

# Sexing sooty terns on Ascension Island from morphometric measurements

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## Keywords

Ascension Island; *Onychoprion fuscata*; morphometric; PCR; sexing; seabird colony.

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## Abstract

Sooty terns *Onychoprion fuscata* are one of the most abundant seabirds but breeding populations in many colonies have diminished. Rapid sexing of sooty terns in the field could be crucial in advancing our understanding of their reproductive biology, and in promoting conservation. However, sooty tern males and females are identical in their plumage and, thus, difficult to sex in the field. Morphometric measurements were taken from 63 adult sooty terns breeding on Ascension Island in 2005. A small blood sample was taken from the brachial vein to determine the bird's sex using standard PCR-based molecular techniques. Males were consistently larger in all morphometric measurements than females but considerable overlap between the sexes resulted in no single measurement being a useful discriminator of sex. A principal components analysis on a correlation matrix of seven morphometric measurements indicated that the first principal component (PC1) was a good 'body size' axis explaining 40.5% of the variance in the original matrix. The suite of head measurements all had high character loadings on PC1 and were, therefore, good indicators of the body size of sooty terns. Tarsus length and wing length were less reliable predictors of sex. Discriminant analyses revealed that a discriminant function incorporating head measurements and wing length allowed 77.8% of sooty terns to be sexed correctly based upon morphometric measurements alone. Further morphometric approaches to sexing should be explored with sooty terns captured in subsequent years.

## Introduction

The ability to sex birds rapidly and effectively is a potent tool in investigations of the biology of birds under threat. For example, Nisbet & Spendelov (1999) reviewed the recovery programme of the north-west Atlantic roseate tern *Sterna dougallii* population. Sex determination of roseate terns revealed that population size was most limited not by displacement and predation by gulls but by skewed adult sex ratios. Sex determination of sooty terns *Onychoprion fuscata* (nomenclature follows Bridge, Jones & Baker, 2005 and the BOU Checklist: <http://www.bou.org.uk/recbrlst1dna.html>), the focal species of this study, may be just as important in its conservation. They are colonially nesting seabirds that have been severely disrupted by human activity (e.g. Feare, 1976) and, specifically, by the introduction of predators (e.g. Ratcliffe, Hughes & Roberts, 1999). Such a colony exists on Ascension Island, where between 1954 and 1994, the population of sooty terns declined from 500 000 to 200 000 birds (Ratcliffe *et al.*, 1999), predominantly because of predation of adults, chicks and eggs by introduced feral cats *Felis catus* and black rats *Rattus rattus*. The cat-eradication programme funded by the Royal Society for the Protection

of Birds was completed in January 2004 and measures to control rats are now under way. Rapid, effective and non-invasive methods to sex sooty terns in the field would further our understanding of their reproductive biology and, as a consequence, facilitate conservation efforts.

In many avian taxa, determining the sex of individuals presents no challenge because of the marked differences between the sexes in plumage traits. However, in other taxa sex determination by plumage traits can be problematic when birds are sexually monomorphic as in the Sternidae (terns and their allies). Sooty terns are sexually monomorphic in plumage traits but there is slight sexual body size dimorphism, typically with males being 2–5% larger than females (del Hoyo, Elliott & Sargatal, 1996). Although there is considerable overlap between males and females in single characters, some success has been achieved in sex discrimination of terns by combining characters using discriminant analysis. For example, Fletcher & Hamer (2003) sexed up to 78% of common *Sterna hirundo* and Arctic *Sterna paradisaea* terns correctly using this statistical approach, but Coulter (1986) sexed only 72% of common terns correctly based upon a discriminant function using culmen length, breadth and depth. Discriminant analysis has been

used as an effective statistical approach to sex birds from morphometric measurements in a number of orders (e.g. Sphenisciformes Zavalaga & Paredes, 1997; Procellariiformes Weidinger & van Franeker, 1998; Pelecaniformes Glahn & McCoy, 1995; Charadriiformes Grecian, Diamond & Chardine, 2003; Passeriformes Sweeney & Tatner, 1996).

Despite the reliability of molecular techniques used to determine sex by DNA extraction from blood samples (Ellegren & Sheldon, 1997), they are not routinely available to most ornithologists who have access to neither trained staff with their associated expertise nor the laboratory facilities to carry out concerted molecular analysis of large numbers of samples. An alternative is a morphometrically based sexing technique that is especially valuable for those species that nest in large colonies where morphometric measurements are routinely taken during the ringing of many birds. Blood sampling (and subsequent laboratory work associated with molecular sexing protocols) does not allow the rapid sexing of such colonially nesting birds and, therefore, does not allow sex discrimination to shape on-the-ground research programmes.

The aim of this study is to determine whether a sample of sooty terns from the breeding population on Ascension Island can be sexed from single morphometric measurements or from a combination of them. While there is considerable overlap between the sexes of some individual morphometric measurements (Schreiber *et al.*, 2002), we wanted to test the discriminatory power of a range of morphometric measurements taken from birds whose sex was confirmed using molecular analysis of DNA (Ellegren & Sheldon, 1997) extracted from their blood samples. The threats to sooty tern colonies will undoubtedly worsen without intensification of conservation efforts; the long-term aim of this study is to refine morphometrically based sexing techniques to optimize rapid and reliable sex determination and, thereby, facilitate application of effective conservation measures for this species.

## Materials and methods

### Study area

Ascension Island is a United Kingdom Overseas Territory and an Important Bird Area (ref number SH001). It is an isolated volcanic island situated in the tropical South Atlantic Ocean (07°57'S, 14°24'W), approximately midway between South America and Africa. The nearest land is St Helena 1300 km to the south-east. The island is roughly triangular in shape and has an area of *c.* 97 km<sup>2</sup>. The breeding distribution of >180 000 pairs of sooty terns on Ascension Island is entirely restricted to the barren coastal plain on the south-western corner of the island. The terns breed sub-annually (Ashmole, 1963), returning to the colonies at Mars Bay and Waterside every 10 months.

### Morphometric measurements

Morphometric measurements were taken by CPW from sooty terns caught at Mars Bay and Waterside colonies in

late October and early November 2005. All birds were breeding adults caught with a long-handled net while feeding young. The following measurements were taken from a sample of 64 birds: (1) wing length – measured with a stopped ruler to the nearest millimetre (Redfern & Clark, 2001); (2) tarsus length – measured as the minimal tarsus length (i.e. length of the tarsometatarsus) with digital callipers to the nearest millimetre (Redfern & Clark, 2001); (3) bill-head – shown in Fig. 1a; (4) bill-skull – shown in Fig. 1b; (5) bill-feather – shown in Fig. 1c; (6) bill depth – shown in Fig. 1d; (7) gonys – shown in Fig. 1e. All bill measurements were taken with digital callipers to the nearest 0.1 mm.

### Molecular sexing

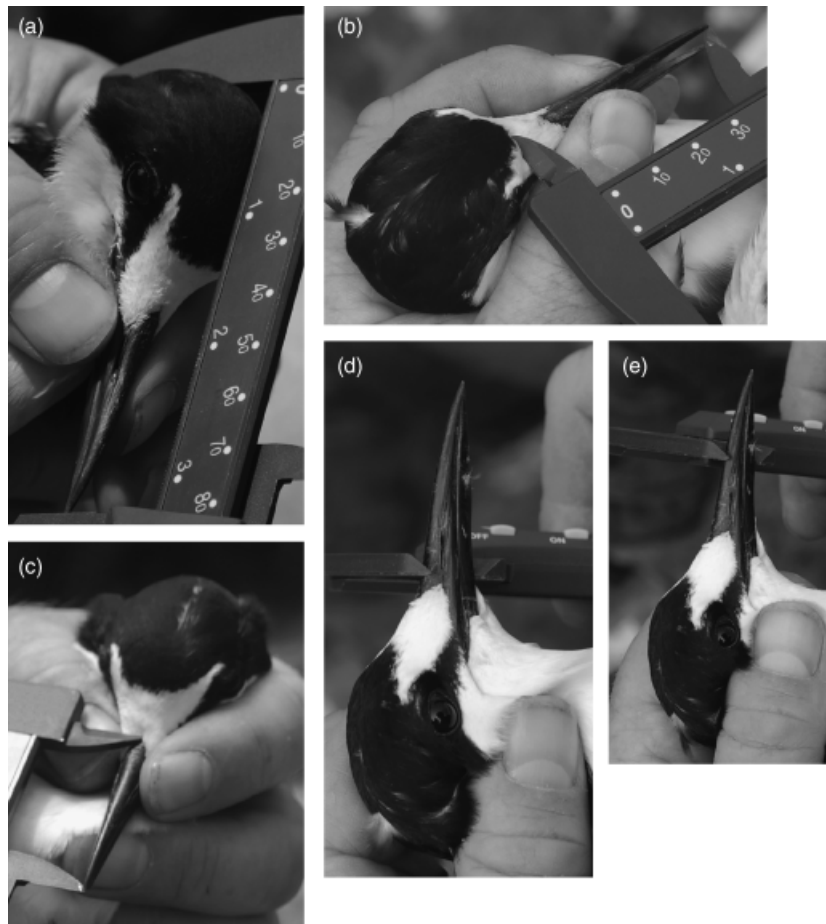
Approximately 250 µL of blood was collected from each bird by venipuncture of the left brachial vein using a hypodermic needle and syringe. Blood was preserved in 25% dimethyl sulfoxide (DMSO) in 5 M NaCl solution and stored in a domestic refrigerator (at <5 °C) before laboratory processing. We used the techniques outlined in Griffiths *et al.* (1998) and optimized the PCR conditions for sooty terns. Genomic DNA was extracted from 5 µL of avian blood with the Qiagen DNeasy Tissue kit and re-suspended in 100 µL EB buffer (Qiagen, Crawley, West Sussex, UK). Molecular sexing was determined in a 50 µL PCR reaction using a Griffiths FAM-labelled P2 primer (FAM-5'-TCTGCATCGCTAAATCCTTT-3') and a non-labelled P8 primer (5'-CTCCCAAGGATGAGRAAYTG-3'). The final reaction conditions were as follows: 10 mM Tris-HCl (pH 9.0), 50 mM KCl and 0.1% Triton-X-100, 1.5 mM MgCl<sub>2</sub>, 200 µM each dNTP, 100 ng of each primer with 0.25 U of Taq polymerase and 250–500 ng genomic DNA per reaction.

PCR was performed on an MWG primus HT thermal cycler (MWG Biotech, London, UK) as follows: initial denaturing at 94 °C for 1 min 30 s, followed by 30 cycles of 48 °C for 45 s, 72 °C for 45 s and 94 °C for 30 s. Finally, this ended with 48 °C for 1 min, 72 °C for 5 min and then holding at 8 °C until further processing.

Ten microlitres of the PCR product was treated with 2 µL ExoSap (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) to remove excess primers and dNTP's before desalting through a Qiagen Dyex spin kit. Finally, before the samples were analysed on the MegaBace 1000 (Amersham Plc, Little Chalfont, Buckinghamshire, UK), ET550-R standard was added to each sample. Samples were denatured at 90 °C for 5 min and then quenched on ice. Samples were injected at 10 kV over 15 s before separation at 750 V for 75 min at 50 °C. The results were analysed using Genetic Profiler version 2.2 (Amersham Biosciences).

### Statistical analysis

We used principal components analysis [PCA – procedure PRINCOMP (SAS Institute Inc., 2002)] to investigate the most appropriate morphometric measurements that gave an



**Figure 1** Photographic plates showing the measurement of (a) bill-head, (b) bill-skull, (c) bill-feather, (d) bill depth and (e) gonyx from sooty terns *Onychoprion fuscatus* ringed on Ascension Island in 2005. Photographs courtesy of Alan Murray, RSPB.

indication of the structural (hereafter referred to as 'body') size of male and female sooty terns. The PCA was based on the correlation matrix among seven morphometric measurements and placed seven independent axes through the dataset to explain maximum variance in the dataset (Manly, 1994). We then used stepwise logistic regression (PROC LOGISTIC; SAS Institute Inc., 2002) on those morphometric measurements revealed by PCA to be the best indicators of body size to construct models that best sexed sooty terns based on biometric approaches. The sexes of birds predicted from these models were compared with their known sexes from molecular sexing techniques and the effectiveness of each model was assessed as the percentage of birds that were correctly sexed.

In order to compare the effectiveness of each of the models, we followed the methods outlined in van Franeker & ter Braak (1993). In brief, the logistic regression approach produces estimates for a constant ( $c$ ) and a regression coefficient ( $a$ ) for all ( $n$ ) characters ( $m$ ), that are significant terms in the logistic regression model that predicts the sex of the birds from which morphometrics are taken. The discriminant function ( $D$ ) from which birds were sexed takes

the form

$$D = a_1m_1 + a_2m_2 + \dots + a_nm_n + c \quad (1)$$

To compare the effectiveness of models in predicting the sex of the birds, discriminant scores ( $DS$ ) were calculated for each using each model by dropping  $c$  and dividing each coefficient  $a$  by  $a_1$  (van Franeker & ter Braak, 1993). The formula for calculating the discriminant score now takes the form

$$DS = m_1 + (a_2/a_1)m_2 + \dots + (a_n/a_1)m_n \quad (2)$$

The  $DS$  for all birds are then used to calculate the cut point in the frequency distribution of discriminant scores of individual terns and, according to whether the focal bird's  $DS$  fell below or above the cut point, it was sexed as either a female or a male, respectively. The cut point for each discriminant score formula based on each discriminant function model was simply calculated as the midpoint between the mean  $DS$  for males and females.

The reliability of the chosen discriminant function for assigning the sex of a given sooty tern was assessed using cross-validation (Fowler, Cohen & Jarvis, 1998) in which a

single bird was removed from the dataset, a discriminant function was derived for the remaining birds and this was used to classify the sex of the removed bird. This process was repeated for the entire dataset of birds and the percentage of correctly sexed birds was calculated.

## Results

Of 64 sooty terns from which blood was obtained for sexing using DNA methods, 63 birds were definitively sexed and subsequently used to investigate whether sex could be determined for birds solely on the basis of their external morphological measurements. In 20 birds, DNA extraction from blood samples was repeated once and in three birds DNA extraction was repeated twice before sex was confirmed unequivocally. Therefore, we had no doubt about the sex of the 63 focal terns (37 males and 26 females) in this study based upon molecular work.

### Body size indicators

Adult male and female sooty terns were not distinct morphologically. There were significant degrees of overlap for each of the seven morphometric measurements (Table 1), and, although males exhibited consistently larger morphometric measurements for all measurements (Table 1), no single morphometric measurement could be used to predict the sex of a sooty tern with any reliability.

Abandoning the univariate statistical approach, a PCA was carried out on the seven morphometric measurements taken from males and females. Principal components were extracted from a matrix of correlations among the morphometric measurements (Table 2), with the first eigenvector exhibiting an eigenvalue far in excess of one and explaining 40.5% of the total variation in the original correlation matrix (Table 3). Character loadings were all positive on the first principal component (PC1) axis, indicating that PC1 scores are predominantly a measure of sooty tern body

size. High degrees of correlation between PC1 and the morphometric measurements indicated that individual terns with a high value on the PC1 axis had long head dimensions. Correlations of PC1 with tarsus length and with wing length were significantly weaker (Table 3). The second principal component (PC2) axis is a balanced one, with wing and tarsus length loading positively and bill-feather loading negatively (Table 3). In morphometric studies such as this, PC2 is an axis reflecting body shape (Rising & Somers, 1989). Together, the first two eigenvectors explained 59.5% of the total variation. Figure 2 shows PC2 plotted against PC1 for male and female sooty terns and it shows that there is considerable variation along the body-size axis, with a preponderance of males being larger in structural size than females.

The PCA reduced the seven-dimensional space to a three-dimensional one with the loss of only 28.9% of the original variance. With all head measurements well correlated with PC1, will they allow effective differentiation between the sexes?

### Prediction of sooty tern sex from morphometric measurements

Stepwise discriminant analyses were carried out to incorporate five [all head measurements – equation (3)], six [all head measurements and wing length – equation (4)] and seven [all head measurements, wing length and tarsus length – equation (5)] morphometric measurements into a single discriminant function ( $D$ ). These models yielded the following equations:

$$D = (0.46 \times \text{bill-head}) + (2.92 \times \text{gonys}) - 60.56 \quad (3)$$

$$D = (0.48 \times \text{bill-head}) + (2.82 \times \text{gonys}) + (0.08 \times \text{wing length}) - 86.00 \quad (4)$$

**Table 1** Means, standard deviations (sd) and ranges of seven morphometric measurements of 37 male (M) and 26 female (F) sooty terns *Onychoprion fuscatus* ringed on Ascension Island in 2005

| Morphometric       | Sex | Mean  | sd   | Range     | $P$     |
|--------------------|-----|-------|------|-----------|---------|
| Wing length (mm)   | M   | 294.9 | 6.56 | 285–314   | <0.05   |
|                    | F   | 291.7 | 6.45 | 280–303   |         |
| Tarsus length (mm) | M   | 25.5  | 1.14 | 24–28     | <0.01   |
|                    | F   | 24.8  | 0.85 | 23–27     |         |
| Bill-head (mm)     | M   | 87.3  | 1.96 | 82.7–91.7 | <0.001  |
|                    | F   | 85.2  | 1.71 | 82.1–88.6 |         |
| Bill-skull (mm)    | M   | 50.8  | 2.08 | 42.2–55.4 | NS      |
|                    | F   | 49.7  | 2.18 | 45.9–53.2 |         |
| Bill-feather (mm)  | M   | 43.7  | 2.47 | 32.4–47.6 | NS      |
|                    | F   | 42.8  | 3.12 | 34.8–52.2 |         |
| Bill depth (mm)    | M   | 9.4   | 0.54 | 8.2–10.7  | <0.05   |
|                    | F   | 9.1   | 0.57 | 8.0–10.4  |         |
| Gonys (mm)         | M   | 7.6   | 0.38 | 6.7–8.6   | <0.0001 |
|                    | F   | 7.2   | 0.24 | 6.5–7.7   |         |

$P$ -values are from  $t$ -tests between the sexes for each morphometric measurement mean; NS, not significant.

**Table 2** Correlation coefficients among the seven morphometric measurements taken from 63 male and female sooty terns *Onychoprion fuscatus* ringed on Ascension Island in 2005

| Morphometric  | Wing length | Tarsus length | Bill-head         | Bill-skull        | Bill-feather      | Bill depth        | Gonys             |
|---------------|-------------|---------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Wing length   | 1.00        | 0.23          | 0.14              | -0.04             | -0.09             | 0.12              | 0.13              |
| Tarsus length |             | 1.00          | 0.26 <sup>a</sup> | 0.09              | -0.02             | 0.25 <sup>a</sup> | 0.12              |
| Bill-head     |             |               | 1.00              | 0.70 <sup>d</sup> | 0.45 <sup>c</sup> | 0.43 <sup>c</sup> | 0.56 <sup>d</sup> |
| Bill-skull    |             |               |                   | 1.00              | 0.48 <sup>d</sup> | 0.50 <sup>d</sup> | 0.36 <sup>b</sup> |
| Bill-feather  |             |               |                   |                   | 1.00              | 0.15              | 0.19              |
| Bill depth    |             |               |                   |                   |                   | 1.00              | 0.46 <sup>c</sup> |
| Gonys         |             |               |                   |                   |                   |                   | 1.00              |

<sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ ; <sup>d</sup> $P < 0.0001$ .

**Table 3** Character loadings on principal component axes (PC1, PC2 and PC3) for a principal component analysis (PCA) extracted from a correlation matrix of seven morphometric measurements taken from 63 sooty terns *Onychoprion fuscatus* ringed on Ascension Island in 2005

| Morphometric         | Principal component axis |       |       |
|----------------------|--------------------------|-------|-------|
|                      | PC1                      | PC2   | PC3   |
| Wing length          | 0.10                     | 0.62  | 0.15  |
| Tarsus length        | 0.19                     | 0.54  | 0.53  |
| Bill-head            | 0.52                     | -0.03 | 0.11  |
| Bill-skull           | 0.49                     | -0.25 | 0.08  |
| Bill-feather         | 0.33                     | -0.46 | 0.50  |
| Bill depth           | 0.41                     | 0.18  | -0.38 |
| Gonys                | 0.41                     | 0.11  | -0.53 |
| Eigenvalue           | 2.83                     | 1.33  | 0.82  |
| % variance explained | 40.5                     | 19.0  | 11.7  |

$$D = (0.40 \times \text{bill-head}) + (3.05 \times \text{gonys}) + (0.63 \times \text{tarsus length}) + (0.08 \times \text{wing length}) - 95.75 \quad (5)$$

Where  $D > 0$ , birds were classified as males and where  $D < 0$ , they were classified as females. Models (a), (b) and (c) reliably predicted the sex of the birds in 73.0, 77.8 and 74.6%, respectively, of birds. In order to compare discriminant function models, we calculated  $DS$  of birds using each model and compared the percentage of birds that fell correctly on either side of a cut point (Table 4). The most potent discriminant formula for predicting the sex of the birds from morphometric measurements was that based upon head measurements and wing length [equation (4)]. It categorized 50 of 63 of sooty terns correctly according to sex (Fig. 3). Cross-validation resulted in 69.8% of the omitted birds being correctly assigned as males or females based upon discriminant functions derived from all other birds in the dataset.

## Discussion

In our sample of sooty terns on Ascension Island, there was significant overlap of single morphometric measurements between the sexes (Table 1). Thus, none can be used solely to discriminate between the sexes. In this study, sooty terns on Ascension Island show morphometric measurements similar to conspecifics from the Dry Tortugas, Johnston Atoll and

Christmas Island populations (see Table 1 in Schreiber *et al.*, 2002) and are typical of other sternids (del Hoyo *et al.*, 1996). The PCA of morphometric measurements indicated that PC1 was a good measure of body size (Rising & Somers, 1989), with all character loadings on PC1 being positive (Table 3). PCA specifically revealed a high degree of correlation between the head measurements and PC1 (character loadings between 0.33 and 0.52), which suggested that they would be good indicators of the overall body size of sooty terns. In contrast, weak correlations of PC1 with tarsus length and wing length (character loadings of 0.19 and 0.10, respectively) indicated that they would be less reliable indicators of body size. Based upon the overall separation of individual PC1 scores for male and female terns, we conclude that males are slightly larger than females (Fig. 2). The high degree of overlap in these scores, however, suggests that PCA allows sex discrimination with only a moderate degree of reliability.

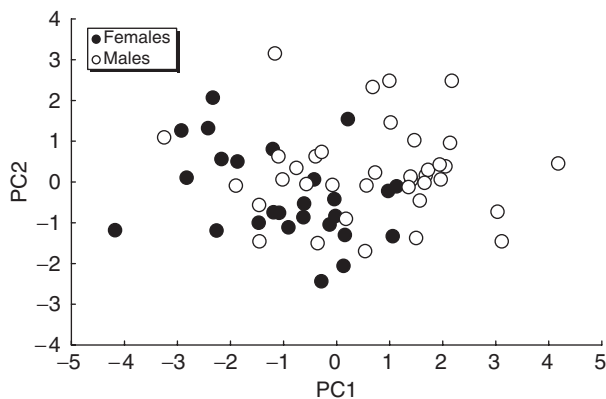
When morphometric measurements (head measurements, tarsus length and wing length) were incorporated into discriminant functions [equations (3–5)], between 73 and 77.8% of sooty terns were correctly sexed. The latter was based upon a discriminant analysis run on five head measurements and wing length. Of those birds that were incorrectly sexed, six of 26 (23.1%) were female and eight of 37 (21.6%) were male and, therefore, there appeared to be no sex bias in errors in the technique. We have demonstrated

that the sex of sooty terns on Ascension Island can be determined from morphometric measurements with some accuracy. Similar levels of accuracy have been found in other studies of tern species (e.g. Coulter, 1986; Chardine & Morris, 1989; Devlin, Diamond & Saunders, 2004), but, like the latter, the discriminatory power of such an approach was less than in similar studies of larids (e.g. Hanners & Patton, 1985; Mawhinney & Diamond, 1999). A check on the potency of the discriminant function was provided by cross-validation which demonstrated that the sex of a bird could be predicted from a discriminant function derived from the remaining birds in < 70% of the cases.

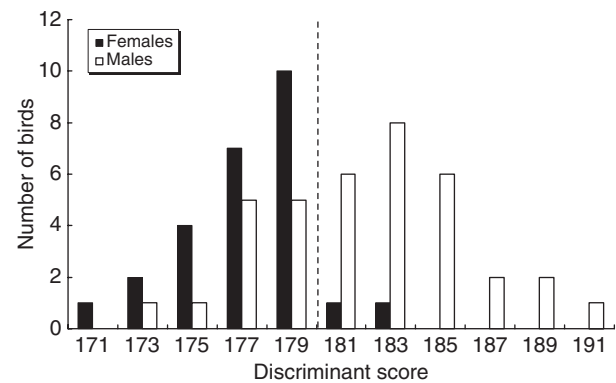
Our conclusions are similar to those of other studies that have suggested that for some avian taxa, it is impossible to sex birds with any certainty using morphometric measurements alone (e.g. Gunnarsson *et al.*, 2006 and references therein). We could have tried taking more morphometric measurements in addition to those in this study, but such measurements focus primarily on subtleties in the anatomical structure of the head (Redfern & Clark, 2001; Gosler, 2004), and the suite of head measurements that were taken were highly correlated with one another (Table 2). Therefore, we would not have improved the separation of the sexes based upon morphology as demonstrated by the PCA (Fig. 2). The best discriminant function model incorporated wing length, a measurement that was the least well correlated of all measurements with PC1 (Table 3) and one that is difficult to measure precisely in the field because the curved

wing requires flattening and the distal end of the second phalanx can be difficult to locate. Despite other studies also finding wing length to be a poor indicator of structural size in birds (as suggested by Rising & Somers, 1989; Reynolds, 1996), it is difficult to justify its replacement with tarsus length based upon the findings in the present study.

This is the first study to attempt to discriminate the sex of sooty terns from morphometric measurements alone and the lack of high discriminatory power is no surprise in a study of this taxon. Further research should involve increased collection of morphometric measurements from sooty terns on Ascension Island and in breeding colonies elsewhere in order to verify the findings of our study. This study and continued investigations on live birds in the field should be preferred over those using museum specimens because of differential inter-specimen shrinkage in the latter (see Winker, 1993, and references therein). We will continue to invest in approaches that try to sex sooty terns through means other than molecular analysis. Nevertheless, we have successfully refined techniques to make molecular approaches possible in this study, DNA can be readily obtained from tissues other than blood (e.g. feathers), and it will be essential to use molecular and morphometric approaches in concert if we are to develop the latter as a non-invasive sexing technique for this species. Rapid and reliable sexing of birds in the field would certainly allow more detailed study of their breeding behaviour on Ascension Island. This could be fundamental in the long-term conservation of this



**Figure 2** Plot of the first (PC1) and the second (PC2) principal components from a principal components analysis of seven morphometric measurements taken from 63 sooty terns *Onychoprion fuscata* ringed on Ascension Island in 2005.



**Figure 3** Frequency distributions of discriminant scores (*DS*) of sooty terns *Onychoprion fuscata* on Ascension Island based upon the following formula:  $DS = \text{Bill-head} + (5.886 \times \text{gonys}) + (0.173 \times \text{wing length})$ . The vertical dashed line represents the cut point, the left of which birds are categorized as females and the right of which as males. Birds were correctly categorized as male or female in 79.4% of the cases.

**Table 4** Formulae for the calculation of discriminant scores for sooty terns *Onychoprion fuscata* on Ascension Island

| Discriminant function model | Formula                                                                       | Cut point | % of birds correctly classified |
|-----------------------------|-------------------------------------------------------------------------------|-----------|---------------------------------|
| (3)                         | Bill-head + (6.397 × gonys)                                                   | 133.74    | 76.2                            |
| (4)                         | Bill-head + (5.886 × gonys) + (0.173 × wing length)                           | 180.59    | 79.4                            |
| (5)                         | Bill-head + (7.625 × gonys) + (1.575 × tarsus length) + (0.200 × wing length) | 241.17    | 77.8                            |

Formulae are calculated from discriminant function models (3–5) (see text for details). A discriminant score is calculated for each bird and if it falls above or below the cut point, the bird is classified as a male or a female, respectively.

species in investigations of differential breeding investment and of differential consequences of such investment between the sexes.

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