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SEX DETERMINATION USING CHD-1 GENE AND SEXUAL DIMORPHISM IN THE WHITE-NECKED THRUSH (*TURDUS ALBICOLLIS*) IN THE ATLANTIC FOREST AT ILHA GRANDE, RIO DE JANEIRO, SOUTHEASTERN BRAZIL

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Resumo. – Determinação do sexo pelo gene CHD-1 e dimorfismo sexual no Sabiá-de-coleira (*Turdus albicollis*) na Mata Atlântica da Ilha Grande, Sudeste do Brasil. – A determinação do sexo em aves é um aspecto importante em estudos ecológicos, comportamentais e conservacionistas. No entanto, muitas espécies não podem ter o sexo determinado pela plumagem ou características morfológicas. O Sabiá-de-coleira (*Turdus albicollis*) não tem dimorfismo sexual aparente. No presente estudo, os indivíduos dessa espécie foram capturados em redes ornitológicas e amostras de sangue foram coletadas entre Junho de 1998 e Maio de 1999 em área de Mata Atlântica na Ilha Grande, Rio de Janeiro, Sudeste do Brasil. O sexo de um total de 74 indivíduos foi determinado utilizando-se o gene CHD-1 como marcador. Deste total, 52 foram identificados como machos e 22 como fêmeas, resultando numa razão sexual (2,4:1) desviada para machos. Além disso, modelos de regressão logística foram utilizados para determinar diferenças na massa e características morfológicas entre os sexos. Asa corda e massa corporal foram as características morfológicas utilizadas para distinguir machos de fêmeas. As fêmeas foram mais pesadas e tiveram asas mais curtas que os machos.

Abstract. – Sex determination in birds is an important aspect for behavioral ecology, population and conservation studies. However, many bird species cannot be sexed by their plumage and morphology. The White-necked Thrush (*Turdus albicollis*) has apparently no sexual dimorphism. Individuals of this species were captured and their blood samples taken from June 1998 to May 1999 in Atlantic forest at Ilha Grande, Rio de Janeiro, Southeast Brazil. A total of 74 individuals of White-necked Thrush were sexed from blood samples using a molecular marker (CHD-1 gene). From this total, 52 were identified as males

and 22 as females, resulting in a sex ratio biased toward males (2.4:1). In addition, logistic regression models were used to evaluate differences in body mass and morphological traits between sexes. Wing length (flattened chord) and body mass were the best traits to distinguish males from females. Females were heavier and had significantly shorter wing length than males. *Accepted 7 January 2003.*

Key words: Atlantic forest, CHD-1 gene, sex determination, sex ratio, sexual dimorphism, thrushes, White-necked Thrush, *Turdus albicollis*.

INTRODUCTION

Many bird species cannot be sexed by any morphological trait, whereas others are dimorphic only when adult (Ellegren & Sheldon 1996, Griffiths *et al.* 1996, Khan *et al.* 1998). Sexing birds is of most importance for conservation breeding programs (Ellegren & Sheldon 1996, Miyaki *et al.* 1998), identification of morphological cues (King & Griffiths 1994, Burns 1998), behavioral ecology and evolutionary biology (Ellegren 1996, Lessells & Mateman 1998).

There are many techniques to sex birds such as laparoscopy, karyotyping (Basrur *et al.* 1998), fecal steroid analysis, DNA fingerprinting and molecular techniques based on the CHD-1 gene (Griffiths *et al.* 1996, Ellegren 1996, Kahn *et al.* 1998, Miyaki *et al.* 1998). The CHD-1 gene has been used successfully in many bird species (Griffiths *et al.* 1996, Madsen 1997, Griffiths *et al.* 1998, Lens *et al.* 1998, Lessells & Mateman 1998, Miyaki *et al.* 1998, Sheldon *et al.* 1998) because it is present throughout the class Aves, with the exception of ratites (Griffiths & Tiwari 1996).

Females are the heterogametic sex (ZW) in birds whereas males are homogametic (ZZ) (De Lucca 1978). The W chromosome is specific to the female and gender determination is done based on the absence or presence of the W-linked marker (Griffiths *et al.* 1996). Using PCR techniques to amplify the sex-linked CHD-1 gene, one can easily identify the females, as they will show two bands on agarose or acrylamide gels.

Fisher's theory (1930) predicts that, in

sexually reproducing species, the sex ratio should not deviate from 1:1. However, in many species, the sex ratio is skewed towards one sex (Hunt *et al.* 1980, Ricklefs 1997, Lens *et al.* 1998). A biased sex ratio can occur, among other reasons, due to one sex requiring different degrees of parental investment (Teather 1992), differential nestling mortality (Griffiths 1992), maternal condition (Trivers & Willard 1973), in relation to resources availability (Wiebe & Bortolotti 1992, Komdeur 1996, Bradbury & Blakey, 1998), or different evolutionary benefits from the production of one sex over the other (Komdeur 1996, see Trewick 1997).

Individuals from different sexes might have different life-history characteristics that could affect population dynamics (Lessells & Mateman 1998). For instance, in birds, females usually disperse whereas males stay in their natal territories (Woolfenden & Fitzpatrick 1978). Another example is that in some species, juveniles or even adults of one of the sexes help during the breeding season (Emlen 1991, Alves 1990, Castiglioni 1998).

The White-necked Thrush (*Turdus albicollis*) has no external sexual dimorphism (Sick 1997) and it is the most captured species in the understory of Atlantic forest in the area where the study was conducted, Ilha Grande, Rio de Janeiro State, southeastern Brazil (Alves 2001). The present study aimed to answer the following questions: 1) Does the sex ratio of White-necked Thrushes differ from the expected (1:1)? 2) Is there any morphometric sexual dimorphism in the morphological traits detectable by morphometry? 3)

If so, is there any morphological trait that could be used in the field to sex individuals of this species?

STUDY AREA AND METHODS

Study area. The work was carried out in an area of Atlantic forest near Vila Dois Rios, Ilha Grande (23°11'S, 44°12'W), the biggest island (19,000 ha) of Rio de Janeiro State, southeastern Brazil. Ilha Grande is an island covered by Atlantic rain forest in different successional stages due to prior agriculture. The climate is warm and humid, with mean annual temperature of 23°C (T. Moulton pers. com.). The annual rainfall is 2200 mm (Data obtained from Central Nuclear Almirante Álvaro Alberto meteorological center - CNAAA and sent by environmental meteorological management of Eletrobras Termonuclear S. A. - Eletronuclear).

Field methods. Birds were captured using mist nets twice a month between June 1998 and May 1999. All individuals caught were individually marked with metal and colored rings, weighed (with a Pesola spring balance to the nearest 0.1 g), measured and released. Morphological measurements of tarsus length, bill (length and width), bill base (length and width) and HB (distance between distal part of the head and tip of the bill), were taken with dial calipers to the nearest 0.1 mm. Wing flattened chord and total length of the body were taken using ornithological rules (mm). Blood samples (approx. 100 µl each) from 74 individuals were taken by simple puncture of the tarsal or brachial veins and stored in tubes with absolute ethanol under refrigeration. Five recaptured individuals, that had blood samples taken previously, were found dead in mist nets. These individuals were sexed by dissection (checking the gonads) as being three males and two females. The amplification of the CHD-1 genes from these individu-

als confirmed their previously determined sexes.

Laboratory methods. Genomic DNA was extracted with 50nM NaOH following Khatib & Gruenbaum (1996). DNA concentration was checked in 0.8% agarose gels and the CHD-1 gene was PCR amplified using primers P2 and P8 (Griffiths *et al.* 1998). PCR was performed in a 10 µl total volume, consisting of 4.9 µl of MilliQ water, 1.0 µl of buffer (Pharmacia), 2.0 µl of each dNTP, 10 pmoles of each primer (P2: 5'-TCTG-CATCGCTAAATCCTTT- 3' and P8: 5'-CTCCCAAGGATGAGRAAYTG-3' Griffiths *et al.* 1998), 1.0 µl of DNA and 0.5 U of Taq Polymerase (Pharmacia). A cycle of 95°C for 1 min, 45°C for 30 s, 72°C for 30 s was repeated 35 times and followed by a final extension step at 72°C for 7 min. The products were electrophoresed in 3% agarose gels.

Statistical analysis. To evaluate whether the sex ratio of White-necked Thrushes differs from 1:1, we used a one-sample test of proportion. To check any statistical difference between males and females in morphology, a two-sample independent Student's t-test with equal variances was performed. Traits that differed between sexes (wing length, bill width and weight, Student t-test) were analyzed by logistic regression models, where females were coded as 1 and males as 0. Logistic regression models' results are reported in the scale of chances. Here, the chances are defined as the ratio between the probabilities of female and male. In order to assess how logistic regression predicts sex, from the models' results, we classified as females and males, those individuals with a probability of being a female greater than and male less than 0.5, respectively. Four logistic models were fitted, namely model I (sex = weight), model II (sex = wing length), model III (sex = weight + wing length) and model IV (sex = weight + wing length + bill width). The statistical analyses

TABLE 1. Means (95% confidence interval), sample sizes [n] for body mass, wing length and bill width for male and female White-necked Thrushes, sexed by CHD-1 gene, compared by a two sample Student's t-test with equal variances ($t_{\alpha - a}$, degrees of freedom).

Traits	Mean (95% confidence interval)		Student's t-test (df)	P value
	Males	Females		
Body mass (g)	62.37 (60.97 - 63.78) [49]	67.55 (65.12 - 69.97) [21]	-3.95 (68)	< 0.001
Wing length (mm)	116.62 (115.86 - 117.38) [50]	112.76 (111.48 - 114.05) [21]	5.45 (69)	< 0.001
Bill width (mm)	12.69 (12.44 - 12.94) [49]	13.19 (12.79 - 13.59) [22]	-2.22 (69)	< 0.05

were performed using STATA 7.0 (2000 – STATA Corporation, College Station, Texas).

RESULTS

The five dissected birds had their sexes (three males and two females) confirmed by the CHD-1 gene amplification. A total of 74 individuals were sexed using the CHD-1 gene technique. From these, 52 were males (15 captured during the breeding season from October to January) and 22 females (10 captured during the breeding season). The sex ratio of White-necked Thrushes of Ilha Grande was biased toward males (2.4:1) and differed from the expected 1:1 (one-sample test of proportion, $Z = -3.48$, $P < 0.001$).

To test the hypothesis that sex ratio differs between breeding and non-breeding birds, we performed a two-sample test of proportions. It was found that the population proportion of females:males equal to 1:1 is rejected in months when birds are not breeding ($Z = -3.571$, $P < 0.001$). However, during the breeding season, the hypothesis of a proportion of captured males and females equal to 1:1 could not be rejected ($Z = -1.000$, $P > 0.30$).

Wing length, bill width and body mass differed statistically between males and females (Table 1). Females are heavier, have wider bills and shorter wings than males. Females also have greater variance in body

mass during the breeding season than in the non-breeding season (test of variances in and out of the breeding season, $F = 4.035$, $P < 0.05$).

Table 2 shows the results from the logistic regression models. It results that, for each variable included, the model fit improves. However, concerning the number of individuals correctly classified, model III provides the best results, with 58 of 67 individuals (86.56%) sexed correctly. In models IV, II and I, the proportions were 86.15%, 80.3% and 72.86%, respectively.

In Model I, for each gram of weight increase, there is a 21.3% increase in the chances of an individual being classified as female. Body mass is not the best predictor of sex as only 33.3% (7/21) of females and 89.8% of males (44/49) were correctly sexed.

Concerning wing length (Model II), for each millimeter, there is a 37.3% decrease in the chances of an individual being classified as female. In that model, 57.14% of females and 90% of males were correctly classified.

In model III, among two individuals of equal wing lengths but of weights differing by one gram, the heavier one has a 38.4% increase in the chances of being female. Similarly, for two individuals of equal weights, but with wing lengths differing by one millimeter, the one with longer wings has a 50% decrease in the chances of being a female. Seventy percent of females and 93.61% of

TABLE 2. Chance ratio (SE), *P*-value, model-fit statistics and number of individuals correctly classified for each model.

Variables	Model I	Model II	Model III	Model IV
Weight (g)	1.213 (0.072) <i>P</i> = 0.001	—	1.384 (0.142) <i>P</i> < 0.010	1.323 (0.141) <i>P</i> < 0.010
Wing length (mm)	—	0.627 (0.073) <i>P</i> < 0.001	0.505 (0.097) <i>P</i> < 0.001	0.416 (0.104) <i>P</i> < 0.001
Bill width (mm)	—	—	—	4.564 (2.768) <i>P</i> < 0.050
Sample size (n)	70	71	67	65
Loglikelihood	-35.9681	-31.4277	-20.9699	-17.1638
Pseudo R-squared	0.1588	0.2711	0.4866	0.5722
Correct female	7/21	12/21	14/20	15/20
Correct male	44/49	45/50	44/47	41/45
Correct total	51/70	57/71	58/67	56/65

¹Ratio between the probabilities of female and male.

males were classified correctly according to that model.

In the last model, results can be interpreted likewise. Figure 1 shows the classification line (model III) for males and females. Individuals whose weight and wing length coordinates are under the line are classified as males, otherwise as females.

DISCUSSION

The sex ratio of the White-necked Thrush population studied departed from the expected (1:1). This could be due to the period when sampling was done (August to January), dispersion patterns or even mating system.

The White-necked Thrush, at Ilha Grande, was more abundant from August to October 1998 when males were probably establishing their territories, and at the beginning of the breeding period, when the probability of their being caught in mist nets increased. In Brazil, the breeding season runs from September to January (Sick 1997). However, we captured individuals at Ilha Grande

with brood patches from October to January. Ricklefs (1997) observed the same pattern for the Creamy-bellied Thrush (*Turdus amaurochalinus*). He also found a slight bias in favor of males throughout the year, but more significant during the pre-breeding and breeding periods. In contrast, we captured more male individuals than females during the non-breeding season. Our results suggest that, outside the breeding period, females could disperse out of the area whereas males stay in their territories as proposed by Woolffenden & Fitzpatrick (1978). There was no difference in the proportions of male and female White-necked Thrushes during the breeding season, as expected if monogamy is the mating system. The biased sex ratio found in the present study, considering the whole sample, could also be a characteristic of the *Turdus* genus, since Ricklefs (1997) and Lens *et al.* (1998) also found more males than females for species of this genus.

We also observed that females were heavier, had shorter wings and wider bills than males. Females were heavier, probably because they were to lay their eggs and accu-

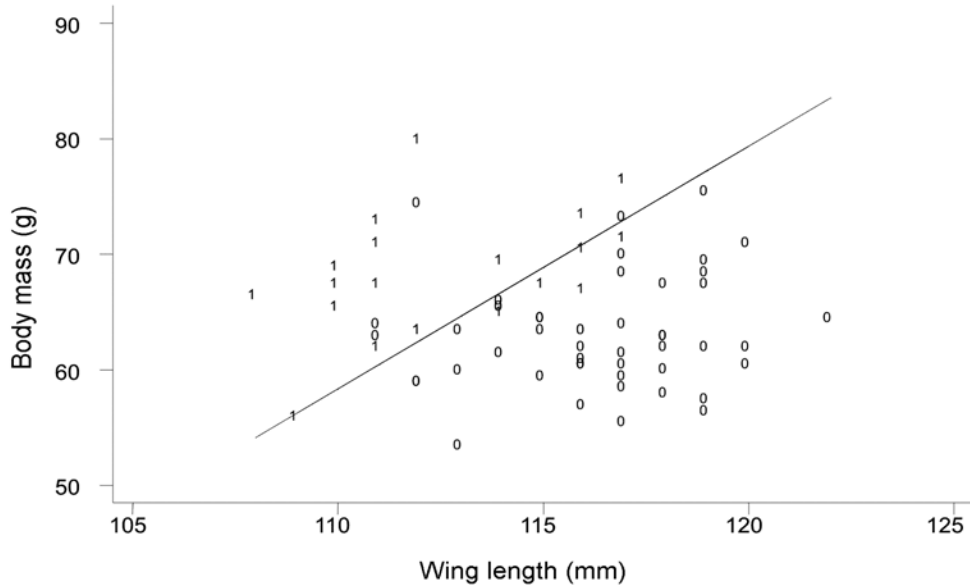


FIG. 1. Body mass and wing length relationship of White-necked Thrushes resulting from Model III. Individuals whose pair of coordinates (body mass and wing length) lies below the line are classified as males, those above it as females. Actual males and females are represented, respectively with 0's and 1's.

mulate fat reserves before breeding. Bill width could be associated with differences in diet. However, we have no data to confirm this hypothesis, and there are no studies regarding the diet of the White-necked Thrush in literature.

The fact that males have longer wings could be associated with parental care. Male White-necked Thrushes could behave similarly to male Blackbirds (*Turdus merula*) which fly more than females once they help their partner feeding the brood while females stay in the nest (Hill *et al.* 1999). Behavioral studies are needed to test this hypothesis. Given the poor knowledge on the biology of the White-necked Thrush, we can only speculate on what might have caused the results we found.

The results of this study show that White-necked Thrushes can be sexed using the CHD-1 gene technique. Besides, a logistic

model using body mass (g) and wing length (mm) can help sex determination in the field.

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