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MALE/FEMALE CLASSIFICATION OF THE PERUVIAN BOOBY

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ABSTRACT.—We studied adult Peruvian Boobies (*Sula variegata*) on two islands in northern Peru to classify males and females using DNA-based techniques. We used this information to (1) assess the extent of size dimorphism in this species, (2) identify males and females using discriminant functions of external characters, and (3) validate use of voice as a reliable method for identifying male and female Peruvian Boobies in the field. Female Peruvian Boobies were 19% heavier and their culmens and wings were 3 and 4% larger than males, respectively. A discriminant function that included body weight and wing chord correctly classified 90% of the birds. Alternatively, 88% of correct identification of males and females was obtained with a function that incorporated only wing chord. Whistles were performed exclusively by males (25/25 of cases), whereas grunts or goose-like honk vocalizations were performed only by females (24/24 of cases). The female-larger size dimorphism of Peruvian Boobies is intermediate in comparison to other *Sula* boobies. Calls and biometry provide a fast, reliable, and inexpensive method for classifying most adult Peruvian Boobies as males or females in the field. We recommend a hierarchical system for classification of male and female Peruvian Boobies: (1) use of vocalizations, (2) use of body weight and wing chord when the bird did not vocalize and was weighed immediately after daybreak or before the first feeding trip of the day, and (3) use of wing chord only when there is uncertainty in temporal variations of body weight.
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Most seabird species do not display male/female-specific differences in coloration of external features such as plumage, feet, or secondary ornaments (Harrison 1983, Redman et al. 2002, Gaston 2004, Ropert-Coudert et al. 2005) making recognition of males and females by field biologists difficult. Seabirds exhibit a wide range of size dimorphism that includes male- (males > females) and female-biased (females > males) species (Fairbairn and Shine 1993, Serrano-Meneses and Székely 2006). The disparity of size between females and males in several seabird taxa has allowed classification using discriminant functions of external characteristics. Discriminant analysis uses morphometric differences between known males and females to calculate a linear function that predicts correct classification of unknown individuals. This method is reliable and accurate in species with a high to moderate dimorphism (Bédard et al. 1995, Zavalaga and Paredes 1997, Torlaschi et al. 2000, Devlin et al. 2004). Male/female-specific calls have also been used for classification in monomorphic birds (Carlson and Trost 1992, Price 2003, Volodin et al. 2003). The dimorphic vocalizations in some species of nocturnal Procellariiformes including shearwaters (Brook 1988, Bourgeois et al. 2007) and storm petrels (Taoka and Okumura 1990) are clear to the human ear allowing individuals to be reliably classified as males or females in the field (James and Robertson 1985).

Size dimorphism varies widely among the 10 extant species of the Sulidae (boobies and gannets). The three species of gannets (*Morus* spp.) and their close relative Abbott's Booby (*Papasula abbotti*) are nearly monomorphic, whereas female *Sula* boobies are 10–38% heavier than males (Nelson 1978). Despite the marked morphological differentiation in boobies, there are no quantitative studies for identification of males and females based on external characters. Dimorphic calls such as high-pitched whistles in males and loud trumpet-like honks in females, have been recognized in five species of boobies (Nelson 1978). Acoustic signaling has been used to identify male and female Nazca (*S. granti*), Masked (*S. dactylatra*), and Blue-footed (*S. nebouxii*) boobies in the field (Anderson 1993, Velando and Alonso-Alvarez 2003, Zavalaga et al. 2007), but this criterion has not been verified in Brown (*S. leucogaster*) and Peruvian boobies (*S. variegata*).

Little information exists regarding male/female-related morphometry of Peruvian Boobies. Murphy (1936), based on data from 14 males and nine females, reported the bill, wing, tail, and tarsus were 4–5% larger in females. The only information on body weight disparity comes from

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comparison of four known-gender adults, where females were 14% heavier than males (Nelson 1978). The calls of Peruvian Boobies appears to differ (males whistle and females grunt), although the pitch intensity and amplitude of the calls is less marked than in some other booby species (Nelson 1978).

Our objectives were to: (1) identify male and female adult Peruvian Boobies using a DNA-based technique, (2) assess the extent of size dimorphism between males and females, (3) provide discriminant functions of external characters to identify males and females, and (4) validate use of voice as a reliable method for classifying males and females Peruvian Boobies in the field.

METHODS

We studied Peruvian Boobies on two islands in northern Peru: Lobos de Tierra (6° 24′ S, 80° 51′ W) between 22 and 30 December 2006 and Lobos de Afuera (6° 57′ S, 80° 41′ W) between 10 and 17 December 2007. Lobos de Tierra is 65 km north of Lobos de Afuera and we pooled the data from the two islands.

Captures and Measurements.—We captured 14 and 35 birds on Lobos de Tierra and Lobos de Afuera, respectively. Peruvian Boobies on the Lobos islands nested in dense groups (~2 nests/m^2) of 100–3,000 nests. We selected birds rearing medium-size chicks and removed them from their nests using a hook attached to a 3-m pole, placed gently around their necks. We qualitatively assessed calls before, during, and after restraining as either of two types: whistles or grunts. Morphological characteristics measured were body weight (BW) recorded early in the morning (0530–0800 hrs), which represented the minimum daily weight, culmen length (CL) was the diagonal distance from the tip to the base of the culmen, and natural wing chord (WC) from the bend in the wrist to the tip of the longest primary without flattening the wing. Birds were weighed with a Pesola® spring scale to the nearest 25 g. The exposed culmen was measured with calipers (accuracy of 0.01 mm), whereas wing chord was measured with a stopping wing ruler (accuracy 1 mm). Measurements were performed by the same person (CBZ) throughout. We obtained three drops of blood from the brachial vein of each individual using a lancet. These were absorbed on filter paper and stored in vials in 70% ethanol. This entire procedure took less than 5 min per individual. Blood samples are archived at Queen's University, Ontario, Canada.

DNA Analysis.—We extracted DNA from blood using a standard proteinase-K phenol/chloroform technique (Friesen et al. 1996). Males and females were identified using primers 2550F and 2178R developed by Fridolfsson and Ellegren (1999). Fragments of two lengths were amplified from female DNA (females are heterogametic) and fragments of a single length were amplified from male DNA. PCR amplifications were conducted in 25 μL of a cocktail containing 10 mM Tris pH 8.0, 3.5 mM $MgCl_2$, 0.4 μM of each primer, 50 mM KCl, 0.2 mM dNTPs, and 0.5 units of *Taq* DNA polymerase (Qiagen Inc., Mississauga, ON, Canada). The temperature profile consisted of an initial denaturization period at 94° C for 3 min followed by annealing for 1 min at 47° C and extension for 90 sec at 72° C. This was followed by 34 cycles of 94° C for 45 sec, annealing at 47° C for 1 min and extension at 72° C for 90 sec. PCR products were subjected to electrophoresis and visualized on 2% agarose gels. All samples were analyzed without knowledge of the suspected gender of the bird.

Statistical Analyses.—We used *t*-tests for male/female-specific comparisons of mean values of morphological characters. We measured the magnitude of the difference in the mean morphological measurement between females and males by calculating a dimorphism index as $DI = 100 \times (F–M)/F$ (Greenwood 2003). Multivariate statistics were conducted using SAS Version 9.1 statistical software (SAS Institute 2004). The stepwise discriminant procedure (PROC STEPDISC) was applied to select the variables (BW, CL, and WC) that had significant influence on classification of males and females. An *F*-test of Wilks' lambda value was used as a criterion to enter the variable contributing the most or to remove the variable contributing the least discriminatory power to the model (i.e., the variable caused a significant reduction in Wilks' lambda). The equality of group covariance matrices (a requirement of discriminant analysis) was tested with Box's *M*-test (Manly 2005). A discriminant analysis procedure (PROC DISCRIM) was used to calculate discriminant functions after identifying variables that contributed to the model. Individuals were classified as females or males on the basis of the discriminant score (D). The percentage of correct classification was calculated by a cross-validation or jackknife procedure

TABLE 1. Differences in morphological characters of breeding Peruvian Boobies on isla Lobos de Tierra ($n = 14$) and Lobos de Afuera ($n = 35$), Peru. Mean ± SD, range in parentheses, and 95% confidence interval in brackets. DI = difference in percentage (F–M)/M × 100, where F is female measurement and M is male measurement.

	Females ($n = 24$)	Males ($n = 25$)	DI (%)	*t*-test	*P*
Body weight (g)	1543 ± 105 (1300–1675) [1499–1587]	1290 ± 75 (1200–1450) [1259–1321]	19	9.65	<0.001
Culmen length (cm)	9.61 ± 0.25 (9.19–10.14) [9.50–9.72]	9.18 ± 0.34 (8.47–9.94) [9.04–9.32]	4	4.95	<0.001
Wing chord (cm)	40.25 ± 0.57 (39.1–41.5) [40.49–40.01]	39.0 ± 0.68 (38.0–40.5) [39.28–38.72]	3	7.03	<0.001

(PROC DISCRIM CROSSVALIDATE), i.e., by repeating the analyses leaving out a single bird each time (leave-out-one test) and then classifying that individual using the function derived from the remaining birds (Phillips and Furness 1997, Chardine 2002, Devlin et al. 2004). The posterior probability (PP) of membership of each bird (the probability that an individual with a particular value of D is, or is not, likely to be a female or male) was calculated using SAS (PROC DISCRIM) following Bayes' Rule. The calculated values of PP and D for each bird were then fitted to a logistic curve:

$$\mathrm{PP} = \frac{1}{1 + \exp(k\mathrm{D} - \mathrm{c})}$$

where *k* and *c* are constants calculated using the non-linear procedure (PROC NLIN). This function allowed calculation of cut-off values for discriminant scores that had 50% or higher probabilities to classify an adult as a female or male. This function can calculate the level of classification accuracy of an individual for any given D score.

We performed a stepwise discriminant procedure including only culmen and wing length in the analysis to create a function that could be used to classify adult males and females caught at different times of the day and seasons. Significance levels were set at $P < 0.05$.

RESULTS

Peruvian Boobies captured on Lobos de Tierra and Lobos de Afuera islands comprised nine males and five females, and 16 males and 19 females, respectively, based on DNA analysis.

External Measurements and Discriminant Analysis.—Female Peruvian Boobies were 19% heavier and their culmens and wings were 3 and 4% larger than males, respectively (Table 1). There was low overlap between measurements of the three characteristics of females and males as evidenced by 95% confidence intervals (Table 1).

Stepwise discriminant analysis selected only body weight and wing chord as variables that best classified males and females (Wilks' lambda = 0.29, $\chi^2 = 56.8$, df = 2, $P < 0.001$; Box's $M = 5.089$, $F = 1.62$, $P = 0.18$) following the unstandardized linear equation:

$$\mathrm{D}_1 = -122 + 0.02643(\mathrm{BW}) + 2.1288(\mathrm{WC}). \qquad \text{Function1a}$$

All boobies with discriminant score ≤ -0.21 were classified as males and ≥ -0.21 as females when the posterior probability was set at 0.5 (Function 1b). There was low overlap between males and females (Fig. 2). The leave-one-out test correctly classified 90% of the birds using Function 1a. Once the discriminant score of an unknown bird was calculated with Function 1a, the posterior probability of classification for any D value could be obtained using the equation:

$$\mathrm{PP}_1 = \frac{1}{1 + \exp(1.0002\mathrm{D}_1 + 0.2103)}. \qquad \text{Function1b}$$

Boobies could be classified as males or females if $\mathrm{D}_1 \leq -1.308$ and $\mathrm{D}_1 \geq 0.88$, respectively, when the posterior probability was set at $\mathrm{PP}_1 \geq 0.75$ in Function 1b (Fig. 1). The correct classification was 88% when PP_1 for either group was ≥ 0.75.

The second discriminant function, when body weight was excluded from the analysis, that best separated males and females included only wing chord (Wilks' Lambda = 0.49, $\chi^2 = 33.3$, df = 1, $P < 0.001$; Box's $M = 0.76$, $F = 0.74$, $P = 0.39$):

$$\mathrm{D}_2 = -126 + 3.20017(\mathrm{WC}). \qquad \text{Function2a}$$

The cut-off point for correct classification of males and females when the posterior probability of each was 0.5 was 0.8134. The cross validation

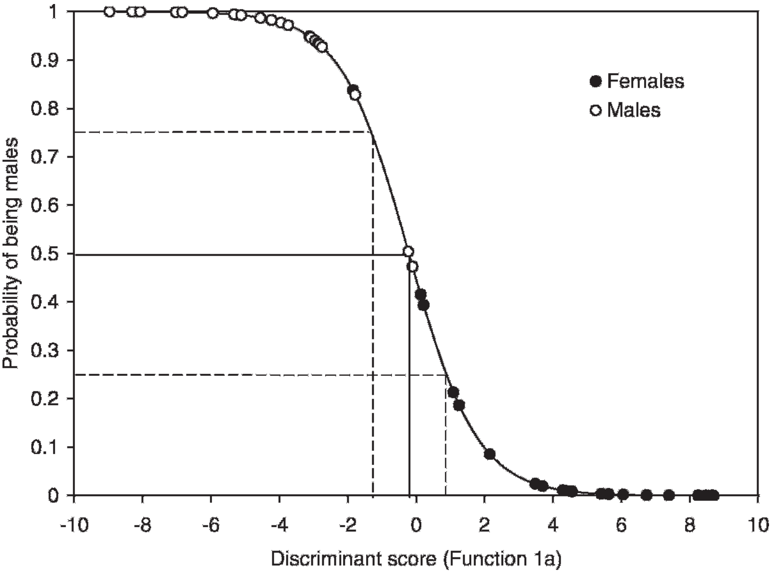

FIG. 1. Posterior probability of being male based on body weight and wing chord of 24 female and 25 male Peruvian Boobies from islas Lobos de Tierra and Lobos de Afuera, Peru. All boobies with discriminant score ≤ -0.21 were classified as males and ≥ -0.21 as females when the posterior probability was set at 0.5 (solid line). The dashed lines indicate the cut-off values of -1.308 and 0.88 when the posterior probability of being a male was set at 0.75 and 0.25, respectively.

method correctly classified 88% of the individuals. The relationship between the posterior probability (PP) of membership and the discriminant score (D) of Function 2a was described by the equation:

$$PP_2 = \frac{1}{1 + \exp(0.9999D_2 - 0.8134)} \quad \text{Function2b}$$

Boobies with $D_2 \leq -0.2831$ were classified as males and $D_2 \geq 1.9169$ as females if the posterior probability of being males or females was set at 0.75. Assigning males and females using Function 2b resulted in the correct classification of 80% when the PP_2 of either group was greater than 0.75 (the classification of birds with $-0.2831 \leq D_2 \leq 1.9169$ was uncertain).

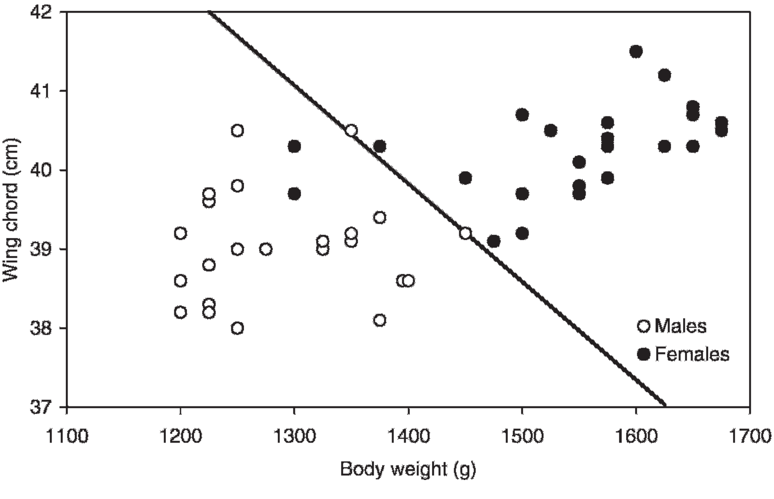

FIG. 2. Discriminant function on the basis of body weight and wing chord of 24 female and 25 male Peruvian Boobies from islas Lobos de Tierra and Lobos de Afuera, Peru. The line is represented by the equation $-0.21 = -122 + 0.02643$(body weight in g) + 2.1288(wing chord in cm). All birds above the line were classified as females.

Vocalizations.—Calls unequivocally identified male and female adult Peruvian Boobies. Whistles were performed exclusively by males (25/25 of cases), whereas grunts or goose-like honk vocalizations were performed only by females (24/24 of cases).

DISCUSSION

We confirmed that calls provide a fast, reliable, and inexpensive method for classifying male and female adult Peruvian Boobies in the field. The marked difference in voice between males and females is unmistakable and easily recognizable by the human ear: high-pitched whistles in males, louder trumpet-like grunts in females. We also observed that the male/female-specific difference in sounds was maintained in different displays described in this species: saluting, wing flailing, jabbing, yes-headshaking, and sky-pointing (Nelson 1978). Dimorphic calls are also given by Blue-footed, Masked, Brown, and Nazca boobies (Anderson 1993, Zavalaga et al. 2007), but call differences are ambiguous (at least to the human ear) in Red-footed (*Sula sula*) and Abbott's boobies, and the three species of gannets (Nelson 1978). This inter-specific pattern of vocalizations is in accord with the molecular phylogeny of sulids, where the five species of boobies with distinct vocal sexual differences are more closely related to each other than to Red-footed or Abbott's boobies, or the three species of gannets (Friesen and Anderson 1997). The proximate explanation for the sexually dimorphic calls is the anatomical difference of the syrinx between adult males and females (Murphy 1936).

The Peruvian Booby is a relatively small sulid, only slightly larger than Brown and Red-footed boobies (Nelson 1978). The extent of dimorphism of Peruvian Boobies is intermediate between the highly dimorphic Blue-footed and Brown boobies, and the less dimorphic Nazca, Masked, and Red-footed boobies. The disparity in size of Peruvian Boobies on the Lobos Islands was sufficiently large to separate males and females in 90% of the cases by simultaneously using body weight and wing chord. However, diurnal fluctuation of individual body weight can significantly decrease the accuracy of correct classification, particularly in breeding Peruvian Boobies that feed more than once during daylight hours (Duffy 1987). We weighed all individuals minutes after daybreak (before the first feeding trip) when body weight is likely at the minimum value of the day. A discriminant function using only wing chord correctly classified 88% of the individuals and can be used as an alternative when birds are weighed at other times during the day. Regional variation in biometry occurs in several species of sulids (Nelson 1978, Ropert-Coudert et al. 2005). There are no significant genetic differences between Peruvian Booby populations in northern and central Peru (SAT, unpubl. data), but the accuracy of our discriminant equations may not necessarily be the same for locations farther south.

We recommend the following hierarchical system of classification for male and female Peruvian Boobies in the field: (1) use vocalizations, (2) use Function 1a when the bird did not vocalize and was weighed immediately after daybreak or before the first feeding trip of the day, and (3) use Function 2a when there is uncertainty in the temporal variations of body weight.

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