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BIOMETRICS AND SEXING CRITERIA OF THE YELLOW-FACED HONEYEATER *Lichenostomus chrysops*

R. H. CLARKE, C. SCHIPPER, R. L. BOULTON, J. G. EWEN and M. F. CLARKE

Department of Zoology, La Trobe University, Bundoora, Victoria, Australia 3086

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Morphometric data on 99 adult and 13 juvenile Yellow-faced Honeyeaters *Lichenostomus chrysops* that were independently sexed using molecular techniques were analysed to investigate size dimorphism between the sexes. Our results support previous studies that have demonstrated Yellow-faced Honeyeaters are sexually dimorphic in size, with males being the larger sex. Discriminant analyses of morphometric data were used to develop a simple method for sexing adult Yellow-faced Honeyeaters in the hand. As five observers collected the measurements our sexing criteria are conservative and should have wide application for field ornithologists working on the species.

INTRODUCTION

Often bird species that are sexually monomorphic in plumage still display some sexual dimorphism in size (Green and Theobald 1989; Paton and Collins 1989). In many cases analyses of measurements taken during banding studies can be used to develop sexing criteria for species that may otherwise prove difficult to sex. This is particularly true in species with subtle size dimorphism, where multivariate techniques have the potential to provide accurate sexing criteria (e.g. Clarke and Heathcote 1988).

One species that is monomorphic in plumage yet displays some sexual size dimorphism is the Yellow-faced Honeyeater *Lichenostomus chrysops*. Several previous studies have documented size dimorphism in this species (Rogers *et al.* 1986; Higgins *et al.* 2001). However, only Rogers *et al.* (1986) present data for live birds. Despite the recognized dimorphism, no method for accurately sexing a large proportion of the population has been developed. This may in part reflect the fact that only univariate methods have been applied. For example, Pyke and Armstrong (1993) concluded they were unable to sex Yellow-faced Honeyeaters on head-bill measurements alone. Rogers *et al.* (1986) used a bimodal distribution of wing length and reported being able to accurately sex only 43 per cent of individuals. This method subdivides a sample of individuals into two populations when a bimodal distribution is found in some character. It then goes on to calculate the mean and standard deviation for each of the larger sex and smaller sex and the sex ratio. Provided one has a sample of known-sex individuals through which the larger or smaller sex can be identified these parameters can then be used to develop a sexing criteria. A limitation of this approach is that the actual sex of many or most individuals is estimated rather than known. The development of modern molecular methods for determining the sex of individuals from a blood or tissue sample (Griffiths *et al.* 1998) creates the opportunity to reliably sex all individuals of a sample prior to examining morphological differences. Our aims were therefore to further quantify the level of sexual dimorphism in the Yellow-faced Honeyeater by applying multivariate statistical analyses to morphometric measurements of birds whose sex had been confirmed genetically, and thus to develop accurate methods for sexing the species in the field.

METHODS

Yellow-faced Honeyeaters (*L. c. chrysops*) captured in mist nets between September 1997 and January 2001 in the Coranderrk Reserve, Healesville, Victoria were subjected to morphometric measurements of wing length (maximum chord) and tail length to the nearest 1 millimetre using a butt-ended ruler and culmen depth (at base of exposed culmen), and total head length to the nearest 0.1 millimetre using dial calipers. Body mass was recorded with a Pesola spring balance to the nearest 0.5 gram. The presence or absence of a brood patch was also recorded. Individuals were aged following the methods of Rogers *et al.* (1986) and Matthew (1999) and only adults were used in our analysis to determine sex. As this work was conducted as part of a larger study on Yellow-faced Honeyeater social behaviour and breeding biology (Clarke *et al.* 2003), most individuals were captured at active nests and thus the proportion of juveniles in our samples are smaller than would be expected. As our aim was to develop sexing criteria that would be widely applicable to field workers, we made no effort to account for the inevitable inter-observer variability that will occur when five researchers contributed measurements. In all cases, where an individual was measured more than once by the same observer, the arithmetic mean of the measurements was used in the analyses.

Blood samples (5–100μL) were collected from all individuals through wing venipuncture of the brachial vein and stored in Queens lysis buffer (Seutin *et al.* 1990). Subsequently we extracted genomic DNA and all individuals were sexed using PCR (Griffiths *et al.* 1996) (see Ewen *et al.* 2001 for further details). This protocol always sexed known males and females correctly (deduced from breeding behaviour and for females, brood patches) and also correctly sexed known pairs of other Meliphagids (Ewen *et al.* 2001). A method for sexing Yellow-faced Honeyeaters on external measurements (wing length, tail length, culmen depth and total head length) was developed using stepwise discriminate analysis employing Rao's V as the selection criteria (SPSS V.10. 1999). Weight was not incorporated into this analysis as it is not a measure of structural size (Piersma and Davidson 1991) and Higgins *et al.* (2001) demonstrated there were significant seasonal fluctuations in Yellow-faced Honeyeater weight. We used methods outlined in (Green and Theobald 1989) to calculate confidence contours and present these graphically so that ornithologists may assign sex in the field. Morphometric differences between the sexes were tested for with t-tests.

RESULTS

Adult male Yellow-faced Honeyeaters were significantly larger than females in all characters measured except culmen depth (Table 1). In contrast juvenile males only

TABLE 1

Biometrics of adult Yellow-faced Honeyeaters from Coranderrk Reserve, Victoria and results of *t*-tests comparing morphometric characters and body mass between the sexes. All individuals were sexed using molecular techniques. Means ± lSD are presented. After Bonferroni correction, *P* values are non-significant if $P > 0.01$.

	Males		Females			*t*-test	
Character	n	Mean	n	Mean	df	t	*P*
Wing length (mm)	53	80.42 ± 2.82	47	76.90 ± 1.97	98	7.14	<0.001
Tail length (mm)	49	73.28 ± 4.48	45	70.54 ± 3.10	92	3.41	0.001
Culmen depth (mm)	42	4.13 ± 0.28	37	4.02 ± 0.27	77	1.77	0.081
Total head length (mm)	57	34.56 ± 0.94	46	33.38 ± 0.78	101	6.79	<0001
Body mass (g)	63	17.32 ± 1.22	58	16.62 ± 1.25	119	3.13	0.002

TABLE 2

Biometrics of juvenile Yellow-faced Honeyeaters from Coranderrk Reserve, Victoria and results of *t*-tests comparing morphometric characters and body mass between the sexes. All individuals were sexed using molecular techniques. Because of the small sample sizes equal variances were not assumed and degrees of freedom were adjusted accordingly (SPSS V10, 1999). Means ± lSD are presented. After Bonferroni correction, *P* values are non-significant if $P > 0.01$.

	Males		Females			*t*-test	
Character	n	Mean	n	Mean	df	t	*P*
Wing length (mm)	5	78.86 ± 2.02	8	75.41 ± 2.13	9.0	2.93	0.017
Tail length (mm)	5	73.00 ± 2.45	8	70.88 ± 1.96	7.2	1.64	0.144
Culmen depth (mm)	5	3.90 ± 0.26	8	3.69 ± 0.10	4.7	1.72	0.149
Total head length (mm)	5	34.82 ± 0.75	8	33.04 ± 0.68	8.0	4.34	0.003
Body mass (g)	5	17.32 ± 1.22	8	16.69 ± 0.96	6.8	2.83	0.026

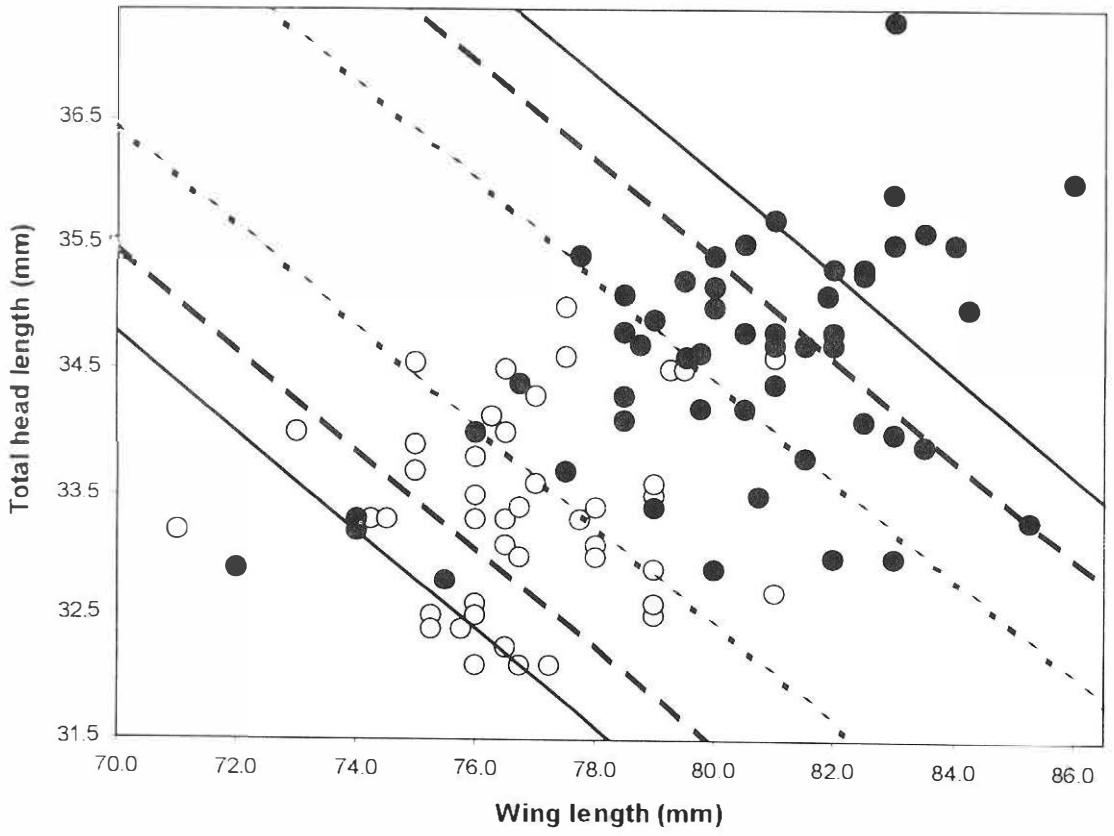

Figure 1. *Size segregation of adult male (●) and adult female (○) Yellow-faced Honeyeaters using total head length and wing length. Solid horizontal lines are the 95 per cent probability contours, broken horizontal lines (– – –) are the 90 per cent probability contours and dotted horizontal lines (-----) are the 75 per cent probability contours of being correctly sexed following Green and Theobald (1989). In all cases the upper bound for females, lower bound for males is shown. Researchers should select the most suitable confidence contours for their purpose (i.e. the level of accuracy required) and birds whose measurements fall between the selected pair of contours should not have their sex assigned.*

displayed significantly larger total head lengths (Table 2). However, sample sizes were small. Four morphometric measurements (wing length, tail length, culmen depth and total head length) of Yellow-faced Honeyeaters were subjected to a stepwise discriminate analysis using Rao's V as the selection criteria. Wing length and total head length (n = 99) were, in combination, the most discriminating variables between the sexes (Fig. 1). The classification function for sex of Yellow-faced Honeyeaters derived using these measurements was:

C = (0.263 × wing length) + (0.658 × Total head length) – 43.060

where C > -0.06 the sex is male and where C < -0.06 the sex is female. A cross validation technique, where each case is classified by the function derived from all other cases, was used to assess the classification function and showed that sex was correctly assigned to 84.8 per cent of birds whose sex was known through molecular analysis. The level of accuracy required when sexing Yellow-faced Honeyeaters will vary depending on the question(s) being asked. We have identified the upper 75, 90 and 95 per cent confidence contours for females and the lower 75, 90 and 95 per cent confidence contour for males (Fig. 1) so that researchers can select the level of accuracy that best suits their requirements. Once a confidence limit is selected birds that fall between the two respective confidence intervals should not have their sex assigned.

DISCUSSION

Our results support previous studies that have demonstrated Yellow-faced Honeyeaters are sexually dimorphic. Male Yellow-faced Honeyeaters are 2.7 per cent to 4.6 per cent larger than females in linear measurements of body size and 4.2 per cent heavier. The means of total head length, wing length and tail length for male and female Yellow-faced Honeyeaters presented here are comparable with those of Rogers *et al.* (1986). Morphometric data presented in Higgins *et al.* (2001) are also similar despite their measurements being gathered from museum specimens. This is surprising as measurements of museum specimens are typically not comparable with measurements of live birds because of shrinkage in specimens (see Higgins *et al.* 2001 pp 37–38 for a review).

It is noteworthy that we identified wing length and total head length as the most discriminant variables for sexing the species as these two characters have been independently analysed with limited success using univariate methods (Rogers *et al.* 1986; Pyke and Armstrong 1993). Our findings highlight the power and value of multivariate analyses when a proportion of the population can be accurately sexed using other techniques. As our results incorporate inter-observer variation from five observers, our method for sexing adult Yellow-faced Honeyeaters is conservative and should have wide application for field ornithologists working on the species. Our sexing criteria are only applicable to the nominate *L. c. chrysops* of eastern Australia as Higgins *et al.* (2001) demonstrated both *L. c. samueli* and *L. c. barroni* are significantly smaller in several characters including wing length. As such, sexing criteria for Yellow-faced Honeyeater populations in both the Mt Lofty Ranges, South Australia and Clarke Range to Atherton Tableland, Queensland do not currently exist.

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