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SEXING MONOCHROMATIC BIRDS IN THE FIELD: CRYPTIC SEXUAL SIZE DIMORPHISM IN BUFF-BREASTED WRENS (*THRYOTHORUS LEUCOTIS*)

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Resumen. – Determinación del sexo de aves monocromáticas en el campo: dimorfismo sexual críptico en *Thryothorus leucotis*. – Trabajar en el Neotrópico trae consigo desafíos especiales para los interesados en determinar el sexo de las aves residentes, pues muchas especies son monocromáticas y aparentemente monomórficas, y los individuos de ambos sexos pueden cantar y defender territorios. En este estudio, comprobamos que es posible determinar confiablemente el sexo de individuos de la especie *Thryothorus leucotis* utilizando medidas sencillas del tamaño corporal. Comparamos el tamaño de machos y hembras conocidos por medio de pruebas *t* pareadas y análisis de regresión logística. A nivel de la población, los sexos se superpusieron en todas las mediciones, aunque en promedio los machos fueron más grandes que las hembras. Al considerar parejas individuales, los machos fueron significativamente más grandes y más pesados que las hembras. El mejor modelo logístico indicó que la medida de la cuerda alar fue suficiente para discriminar entre machos y hembras, clasificando correctamente al 95.5% de los individuos. Tres individuos (dos machos y una hembra) fueron clasificados incorrectamente por el análisis de regresión, pero pudimos determinar su sexo ya que los machos fueron más grandes y la hembra más pequeña que sus respectivas parejas en todas las mediciones. Estos resultados indican que *T. leucotis*, una especie monocromática, presenta dimorfismo sexual críptico en tamaño. Además, nuestros resultados demuestran que es posible determinar el sexo de los individuos con base en mediciones sencillas. Es necesario realizar más estudios en otras especies monocromáticas y presumiblemente monomórficas para determinar la utilidad general de este método.

Abstract. – Working in the Neotropics poses special challenges for those interested in sexing resident bird species. Many species are monochromatic and apparently monomorphic, and both sexes may sing and defend territories. We tested whether monochromatic Buff-breasted Wrens (*Thryothorus leucotis*) could be reliably sexed using simple body size measurements. We compared the size of known males and females using paired t-tests and logistic regression analysis (LRA). At the level of the population, the sexes overlapped in all mensural characteristics; however, on average males were larger than females. When considering individual pairs, males were significantly larger and heavier than their mates. The best model from LRA indicated that measurement of wing chord alone was sufficient to discriminate between males and females, with 95.5% of individuals correctly classified. Three individuals were incorrectly classified by LRA (two males and one female), but could be sexed by comparing them with their mates, as the males were larger

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and the female smaller than their partners for all measurements. These results show that monochromatic Buff-breasted Wrens are cryptically sexual dimorphic in size. Moreover, they highlight that it is possible to determine sex based on simple mensural characteristics. Additional research is needed on other monochromatic and presumed monomorphic species to determine the general utility of this approach. *Accepted 19 April 2006*.

Key words: Body mass, body size, Buff-breasted wren, Panama, sexing technique, sexual size dimorphism, *Thryothorus leucotis*.

INTRODUCTION

Working in the Neotropics poses special challenges for those interested in sexing birds, as researchers lack reliable methods for identifying the sex of individuals of monochromatic species. Such difficulties are compounded by the behavior of many resident species as both sexes may defend territories and both sexes may sing, two features that can be used to identify males in monochromatic temperate species. Sex identification is critical for behavioral studies, but may also be important for studies of population dynamics and community ecology. Some studies have used laparotomy, a procedure in which a small incision is made into the body cavity and the gonads are directly examined (Wingfield & Farner 1976), to assign sex. However, such invasive procedures may not be necessary when careful morphological measurements are taken. Using museum specimens collected from Veracruz, Mexico, Winker *et al.* (1994, 1996) found that on average males of six monochromatic species were larger than females, although some overlap occurred in all characters measured, and that linear discriminant function analysis (DFA) correctly classified the sex of 82–97% of the specimens. Studies on temperate-zone species have had similar success in determining sex in monochromatic species by analyzing mensural characteristics using DFA (e.g., Dunnet & Anderson 1961, Maron & Myers 1984, Hanners & Patton 1985, Sweeney & Tatner 1996, Weicker & Winker 2002).

In this paper, we made body size and mass comparisons within pairs and performed logical regression analysis (LRA) to assign sex in Buff-breasted Wrens (*Thryothorus leucotis*), a monochromatic species resident in the Neotropics in which males and females overlap in size (Wetmore *et al.* 1984). By comparing males and females within pairs, we assessed whether significant sex differences exist at the level of individual pairs, important for researchers studying individual-level questions. We performed LRA to identify those measurements best able to discriminate between males and females. Our study demonstrates that, through the combination of within-pair comparisons and logistic regression analysis, all individuals in our study population could be correctly classified by sex. Importantly, only simple and standard field measurements need be taken for sex assignment by size. This is critical for research on individual-level studies of banded populations, and important for population- and community-level banding programs where it is not practical to use behavioral cues to sex birds after release.

METHODS

Buff-breasted Wrens are found in secondary tropical forests and woodlands from central Panama to northern South America (Ridgely & Tudor 1989). They glean insects from live and dead substrates, and are paired and hold territories year-round. In our study site, birds are found in groups of 2–5 individuals, with offspring remaining on natal territories for several months after independence to over a

TABLE 1. Univariate measurements of body size (mm) and mass (g) of male and female Buff-breasted Wrens, and an index of sexual size dimorphism, cal-culated by dividing mean value for males by the mean value for females. Results of paired t-tests comparing body size and body mass of males and females within pairs.

	Males			Females			Dimorphism	Paired t
	Mean ± SD	Range (n)	CV	Mean ± SD	Range (n)	CV		
Wing chord	62.5 ± 1.7	58.8–65.0 (31)	2.7	58 2 ± 1.4	54.0–61.0 (37)	2.4	1.07	16.760*
Tail length	46.9 ± 2.4	42.0–51.0 (30)	5.0	44.3 ± 2.2	38.0–49.0 (37)	5.0	1.06	7.478*
Bill width	3.4 ± 0.2	3.0–3.7 (31)	4.6	3.3 ± 0.1	3.0–3.6 (37)	3.9	1.03	5.844*
Bill depth	3.6 ± 0.1	3.3–3.8 (31)	3.4	3.4 ± 0.2	2.5–3.7 (37)	6.3	1.06	7.084*
Bill length	12.2 ± 0.6	10.8–13.4 (31)	5.2	11.4 ± 0.5	10.6–12.5 (37)	4.0	1.07	7.384*
Tarsus	24.6 ± 0.7	22.8–26.0 (31)	3.1	23.5 ± 0.6	22.1–24.7 (36)	2.7	1.05	8.972*
Mass	20.2 ± 1.0	18.5–22.8 (31)	5.2	17.9 ± 0.8	16.0–19.5 (35)	4.8	1.13	11.826*

*$P < 0.0001$. The minimum table-wide sequential Bonferroni-corrected significance level is 0.007.

year in some cases (Gill 2004). Buff-breasted Wrens give antiphonal duets with unique male and female parts (Farabaugh 1983; Gill pers. observ.), making it possible to distinguish between the sexes based solely on song. Following banding (see below), we observed all pairs to determine the individuals that gave the male or female sections of duets (Farabaugh 1983). During breeding, we made additional observations of egg-laying (Gill 2003a) and incubation (Gill unpubl.) to confirm the sex assignments. We also made observations of family groups to distinguish between pairs and their offspring in cases where offspring were banded after they retained adult characteristics (i.e., ossified skull and rust-colored iris, Wetmore *et al.* 1984).

From 1997–1999, we monitored a population of Buff-breasted Wrens in a small secondary forest in Gamboa, Republic of Panama (09°07′N, 79°42′W), as part of an intensive study of their breeding biology and behavior. We caught unbanded birds using mist nets and playbacks of duets. All birds were caught in the non-breeding season or in non-breeding condition, and thus, could not be sexed by the presence of brood patches (only females incubate, Gill pers. observ.) or cloacal protuberances. To facilitate individual recognition of wrens, we banded each individual with a unique combination of three colored bands and one aluminum band. During the course of the study, we banded 120 individuals: 61 adults, 15 retained offspring with adult characteristics, and 40 nestlings or recent fledglings.

While handling the birds, we made seven measurements of body size for statistical analyses: lengths (mm) of unflattened wing chord, tail, bill (from the tip of the bill to the distal edge of the nostril), and tarsus; bill width (mm) measured at the distal edge of the nostril; bill depth (mm) measured at the distal edge of the nostril; and body mass (g). Tail length, tarsus length, and mass were not mea-

sured on all individuals, resulting in slightly smaller sample sizes for these characteristics (Table 1). Measurements were made with vernier calipers to the nearest 0.1 mm, with the exception of wing chord and tail measured to the nearest 0.5 mm with a stopped ruler. Body mass was measured to the nearest 0.5 g using a Pesola 30-g scale. SAG measured all individuals. We calculated the mean ± SE for each characteristic, from which we calculated an index of dimorphism, defined as mean male body size or body mass divided by mean female body size or body mass, respectively (Winker *et al.* 1994).

The first objective of our study was to determine whether significant differences in body size and mass existed between males and females that were paired, and whether such size differences could be used to distinguish sex within pairs. The ability to reliably assign the sex of paired birds would facilitate studies that examine sex differences in behavior, such as singing and duetting, mating guarding, and nestling provisioning. To examine this possibility, we performed paired t-tests on body size and mass measurements of males and females by pair. Over the three years of study, 31 males and 37 females combined to produce 51 pairs. Some individuals paired with more than one individual (range: 1–6), accounting for the discrepancy between the number of males and females banded and the number of pairs produced. All pairs were included in the analysis with the result that some individuals were analyzed more than once; however, comparisons were made only within pairs. Because seven mensural characteristics were analyzed, we calculated sequential Bonferroni corrections on significance levels to control for group-wide Type 1 errors (Rice 1989).

Mensural characteristics, to be useful in distinguishing between males and females, must be reliably measured in the field. We examined the reliability of body size and body

mass measurements by making repeated measurements on individuals caught more than once and by estimating repeatability, *R*. Repeatability describes the consistency of repeated measurements, and varies between 0 and 1 with values closer to 1 indicating that measurements are highly repeatable (Krebs 1989). Repeatability is calculated by considering the variance among groups and variance within groups from a one-way ANOVA, with the significance of repeatability determined by the *F*-value of the ANOVA (Krebs 1989). We did not measure all characteristics every time each individual was captured, resulting in unequal sample sizes among the measured traits.

Juveniles as young as 8 months old pair and defend territories on our study area (Gill unpubl.); therefore, estimates of both within-sex variation in size and repeatability could be affected by the inclusion of unidentified juveniles in our sample. To examine the potential effect of growth, we compared body size and mass of known-aged juveniles to their same-sex parent using paired t-tests. We compared juveniles with their parents, rather than with the larger population, to ensure that only known-aged birds were included in the analysis. We banded 15 juveniles less than 1-year old that still resided on their natal territories. Exact age of most juveniles was unknown, but we estimated their ages as 6–9 months old based on mean fledgling date (Gill unpubl.) and date of banding. To assign sex to juveniles, we used vocalizations (n = 13) as well subsequent pairing by juveniles to known-sex birds (n = 9). We could not assign sex to two juveniles, which we then excluded from the analysis.

We used LRA to analyze differences in morphological measurements between sexes (Hosmer & Lemeshow 1989). Because the magnitude of regression coefficients may be influenced by correlations between variables, we calculated Spearman's rank correlations among continuous independent variables and excluded one of any pair of variables that had a Spearman's rank correlation coefficient > 0.4 before performing the LRA. In this study, the variable with the highest repeatability was retained, but this decision could be based on the relative biological importance or ease of measurement of each variable, or some other relevant criterion. We performed LRAs containing variables that were not highly correlated, and compared the resulting models based on their ability to correctly classify individuals to the correct sex. The LRA provided jack-knifed estimates of the correct classification rate for each category of the dependent variable, with a cutoff probability of 0.5. Values less than 0.5 indicated individuals that were female, and those higher than 0.5 were males. We examined model fit using the Hosmer-Lemeshow Goodness-of-fit test.

RESULTS

Male and female Buff-breasted Wrens showed considerable overlap in all of the size and mass characteristics measured (Table 1). However, on average males were slightly larger and heavier than females, resulting in small, male-biased indices of dimorphism. Indices of dimorphism ranged from 1.03 male to female size in bill width, to 1.13 male to female mass. Wing chord and tarsus length were the least variable measurements for both males and females (CV = 2.4–3.1).

At the level of individual pairs, males were significantly larger and heavier than their mates for all characteristics measured (paired t-tests, $P < 0.0001$). Thus, even though mean body size and body mass measurements show overlap between the sexes at the level of the population, within-pair comparisons showed significant sex differences which could be used in assigning sex. There were no significant correlations between paired males and

TABLE 2. Body size (mm) and body mass (g) measurements of juvenile (< 1-year old) male and female Buff-breasted Wrens, and results of paired t-tests comparing size and mass of juveniles with their parents. The minimum table-wide sequential Bonferroni-corrected significance level is 0.007. See Table 1 for body size and body mass measurements of adults.

	Males			Females			Paired t	*P*
	Mean ± SD	Range (n)	CV	Mean ± SD	Range (n)	CV		
Wing chord	61.5 ± 0.4	60.0–63.5 (9)	2.0	54.3 ± 1.9	49.0–58.0 (4)	7.1	3.480	0.0045
Tail length	45.2 ± 0.8	41.0–49.0 (9)	5.4	44.3 ± 1.0	42.0–46.0 (4)	5.1	0.302	ns
Bill width	3.3 ± 0.1	3.1–3.6 (9)	5.7	3.2 ± 0.1	3.1–3.2 (4)	1.8	1.537	ns
Bill depth	3.5 ± 0.6	3.3–3.8 (9)	4.8	3.1 ± 0.2	2.5–3.4 (4)	13.2	1.865	ns
Bill length	12.0 ± 0.2	11.2–12.9 (9)	4.4	11.3 ± 0.2	10.9–11.7 (4)	2.9	0.533	ns
Tarsus	24.5 ± 0.2	23.6–26 (9)	2.7	23.3 ± 0.3	22.8–24.1 (4)	2.6	0.579	ns
Mass	19.4 ± 0.4	17.0–20.5 (9)	5.8	17.5 ± 0.3	17.0–18.0 (3)	2.9	1.274	ns

TABLE 3. Model fit and correct classification rates for logistic regression models on Buff-breasted Wren mensural characteristics.

Independent variables	Hosmer-Lemeshow goodness-of-fit		% correctly classified		
	χ^2	P	Males	Females	Overall
Tail and tarsus	39.66	< 0.0001	86.67	88.57	87.69
Tail and bill depth	0.96	0.9954	83.33	86.11	84.85
Tail and bill width	7.92	0.3401	76.67	77.78	77.27
Wing chord	4.71	0.6953	93.55	97.22	95.52

females in any univariate measure ($r_{46–49}$ < 0.20, P > 0.2 for all analyses).

During the study, 27 individuals (16 males and 11 females) were recaptured 1–3 times (mean ± SE = 1.2 ± 0.4) from which repeated measurements were taken. The mean ± SE time between successive measurements on the same individual was 292.8 ± 205.6 days (range: 16–738 days). Repeatability scores varied considerably among body size and body mass measurements, ranging from 0.567 for tail length to 0.894 for wing chord. All measurements were significantly highly repeatable at P < 0.001. Mean differences between successive measurements varied from 0.03 mm for bill width to 0.70 mm for tail length, differences which are orders of magnitude smaller than those between mean male and female measurements.

We examined 13 pairs of juveniles and their same-sex parents to examine possible age effects on body size and body mass. Several characteristics of juveniles, in particular male and female tail length, male body mass, female wing chord, and female bill depth, were moderately to highly variable among individuals (CV = 5.1–13.2), possibly reflecting different stages of growth. On average, juveniles were smaller than adults in all morphological characteristics (Tables 1 and 2); however, differences were significant for wing chord only (paired t-test, t = 3.480, P = 0.0045).

Virtually all morphological measurements were highly positively correlated. Only the following pairs of measures were not highly correlated: tail length and bill width (Spearman rank correlations, r = 0.183), tail length and bill depth (r = 0.283), and tail length and tarsus (r = 0.390). Bill width, bill depth, and tarsus were all highly correlated (r > 0.55). Therefore, we performed three LRAs with the three pairs of weakly correlated measures as the independent variables and compared model performance. We performed an additional LRA using wing chord alone, as all other variables were highly correlated with this variable, it is easy to measure in the field, and because its repeatability was very high (R = 0.894).

The logistic regression models correctly classified 77–96% of birds to the correct sex (overall percent correctly classified; Table 3). Model fit was good for all models based on the Hosmer-Lemeshow goodness-of-fit test, with the exception of the tail length-tarsus length model. In terms of classification accuracy, the model containing only wing chord correctly classified the greatest number of individuals (95.5% overall). Based on this model, the probability that a bird is a male can be calculated using the following equation: $P(\text{male}) = 1 / 1 + e^{-(-116.94 + 1.94 (\text{wing chord}))}$, where a P value > 0.5 indicates the individual is likely to be a male. Calculating predicted probabilities for all individuals in the popula-
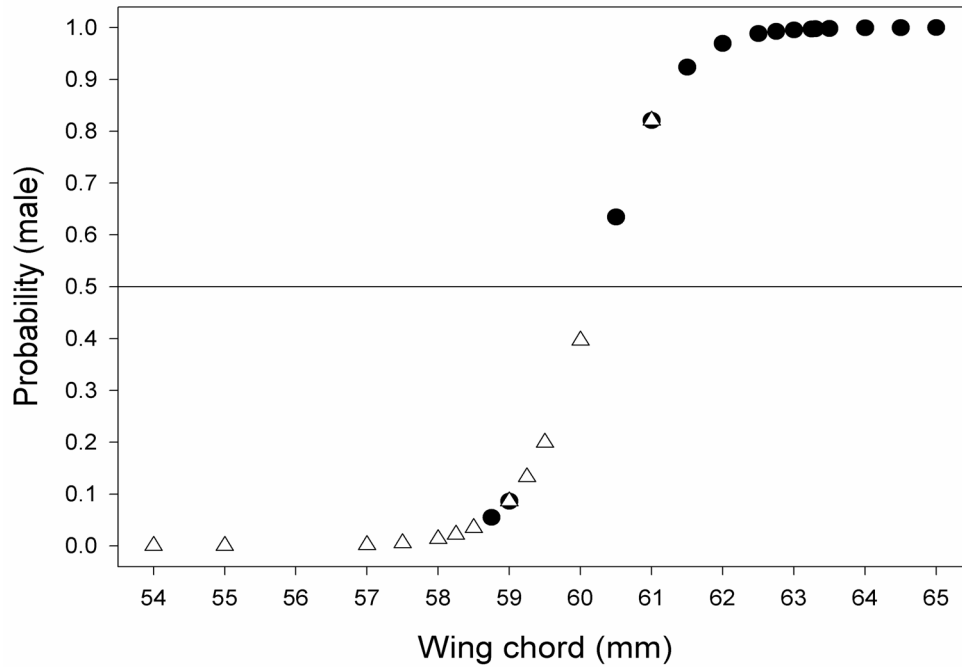
FIG. 1. The probability of classifying individual Buff-breasted Wrens as male based on wing chord length. Individuals above a probability (male) of 0.05 (indicated by the line) were classified by the logistic regression analysis as males, those below 0.05 were classified as females. Male Buff-breasted Wrens are indicated by the closed circles, and females are indicated by the open triangles.

tion using this equation provided almost complete separation between males and females (Fig. 1). Most females had a wing chord of less than 58.5 mm, whereas most males had a wing chord of greater than 61.5 mm. The 3 mm overlap in wing chord between the sexes contained a total of nine individuals (13.2% of 68 individuals), three (two males and one female) of which were incorrectly classified, indicating that model fit was not perfect.

DISCUSSION

Our analyses of mensural characteristics in Buff-breasted Wrens highlight two methods of identifying males and females in the field. First, we found that despite overlap between the sexes in body size and body mass at the level of the population, within pairs males were larger and heavier than females. In practice, this means that when both members of a pair have been caught and measured, sex can be assigned simply and reliably on the relative size of paired individuals.

Second, from the pooled sample of males and females, LRA produced an equation based on a single measurement that was almost always successful in correctly classifying sex. Using wing chord alone, 95.5% of individuals were correctly classified as male or female. This simple model provided the best discrimination ability of all the models examined, and was able to clearly distinguish between the two sexes. Thus, when only one member of a pair has been caught, when deal-

ing with floaters, or in population banding programs, sex can be assigned with high probability by measuring wing chord and calculating the probability of the individual being male. An analysis of known-sex museum specimens would be useful in generating a model before researchers engage in data collection in the field.

The variables that are best able to distinguish between males and females will depend not only on the probabilities that they can distinguish sex in unknown individuals, but also on the repeatability of their measurement (Sweeney & Tatner 1996). The length of wing chord was the most repeatable, and thus reliable, measurement. Given the ease with which this characteristic is measured and its significance in the paired comparisons and logistic regression, wing chord length would be most useful in distinguishing between the sexes in Buff-breasted Wrens. As an overall indicator of body size, tarsus length is a much better predictor than wing chord (Rising & Somers 1989, Freeman & Jackson 1990). Therefore, depending on the question of interest, inclusion of this additional measurement may be warranted.

The mean time between successive measurements was almost one year. A shorter duration between successive measurements would be more likely to control for growth and feather abrasion (Sweeney & Tatner 1996), leading to stronger estimates of repeatability. In this study, the influence of growth on repeatability estimates is unknown because the wrens could not be aged once they attained adult characteristics. In a limited sample of known-aged juveniles, we found that the wing chord of juveniles was significantly smaller than their same-sex parent. Since Buff-breasted Wrens as young as 8 months pair and defend territories (Gill unpubl.), there is a real possibility that growth influenced reliability estimates. The observed overlap in wing chord length between males and females could be due to such an age-related effect, although it did not affect our ability to reliably assign sex.

The results of this study show that, despite overlap between the sexes in body size, sex in monochromatic Buff-breasted Wrens can be assigned reliably based on simple mensural characteristics. This information was used in our behavioral studies to make sex identifications and to avoid invasive sexing techniques (e.g., Gill 2004, Gill *et al.* 2005, Gill & Stutchbury 2005). It will be important to assess the generality of these findings to other monochromatic and presumed monomorphic resident species, either using museum specimens (e.g., Winker *et al.* 1994, 1996) or free-living birds (this study). By assigning sex to birds caught during population- and community-level studies, researchers may gain further insight into population dynamics and the viability of threatened species.

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