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Hybridization Between Blue-footed (*Sula nebouxii*) and Peruvian (*Sula variegata*) Boobies in Northern Peru

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Abstract.—Recent reports have suggested that hybridization occurs between Blue-footed (*Sula nebouxii*) and Peruvian (*Sula variegata*) Boobies in northern Peru, but none included morphometric or genetic analyses of apparent hybrids. Over three field seasons, morphological measurements and blood samples were taken from four apparent Peruvian x Blue-footed Booby hybrids on Lobos de Tierra Island, and one apparent hybrid on the Lobos de Afuera islands. Feather samples were collected from one representative of each parental species and one hybrid. Morphological variation of apparent male hybrids was compared to 25 male Peruvian Boobies and 140 male Blue-footed Boobies, and variation in 540 base pairs of the mitochondrial control region and 8 microsatellite loci for all hybrids was compared to 141 Peruvian Boobies and 168 Blue-footed Boobies. Molecular sexing indicated that four hybrids were male and one was female. Male hybrid boobies showed weak transgressive segregation, all hybrids possessed Peruvian Booby mitochondrial DNA and microsatellite alleles from each parental species. Hybridization between these species appears rare and restricted to the islands where both species breed, and pre and post-mating barriers appear well established. *Received 25 December 2009, accepted 26 February 2010.*

Keywords.—booby, genetics, hybridization, microsatellite, morphometrics.

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Blue-footed (*Sula nebouxii*) and Peruvian (*Sula variegata*) Boobies are sister species within the Sulidae (Friesen and Anderson 1997). The Blue-footed Booby breeds in the Gulf of California, along the coasts of Ecuador and northern Peru, and in the Galapagos, and the Peruvian Booby breeds on coastal islands and headlands along the coasts of Peru and Chile (Fig. 1). The geographic ranges of these species overlap in northern Peru on Lobos de Tierra Island and the Lobos de Afuera islands (Fig. 1, Duffy 1987). Though both species breed on each island, the number of Blue-Footed Boobies is at least one order of magnitude larger than that of Peruvian Boobies on Lobos de Tierra Island (Nelson 1978, Figueroa and Stucchi 2008). Morphologically, each species is very distinct: sexually mature Blue-footed Boobies have bright blue feet and legs, yellow irises, and predominantly brown head and neck feathers, while sexually mature Peruvian Boobies have grey feet and legs, red irises, and white head and neck feathers (Fig. 2C, 2A).

Recently, Ayala (2006) and Figueroa and Stucchi (2008) reported hybridization between these species on both Lobos de Tierra Island and the Lobos de Afuera islands. Each year for three years on Lobos de Tierra Island, Ayala (2006) recorded between three and six individuals that appeared by morphology to be hybrids. These birds possessed orange irises, light brown head feathers, and grey legs, and all but one of these individuals was observed breeding (successfully laying eggs and raising chicks) with a male Blue-footed Booby (Ayala 2006). In one case the apparent hybrid individual was male, and was breeding with a female Peruvian Booby (Ayala 2006). Figueroa and Stucchi (2008) observed three similar apparent hybrid individuals on the Lobos de Afuera islands. These individuals were all male and were always observed courting female Blue-footed Boobies. The putative hybrids appeared from morphology to be the product of recent hybridization, and Ayala (2006) suggested that the rarity of apparent hybrids indicated that pre-mating and post-mating barriers were well established between these species.

Both Ayala (2006) and Figueroa and Stucchi (2008) highlighted the need for a

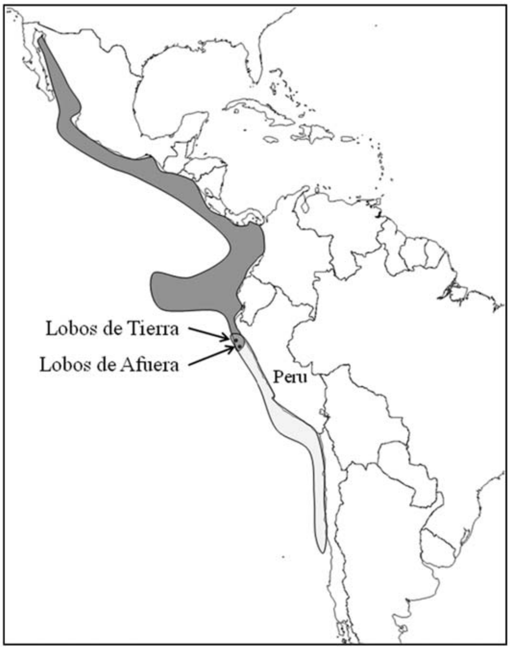

Figure 1. Locations of Lobos de Tierra Island and the Lobos de Afuera islands in northern Peru. Approximate distributions of Blue-footed (dark grey area) and Peruvian (light grey area) Boobies, from Nelson (1978). Black circles indicate island locations and are not representative of actual island size.

genetic analysis of the apparent hybrids, and neither of the previous studies included morphometric analyses. The authors of the present study captured five apparent hybrids over the course of three field seasons on Lobos de Tierra Island and the Lobos de Afuera islands. Using eight microsatellite loci, 540 base pairs (bp) of the mitochondrial control region, and measurements of weight, culmen length, and wing chord, the authors attempted to better characterize apparent hybridization between Blue-footed and Peruvian Boobies.

METHODS

Apparent hybrid boobies were captured opportunistically during three field seasons: two seasons on Lobos de Tierra Island (6°24'S, 80°51'W) and one on the Lobos de Afuera islands (6°57'S, 80°41'W), when the authors were studying Blue-footed Booby and Peruvian Booby foraging ecology (Zavalaga *et al.* 2007, Zavalaga *et al.* 2009) and collecting blood samples for population genetic analysis of Peruvian Boobies (Taylor *et al.* unpubl. data) (Fig. 1). A bird was determined to be an apparent hybrid if the eye color, foot color, and neck plumage were inconsistent with sexually mature Blue-footed or Peruvian Boobies.

Four individuals were captured on Lobos de Tierra Island: a single individual in July 2002 and three individuals in February 2003. A single individual was captured on the largest of the Lobos de Afuera islands in December 2007. Morphological measurements were taken from each individual including body weight (g), culmen length (cm), and wing chord (cm). Individuals were allowed to regurgitate, a normal response of sulids to capture, prior to body weight measurement. All measurements were taken by CBZ as in Zavalaga *et al.* (2009). A small blood sample was taken from each individual via brachial vein puncture using a lancet and was stored in 70% ethanol. Original blood samples are archived at -80°C at Queen's University, Kingston.

DNA was extracted using a standard proteinase-K phenol/chloroform technique (Sambrook and Russell 2001). Individuals were sexed using a molecular marker (Zavalaga *et al.* 2009). 540 bp of the mitochondrial control region were sequenced from each individual following Morris-Pocock *et al.* (2010a), and eight microsatellite loci were amplified in each individual and sized following Taylor *et al.* (2010) and Morris-Pocock *et al.* (2010b). Variation was compared to reference control region sequence and microsatellite alleles from range wide surveys of both Peruvian (N = 141) and Blue-footed (N = 168) Boobies (Taylor *et al.* unpubl. data).

To test the power of the microsatellite data for determining the hybrid category of individuals, multilocus genotypes were simulated using HYBRIDLAB (Ver 1.0, Nielsen *et al.* 2006), for each of the parental species (N = 30), F1 hybrids (N = 15), F2 hybrids (N = 15), first generation Blue-footed Booby backcrosses (N = 10), and first generation Peruvian Booby backcrosses (N = 10) starting from equally sized parental populations generated from existing data. These simulated data sets were then analyzed in STRUCTURE (Version 2.2, Pritchard *et al.* 2000) and NEWHYBRIDS (Version. 1.1 beta, Anderson and Thompson 2002) to determine if the ancestry of simulated hybrids could be inferred reliably.

STRUCTURE was run using the admixture model (because the species were exchanging genes in the simulated populations) and correlated allele frequencies (because the species are very closely related) with a burn-in of 25 000 steps and 250 000 steps after the burn-in (determined from test runs to be sufficient run lengths for parameter stabilization and convergence).

Analyses with NEWHYBRIDS were run using six genotype frequency classes (above) and Jeffery's-type priors for both the mixing proportion (π) and for the allele frequencies (θ) of the mixing proportion (Anderson and Thompson 2002). Genotype frequency classes are specified by the program as the expected proportions of the loci that have alleles from each parental population based on a given level of hybridization. For example, an F1 individual has a genotype frequency class of 0.5 because half of the genes come from each parental population. Jeffery's-type priors are provided by the program and can be more informative than uniform priors because they do not downweight the importance of alleles that are rare in one species and are absent from the other (Anderson and Thompson 2002). Using a uniform prior did not significantly alter the results, indicating that hybrids were not categorized based on rare alleles in this dataset. Final runs of the program had a burn-in of 30,000 cycles followed by an additional 300,000 cycles

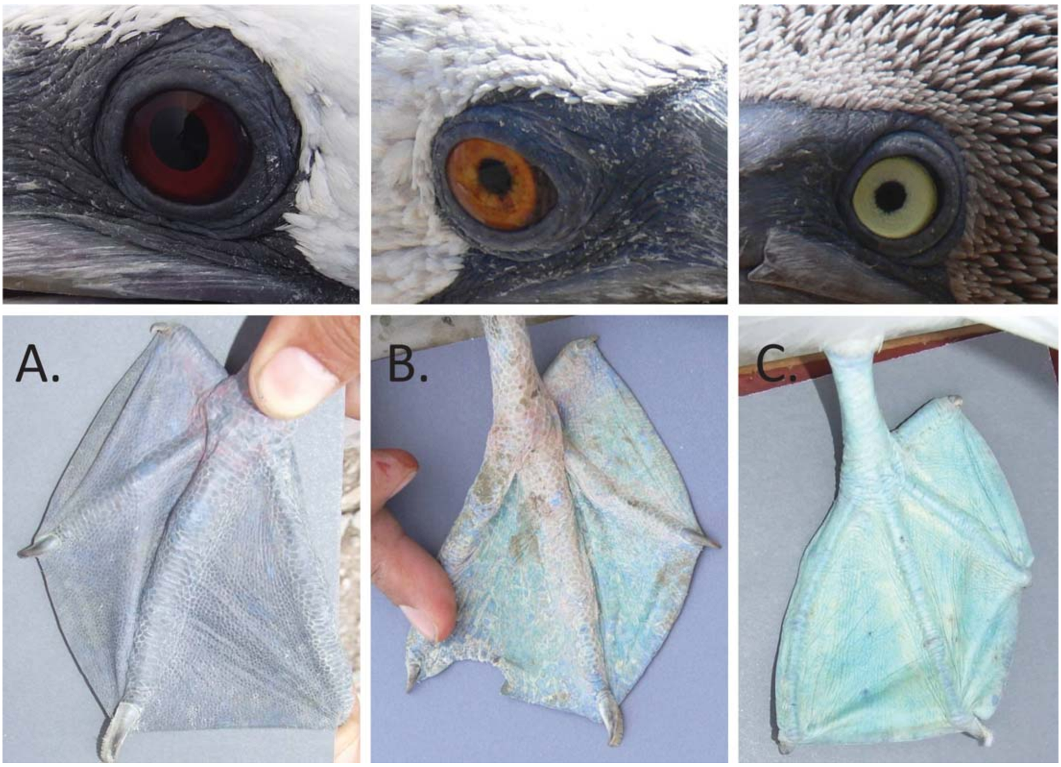

Figure 2. Eye and foot color comparison images: A) Peruvian Booby eye and foot B) hybrid Peruvian x Blue-footed Booby eye and foot C) Blue-footed Booby eye and foot

after the burn-in (determined from test runs to be sufficient for repeatable posterior probability estimates).

Weight, culmen length, and wing cord were compared between male hybrids (N = 4), and male Blue-footed Boobies from Lobos de Tierra (N = 140) and male Peruvian Boobies from Lobos de Afuera (N = 25) (Zavalaga *et al.* 2007, 2009) using unequal variance *t*-tests. Unequal variance *t*-tests are more appropriate and reliable than Student's *t*-tests when samples have unequal variance (Ruxton 2006).

RESULTS

Hybrids included in this study had morphology intermediate between Blue-footed and Peruvian Boobies; orange irises, mottled blue and grey feet (Fig. 2B), and intermediate nape and crown feather color and structure (Fig. 3). Four were male and one was female. All apparent hybrids possessed mitochondrial DNA haplotypes that otherwise only occurred in Peruvian Boobies, or grouped with Peruvian Booby haplotypes to the exclusion of all Blue-footed Booby haplotypes on a mitochondrial gene tree, and possessed a mixture of microsatellite alleles that occur in Peruvian and / or Blue-footed Boobies (Table 1, Table 2). Only one of the eight microsatellites used, Sn2A-36, was fixed for a different allele in each parental species (Table 2). With the exception of the single female, all hybrids were heterozygous at Sn2A-36.

Analyses of the simulated data using STRUCTURE and NEWHYBRIDS revealed that the microsatellite loci do not provide sufficient power to determine hybrid category. As such, the hybrids could not be classified as F1, F2, or backcross individuals with high certainty and the results are not included here. The authors are currently testing more microsatellite loci in order to increase the power of the dataset for inferring ancestry.

Male hybrids were significantly heavier (unequal variance *t*-test: $t_7 = 2.37$, $P < 0.05$) and had longer culmens (unequal variance *t*-test: $t_4 = 2.48$, $P < 0.05$) than male Peruvian Boobies; however, wing chord was similar be-

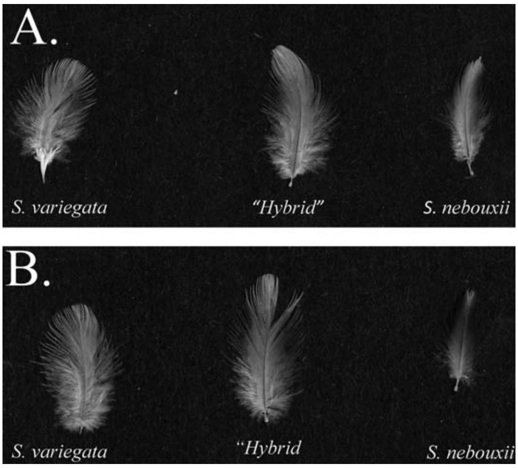

Figure 3. A) Example crown feathers from Peruvian Booby, hybrid Peruvian × Blue-footed Booby (*"hybrid"*), and Blue-footed Booby B) Example nape feathers from Peruvian Booby, hybrid Peruvian × Blue-footed Booby, and Blue-footed Booby

tween hybrids and Peruvian Boobies (unequal variance *t*-test: t_3 = 1.83, P > 0.05) (Fig. 4). There were no significant differences in any body measurements between male hybrids and male Blue-footed Boobies, despite male hybrids being slightly heavier than male Blue-footed Boobies (unequal variance *t*-test: weight, t_4 = 1.45, P > 0.05; culmen length, t_3 = -2.23, P > 0.05; wing chord, t_3 = 0.11, P > 0.05) (Fig. 3).

DISCUSSION

The apparent hybridization documented by Ayala (2006) and Figueroa and Stucchi (2008) between Blue-footed and Peruvian Boobies in northern Peru has been confirmed from both morphology and genetic analysis in the present study. The results presented here indicate that at least some hybrids are fertile, highlight that successful heterospecific pairs appear to form predominantly between female Peruvian Boobies and male Blue-footed Boobies, and provide some support for both Haldane's Rule and transgressive segregation.

All accounts indicate that hybrids are fertile. The female hybrids reported by Ayala (2006) as well as the single female included in this study were paired with male Blue-foot-

Table 1. Collection site, sex, mitochondrial DNA (mtDNA) type based on 540 base pairs of control region sequence, and nuclear genotype at eight microsatellite loci for five apparent hybrid Peruvian × Blue-footed Boobies. Missing data denoted by "?", PEBO = Peruvian Booby.

				Genotype							
Specimen	Collection site	Sex	mtDNA type	Ss1B-88	Ss1B-100	Ss2B-138	Sn2B-68	Sv2A-53	Sn2B-83	Sv2A-47	Sn2A-36
H1	Lobos de Tierra	M	PEBO	220, 220	197, 201	353, 357	170, 170	320, 326	178, 180	293, 297	336, 340
H2	Lobos de Tierra	M	PEBO	222, 224	197, 201	353, 357	170, 174	320, 330	178, 180	?	336, 340
H3	Lobos de Tierra	M	PEBO	222, 222	201, 201	350, 353	168, 172	314, 320	178, 178	293, 293	336, 340
H4	Lobos de Tierra	M	PEBO	218, 224	197, 199	357, 359	170, 172	320, 330	180, 180	285, 293	336, 340
H5	Lobos de Afuera	F	PEBO	222, 224	201, 201	353, 357	170, 170	308, 318	178, 182	293, 295	336, 336

Table 2. Microsatellite alleles found in range-wide surveys of Blue-footed (BFBO) and Peruvian (PEBO) Boobies for loci used in the present study (Taylor *et al.* unpubl. data.).

	Alleles present	
Locus	BFBO	PEBO
Ss1b-88	200, 216, 218, 220, 222	220, 222, 224, 226, 228
Ss1B-100	191, 197, 199, 201, 203	193, 195, 197, 201, 203, 205, 207, 297
Ss2B-138	351, 353, 355, 357, 359	349, 353, 355, 357, 359, 361, 363
Sn2B-68	168, 170, 172	168, 170, 172, 174, 176
Sv2A-53	300, 304, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 336, 338	302, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336
Sn2B-83	178, 180, 182, 184	172, 176, 178, 180, 182, 184
Sv2A-47	291, 293, 295, 297, 299	285, 287, 289, 291, 293, 295, 297, 299
Sn2A-36[1]	336	340

[1] 30 individuals of each parental species were genotyped.

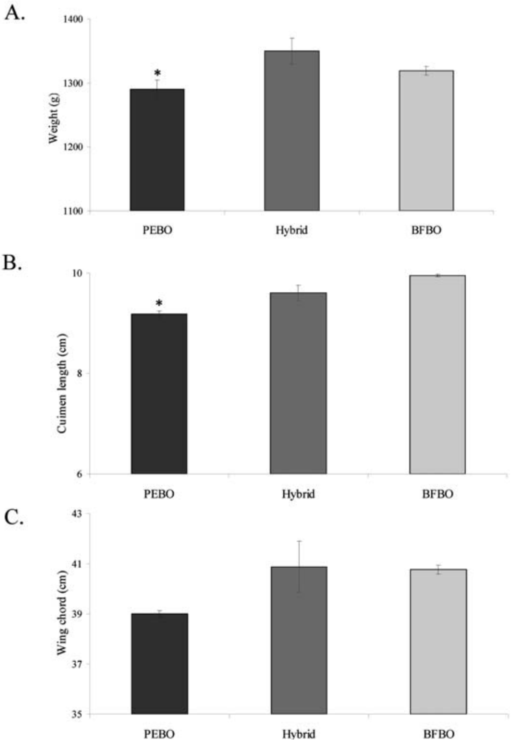

Figure 4. Average (A) weight (g) (B) culmen length (cm) and (C) wing chord (cm) ±SE of male hybrid Peruvian × Blue-footed Boobies ("Hybrid") compared to average measurements from male Peruvian (PEBO) and Blue-footed (BFBO) Boobies. * indicates mean is significantly different from hybrid value ($P < 0.05$).

ed Boobies and were incubating eggs or raising chicks. Though hybrids appear fertile, preliminary evidence suggests that there is no widespread mitochondrial DNA introgression between species (Taylor *et al.* unpubl. data). Offspring resulting from backcross pairings thus appear to be isolated and potentially less fit than the parental species. A more informative panel of markers will allow better detection of introgression.

The observation that all hybrids included in this study have Peruvian Booby mitochondrial DNA indicates that mate choice in this hybrid zone favours pairing between female Peruvian Boobies and male Blue-footed Boobies. Female choice can lead to unidirectional introgression in a number of cases, especially when one species is rare or the males of one species exhibit a more elaborate courtship display (Wirtz 1999). Both of these factors could be influencing mate choice in this hybrid zone: Peruvian Boobies are the rare species, especially on Lobos de Tierra, and male Blue-footed Boobies exhibit a more elaborate courtship display than male Peruvian Boobies.

Haldane's Rule (Haldane 1922) states that viability and/or fertility of hybrids will be more severely reduced in the heterogametic sex, i.e. in female birds (Tegelström and Gelter 1990). The present study at least partially supports this claim: four of five hybrids were male. However, given (1) the small sample size of this study, (2) Ayala's (2006) observations that the majority of hybrids were female, and (3) the inability of either study to determine hybrid ancestry (F1 vs. backcross individuals), these results should be interpreted with caution (Wu and Davis 1993).

The female hybrids observed by Ayala (2006), and the single female hybrid found in the present study, could be the result of backcross mating. In lepidopterans, another group with heterogametic females, backcrossing (by male F1s) can produce viable and fertile females of mixed ancestry even when F1 females are infertile (Naisbit *et al.* 2002). The female sampled in this study appeared to be the result of a backcross mating; as she was the only individual homozygous at locus Sn2A-36. Analysis of more informative markers and more individuals at locus Sn2A-36 should allow the hybrid class to be determined with more certainty, and will also help determine adherence to Haldane's rule in this hybrid zone.

Male hybrids were significantly heavier and had significantly longer culmens than male Peruvian Boobies, and their wing chord measurements were larger, but not significantly so. The production of extreme phenotypes in hybrids, transgressive segregation, may be important in the establishment of hybrid lineages (Rieseberg *et al.* 1999). However, the absence of widespread hybridization between Peruvian and Blue-footed Boobies suggests that selection against hybrids is stronger than any benefits arising from transgression (Rieseberg *et al.* 1999).

Hybridization between Blue-footed and Peruvian Boobies appears rare given the relatively few reports in the literature (Ayala 2006, Figueroa and Stucchi 2008), likely due to the limited geographic overlap between the species. Blue-footed and Peruvian Boobies may have diverged from their common ancestor with gene flow via parapatric speciation (Friesen and Anderson 1997): the rarity of morphological hybrids, the fact that hybrids observed appear to be of recent origin, and apparent absence of mitochondrial introgression in either species (Taylor unpubl. data) all indicate that pre-mating and post-mating barriers are well established, and lend support to this hypothesis. The authors are currently testing whether Blue-footed and Peruvian Boobies diverged from their common ancestor with limited gene flow due to strong ecological selection across an environmental gradient.

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