in BOTTLENECK, the more conservative tests implemented by the program, failed to detect significant heterozygosity excess, which would be indicative of a recent population bottleneck ($P_{sign} = 0.29$, $P_{Wilcoxon} = 0.76$) (Cornuet and Luikart 1996).

Discussion

Peruvian Boobies appear to be genetically panmictic across their geographic range, and the total absence of population differentiation in this species is thus far unique among the Sulidae (Table 2; Steeves et al. 2003; Morris-Pocock et al. 2010a; Taylor et al. in press). Peruvian Boobies also exhibit high genetic diversity compared to other sulids and top avian predators (Steeves et al. 2003; Brown et al. 2007; Morris-Pocock et al. 2010a). The first result is consistent with the prediction that the natural stressors present in an unpredictable system such as the Humboldt Current upwelling, and perhaps to a smaller extent the anthropogenic stressors present throughout the past 150 years, induce frequent dispersal between colonies. The second result is not consistent with the prediction of eroded genetic variation following population bottlenecks, suggesting that population size during declines was adequate to preserve rare alleles.

Although we found evidence of genetic panmixia across the range of the Peruvian Booby, nuclear markers suggested a correlation between genetic and geographic distance. The correlation remains even when the southernmost colony sampled during this study, Isla Pajaros (Fig. 1), is removed from the analysis. This could suggest that

dispersal is primarily a function of the distance between colonies: individuals may be more likely to breed at nearby colonies than at other colonies throughout the 3,500 km linear range, following the stepping stone model of gene flow (Wright 1943). However, given that the pairwise estimates of F_{ST} are not significantly different from zero, we interpret this result with caution. Other studies of marine organisms have found similarly low levels of population differentiation, but significant correlations between genetic differentiation and geographic distance, across large geographic distances (Maes and Volckaert 2002; Couceiro et al. 2007).

Recent work on pelagic pantropical sulids has detected high levels of population genetic differentiation, even on very small geographic scales (Steeves et al. 2003; Morris-Pocock et al. 2010a). These results are surprising given the high mobility of sulids, and appear to be the result of natal philopatry. However, analyses of Blue-footed Boobies revealed only weak differentiation (Taylor et al. in press). This weak structure was attributed to higher dispersal and lower colony fidelity in Blue-footed Boobies compared to the pantropical sulids, potentially the result of selection imposed by a variable feeding environment (Taylor et al. in press). The fact that Peruvian Boobies also exhibit weak population genetic structure is not surprising given their similarities to Blue-footed Boobies for key ecological traits (e.g., specialization to cold-water upwelling systems), which may increase dispersal and reduce colony fidelity. Unfortunately, an absence of comprehensive banding studies prevents direct measurements of Peruvian Booby dispersal; however, our results and evidence of long distance dispersal during ENSO events (Aid et al. 1985) indicate that Peruvian Boobies, like Blue-footed Boobies, disperse widely.

As an endemic seabird of the Humboldt Current upwelling system, the Peruvian Booby has experienced a unique environment throughout its evolutionary history compared to other boobies (Coker 1920; Murphy 1925; Nelson 1978). Given the regularity with which ENSO events occur, their impacts may have played an important evolutionary role in this species, as also suggested by Luna-Jorquera et al. (2003). Individual survival and successful reproduction during ENSO events would depend on dispersal and on the ability of individuals to breed at non-natal colonies. Dispersal of even a single individual per generation between colonies is enough to effectively homogenize genetic variation at neutral markers (Wright 1931; Mills and Allendorf 1996). ENSO events, heavy ectoparasite infestation (Duffy 1983b), or both, probably promote movement between colonies and prevent allele frequency and haplotype differences from establishing. Further, the regular distribution of suitable breeding sites along the length of the Humboldt Current upwelling system (Nelson 1978) could potentially facilitate range-wide genetic panmixia. Suitable breeding sites for Blue-footed Boobies are not distributed in the same manner and range wide panmixia is not found in Blue-footed Boobies (Taylor et al. in press).

Combined with these natural phenomena, anthropogenic influences throughout recent history may have reduced population genetic differentiation: during the mid- to late 1800s, intense guano harvesting likely promoted dispersal between islands (Coker 1920). However, even in the absence of anthropogenic influences, contemporary population differentiation among Peruvian Booby colonies would likely be low for the reasons discussed above. The dispersive nature of Peruvian Boobies, and the fact that the sister species, the Blue-footed Booby, also exhibits high levels of dispersal compared to other members of the Sulidae, both provide support for the inference that dispersal in ancient Peruvian Booby populations was high. Similarly low levels of population differentiation were recently reported between ancient and modern northern fur seal (*Callorhinus ursinus*) populations, along with the finding that historical and contemporary dispersal were high (Pinsky et al. 2010).

If the population fluctuations of this species had been sufficient to cause loss of rare alleles by genetic drift, then contemporary populations should have exhibited low genetic diversity and the signature of a recent population decline. This was not the case. Results from analyses with BOTTLENECK did not detect the signature of a recent genetic bottleneck, and diversity in the mitochondrial control region is similar to that in Blue-footed Boobies, Brown Boobies, and Red-footed Boobies (Taylor et al. in press; Morris-Pocock et al. 2010a). Control region diversity is substantially higher than in another top predator, the Peregrine Falcon (*Falco peregrinus*) (five haplotypes in 184 individuals), whose population contracted recently (Brown et al. 2007). Allelic diversity in microsatellites is also very high and similar between Peruvian, Blue-footed (Taylor et al. in press), Brown, and Red-footed Boobies (Morris-Pocock et al., unpublished data), and Humboldt Penguins (Schlosser et al. 2009). (Note, however, that different loci were screened in different species, some loci involved heterospecific primers, and all studies chose loci that were variable a priori.) The high level of genetic diversity that we detected is not surprising. Even during population lows, Peruvian Boobies numbered at least 1 million birds (Tovar and Galarza 1983; Cushman 2005). The substantial genetic diversity is likely the outcome of high dispersal and relatively large population size. Similar findings exist for other Humboldt Current endemics (Cassens et al. 2005; Schlosser et al. 2009).

Our results represent the first genetic data for Peruvian Boobies and illustrate how specialization to an unpredictable food resource may influence genetic differentiation and diversity. We suggest that environmental instability,

ectoparasitism, and the wide availability of suitable breeding locations associated with this cold-water upwelling system have influenced Peruvian Booby population differentiation in ways similar to Blue-footed Boobies, but unknown in other members of the Sulidae. Anthropogenic factors may have augmented these patterns in Peruvian Boobies. Given that Peruvian Boobies are restricted to the Humboldt Current, a marine ecosystem heavily utilized by humans (Duffy 1983c, 1994; Cushman 2005; Birdlife International 2008; Goya 2000) and an ecosystem that will likely be affected by climate change (Thatje et al. 2008; Wang et al. 2010), our results are encouraging in terms of genetic resilience of the species.

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