

Sexual dimorphism and determination of sex by morphometrics in Blue-fronted Amazons (*Amazona aestiva*)

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Abstract. On current knowledge, it has not been possible to determine the sex of Blue-fronted Amazons (*Amazona aestiva*) in the hand, as males and females were thought not to differ in the colour of plumage or size. We used discriminant function analysis to develop equations for determining the sex of Blue-fronted Amazons using in-hand measurements. We took seven measurements of size and one measure of plumage colour (percentage of yellow on the head) from 202 birds in north-western Argentina. The sex of each individual was determined using DNA-based genetic techniques. For all size measurements, males averaged larger than females. Males also had a higher percentage of yellow plumage on the head than females. The percentage of yellow plumage (64%) and body mass (12%) had the highest degree of dimorphism, but also showed high within-sex coefficients of variation, correctly classifying 73% and 72% of the individuals respectively. Although bill-depth had a low degree of dimorphism (7%), it had the lowest coefficient of variation (4%) resulting in the most accurate single-measurement for sexing this species (80% of the individuals). A cross validation process revealed that a discriminant function including three measurements (bill-depth, tibial length and head-width) was more accurate and reliable for determination of sex than single measurements, classifying correctly 85% of the individuals. Our results show that Blue-fronted Amazons are sexually dimorphic for most characteristics of body size and show sexual dichromatism in the colour of the plumage of the head, which allows determination of sex of individuals by morphological variables that can be easily measured in the field.

Additional keywords: *Amazona aestiva xanthopteryx*, discriminant analysis, sexual dichromatism.

Introduction

Determining the sex of individuals in natural populations is important for studying population dynamics, population structure, habitat use, behaviour and mating systems, and for making management decisions (Hughes 1998). Unfortunately, for many species of bird it is difficult to determine sex using morphometrics or patterns of plumage colouration. This is particularly true for parrots, where sexual size-dimorphism has been described in only four genera (Forshaw 1989). Sexual differences in plumage colouration are more common in parrots in the Old World (65% of 184 species) than in the New World (13% of 148 species), where most sexually dimorphic species are parrotlets (e.g. *Forpus*, *Touit*) and parakeets (e.g. *Bolborhynchus lineola*, *Psilopsiagon aurifrons*). These species have females that are generally duller than males and lack some of the prominent markings of males (Forshaw 1989).

The members of the genus *Amazona* are generally considered monomorphic despite the suggestion that there is some degree of sexual size-dimorphism and dichromatism (Bosch and Wedde 1981; Forshaw 1989; Low 2005; Forshaw 2006; Santos *et al.* 2006). Sexual differences have not been quantified in a useful manner for field observations and studies and so the most common methods used to determine sex in this group have

been laparoscopy or DNA-based genetic techniques (Miyaki *et al.* 1997).

The possibility of determining the sex of a species in the hand using simple measurements may improve our ability to study sex-specific movements and behaviours in the field (Budden and Beissinger 2004). One successful approach in sexing many bird species involves discriminant analysis using morphological measurements (e.g. Donohue and Dufty 2006; Svagelj and Quintana 2007; Reynolds *et al.* 2008). The usefulness of measurements of body size as predictors of sex improves with increasing sexual size-dimorphism and with decreasing variation within sexes (Weidinger and van Franeker 1998; Fletcher and Hamer 2003). Discriminant function analyses based on morphometrics have been used in a few Old World parrots (Bond *et al.* 1991; Butler and Gosler 2004) to successfully separate the sexes.

In this work, we undertook the first quantitative examination of sexual dimorphism in an Amazon parrot, the Blue-fronted Amazon (*Amazona aestiva*). We determined within- and between-sex variation of seven measurements of body size and one measure of plumage colouration of wild adults sexed by DNA-based genetic techniques and performed discriminant analyses to obtain reliable and unbiased functions for sexing

this species. Since size dimorphism has commonly been associated with intersexual differences in feeding ecology and social behaviour (Snyder and Wiley 1976), its demonstrated presence in Blue-fronted Amazons would encourage studies on several features of their biology. In addition, there are practical benefits from being able to reliably sex birds in the field for studies of ecology and social behaviour.

Materials and methods

Study species

Blue-fronted Amazons occur in savannas and dry and semi-humid forests of central South America (e.g. eco-regions of Chaco, Caatinga, Cerrado, Pantanal; Forshaw 2006). Populations of this species are declining, mainly as a result of habitat transformation (deforestation and selective logging) and capture for the pet trade (Bucher *et al.* 1992; Collar 1997; Fernandes Seixas and Mourao 2002). This species comprises two subspecies. The main morphological difference between the subspecies is the colour of the shoulder, which changes from red in eastern populations (*A. a. aestiva*, endemic of Brazil) to yellow in western populations (*A. a. xanthopteryx*), but this character also seems to vary among individuals at any particular locality (Darrieu 1983; Forshaw 1989). The subspecies described for our study area (*A. a. xanthopteryx*) ranges from northern to southern Bolivia, and southern Mato Grosso, Brazil, south through Paraguay to northern Argentina (Forshaw 1989).

Data collection

Blue-fronted Amazons were captured during July 2005 at Sierra de Santa Bárbara and neighbouring areas (23°30'S, 64°35'W) in Jujuy Province, northern Argentina, by officials of the Fauna Authority of the Province of Jujuy. During the non-breeding season (from April to August) Blue-fronted Amazons forage in flocks and usually visit farmlands (Bucher *et al.* 1992). In Argentina, the National Management Plan for this species authorises the use of mist-nets to capture wild individuals that forage in citrus plantations to sell them for the pet trade (Bolkovic and Ramadori 2006). All captured birds were more than 1 year old; birds that are less than 1 year old have dark-brown irides, while birds that are more than 1 year old have orange irides (Forshaw 1989). We captured and handled 202 Blue-fronted Amazons to obtain measurements of body size and photographs of the head. We took the following measurements: maximum bill-depth; maximum bill-width; length of the head, from the tip of the bill to the posterior ridge formed by the parietal-supraoccipital junction; width of the head between external tips of left and right squamosal; length of tibia; and length of the tarsus from the middle of the mid-tarsal joint to the distal end of the tarso-metatarsus. We measured the birds' right tibia and tarsi. For measurements of bill, head, tibia and tarsus we used Vernier callipers (± 0.1 mm). We also recorded body mass using a spring scale (± 10 g). All measurements were taken by the same person (I. Berkunsky) to avoid any bias between observers. For detailed description of these standard measurements, see Winker (1998). We collected these measurements, as they are the most commonly used in field studies (Masello and Quillfeldt 2003). Other researchers have used the length of the culmen along its dorsal curvature

(Bond *et al.* 1991), or the middle toe (Butler and Gosler 2004) to successfully determine the sex of birds, but these measurements are not commonly taken in studies of parrots.

We took photographs of the left side of the head with a Nikon Coolpix 5 Megapixel digital camera without flash, and saved them in JPEG format. We used Adobe Photoshop software to delimit the contour of the head and to determine the percent of its surface with yellow plumage. Percent of yellow plumage was calculated as the number of pixels with yellow colour over the number of pixels of the head. We also took a small blood sample (20–50 μ L) through brachial vein puncture with a 29G needle. Blood was collected with one 80 μ L heparinised capillary tube, immediately mixed with 0.5 mL of lysis buffer (100 mM Tris pH 8, 10 mM NaCl, 100 mM EDTA, 2% SDS) and stored at room temperature until analysis.

We sexed all individuals by amplification of a size-different intron within the highly conserved chromo-helicase-DNA binding protein (CHD) gene located on the avian sex chromosomes (Ellegren 1996). DNA was extracted from blood samples using a standard salting-out protocol (Miller *et al.* 1988) and amplified using F2 and R2 primers (Quintana *et al.* 2003), setting the annealing temperature at 50°C. Polymerase chain reaction (PCR) products were separated in 2% agarose gels stained with ethidium bromide. The presence of one band indicated males (ZZ), whereas two bands indicated females (ZW).

Statistical analyses

We used one-way ANOVA to determine whether the external morphology varied with sex. All measured variables were normally distributed (Lilliefors tests; n.s.). Statistical analyses were carried out using *Statistica* (StatSoft 2001). For each variable we calculated the sexual dimorphism index as: $SDI = (\bar{X}_m - \bar{X}_f) / \bar{X}_f \times 100$ (Weidinger and van Franeker 1998), where \bar{X}_m and \bar{X}_f are the mean values of males and females, respectively. We also calculated the coefficient of variation ($CV = s.d. / \bar{X} \times 100$) for each sex (Fletcher and Hamer 2003) to indicate the degree of within-sex variability of each character (Sokal and Rohlf 1995).

We performed a principal components analysis (PCA) to analyse if males and females showed any degree of grouping in the multidimensional space of variables, though we excluded body mass from the analysis. We randomly split adults into two groups, one to provide the discriminant function value and the other to test its accuracy. To obtain discriminant functions, we used the measurements of 88 parrots (44 males and 44 females). We evaluated the performance of each single-variable as a discriminant variable (univariate discriminant analysis). We also applied discriminant analyses to obtain the combinations of characters (discriminant function) that best distinguished the sexes (Tabachnick and Fidell 1996; Phillips and Furness 1997). We constructed two discriminant functions, one including morphometric variables and the other including morphometric variables and percentage of yellow plumage. For each discriminant analysis, we calculated the associated cut-off value (Phillips and Furness 1997). Parrots with a discriminant score (measurement values for univariate analysis) higher than the cut-off value were classified as males and those with a lower

score as females. Following the criteria proposed by Phillips and Furness (1997), cut-off values were those discriminant score values corresponding to a posterior probability value of 0.5 for each group. We obtained cut-off values by fitting data (each discriminant score and the associated probability of belonging to one sex) to logistic curves (Phillips and Furness 1997).

If discriminant analyses are validated with the same sample used to generate them, correct classification rates tend to be overestimated (Tabachnick and Fidell 1996). Therefore, we performed a cross-validation process to classify a new sample of 114 Amazons (70 males and 44 females) captured at the same site.

We provide the cut-off values and discriminant functions obtained. We also report the significance level (*P* value), Wilks' lambda, and the percentage of correctly classified individuals for each sex and for all birds pooled together.

Results

Males were significantly larger than females for all measurements of body size and also had a higher percentage of yellow plumage on the head than females (all *P* < 0.001; Table 1). The measurements that showed greater levels of sexual dimorphism were yellow plumage on the head and body mass, while the least dimorphic was head-length. However, yellow plumage and body mass also showed high within-sex variation. The measurements that showed the least within-sex variation were head-length and bill-depth (Table 1). The principal component analysis for the correlation matrix of morphometric and plumage colour measurements indicated that the first principal component (PC1) was a good 'body size' axis that explained 53% of the variance in the original matrix (Table 2). Character loadings of morphological measurements indicated that individual parrots

Table 1. Measurements of males and females and proportion of coloured plumage on the head, coefficients of variation (CV) and sexual dimorphism index (SDI) of wild Blue-fronted Amazons (*Amazona aestiva xanthopteryx*)

All measurements are in mm, except body mass (g) and proportion of yellow plumage on the head (%), and show mean ± s.d., with range in parentheses. All variables differed between sexes (see text; one-way ANOVA, all *P* < 0.001)

Body measurement	Males	Females	CV	CV	SSD
	(n = 114)	(n = 88)	males (%)	females (%)	(%)
Body mass	431 g ± 46 (330–645)	386 ± 40 (295–555)	10.3	10.6	12
Head-length	61.6 ± 1.9 (57–65)	58.4 ± 2.4 (53–63.5)	2.9	4.1	5
Head-width	30.8 ± 1.5 (27.5–35)	28.9 ± 1.5 (25–32)	4.8	5.1	7
Bill-depth	31.5 ± 1.0 (29.0–35.0)	29.4 ± 1.2 (26.0–32.0)	3.3	4.3	7
Bill-width	17.5 ± 0.8 (16.0–19.5)	16.4 ± 0.9 (14.0–19.0)	4.4	5.8	7
Tarsal length	30.4 ± 1.3 (28.0–34.0)	28.6 ± 1.4 (25.0–32.0)	4.1	5.2	6
Tibial length	72.8 ± 2.6 (64.0–79.0)	68.7 ± 2.8 (62.0–74.0)	3.7	4.2	6
Percentage of yellow plumage	26.1% ± 14.7 (0–67)	15.9% ± 13.4 (0–58)	49	85	64

with a low value on the PC1 axis were larger. The second principal component (PC2) and the third principal component (PC3) were correlated with yellow plumage and measurements of bill respectively (Table 2). Together, the first two eigenvectors explained 66% of the total variation. Figure 1 shows PC2 plotted against PC1 for male and female Blue-fronted Amazons and it shows that there is considerable variation along the body-size axis, with males being larger than females.

All single measurements were significant predictors of sex of Amazons (all *P* < 0.0001; Table 3). Bill-depth was the most accurate single variable and correctly classified 80% of the parrots (Table 3). Parrots with bill-depth values >30.3 mm (cut-off value) were classified as males whereas those with lower values were classified as females. Bill-width (cut-off value 16.9 mm) and tibial length (cut-off value 70.7 mm) were also accurate predictors of sex, each correctly classifying 79% of

Table 2. Character loadings on principal component axes (PC1, PC2 and PC3) for a PCA extracted from a correlation matrix of six measurements of body size (excludes body mass) and one measure of plumage colour from 202 wild Blue-fronted Amazons (*Amazona aestiva xanthopteryx*) captured in northern Argentina

Character	Principal component axis		
	PC1	PC2	PC3
Head-length	-0.79	0.13	0.21
Head-width	-0.72	0.00	-0.54
Bill-depth	-0.79	0.08	-0.13
Bill-width	-0.78	0.07	-0.27
Tarsal length	-0.74	0.27	0.47
Tibial length	-0.79	-0.06	0.16
Percentage of yellow plumage	-0.42	-0.88	0.13
Eigenvalue	4.19	0.89	0.75
Percentage of variance explained	53	13	10

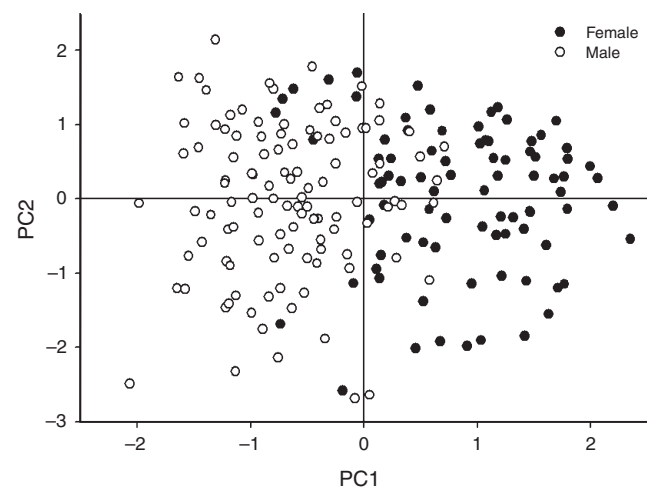


Fig. 1. Plot of factor scores, based on correlations, for the first (PC1) and the second (PC2) principal components from a principal components analysis of six morphometric and one plumage colour variables measured in 202 wild Blue-fronted Amazons (*Amazona aestiva xanthopteryx*) captured in northern Argentina.

Table 3. Accuracy of sexing Blue-fronted Amazons (as percentage of correctly classified birds) using single measurements and two discriminant functions (DF)

The initial sample for the discriminant analyses comprised 88 individuals, the sample for cross-validation (see text) comprised 114 parrots. All discriminant analyses were significant ($P < 0.0001$). DF₁, discriminant function including tibial length, bill-depth and head-width; DF₂, discriminant function including tibial length, bill-depth, head-width and percentage of yellow plumage on the head

	Wilks' lambda	F value	Original sample			Cross-validation sample		
			Males (n=44)	Females (n=44)	Total (n=88)	Males (n=70)	Females (n=44)	Total (n=114)
Body mass	0.755	$F_{1,87} = 28.2$	65	80	73	68	66	67
Head-length	0.662	$F_{1,87} = 44.4$	86	69	77	90	61	79
Head-width	0.654	$F_{1,87} = 46.0$	77	76	76	78	64	73
Bill-depth	0.595	$F_{1,87} = 59.1$	86	76	80	90	82	87
Bill-width	0.746	$F_{1,87} = 29.5$	87	71	79	84	57	73
Tarsal length	0.758	$F_{1,87} = 27.8$	73	71	72	82	75	79
Tibial length	0.592	$F_{1,87} = 59.8$	84	73	79	87	70	80
Percentage of yellow plumage	0.810	$F_{1,87} = 20.1$	67	76	72	62	55	59
DF ₁	0.442	$F_{3,84} = 35.8$	89	82	85	89	84	86
DF ₂	0.403	$F_{4,83} = 30.6$	88	87	88	91	78	86

the parrots (Table 3). Body mass and percentage of yellow on the head had less discriminatory power (Table 3, Accessory publication, see <http://www.publish.csiro.au/nid/96.htm>). For all single measurements, cross-validation provided slightly different classifications than discriminant analyses, with a decrease in the accuracy for body mass, percentage of yellow, and head- and bill-width, and an increase in the accuracy for bill-depth and for head-, tibial and tarsal lengths (Table 3).

We constructed two significant discriminant functions using forward discriminant analyses. The first analysis was conducted with all morphometrical variables and resulted in one discriminant function that included bill-depth, tibial length and head-width as discriminatory variables ($P < 0.0001$). The resulting function was: $DF_1 = (\text{bill-depth} \times 0.40) + (\text{tibial length} \times 0.18) + (\text{head-width} \times 0.24) - 34.00$, and correctly sexed 85% of the individuals. A second analysis with all morphometrical variables plus the plumage colour variable (percentage of yellow plumage in the head) resulted in a slightly better significant discriminant function: $DF_2 = (\text{bill-depth} \times 0.39) + (\text{tibial length} \times 0.16) + (\text{head-width} \times 0.28) + (\text{percentage of yellow} \times 2.73) - 31.64$. This function correctly sexed 87.5% of the adult parrots (Table 3), misclassifying only five males and eight females (Fig. 2). The cross-validation process provided a similar classification as the one produced by the discriminant function (Table 3), with 86% of the parrots correctly classified by sex in both functions.

Discussion

Our results show that Blue-fronted Amazons are sexually dimorphic for most measurements of body size and sexually dichromatic for colour of the plumage of the head. On average, males were larger, heavier and had a higher percentage of yellow plumage on the head than females. Although the percentage of yellow plumage and body mass showed the greatest differences between sexes, bill-depth was the most accurate single variable to distinguish between males and females as it had the lowest within-sex coefficient of variation.

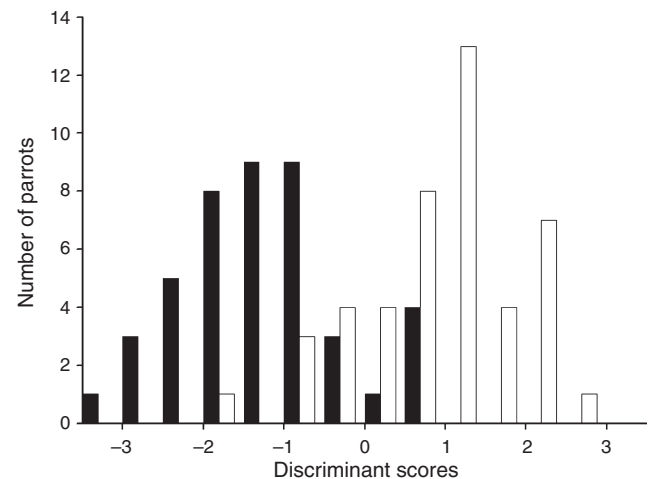


Fig. 2. Frequency distributions of discriminant scores of male (black bars) and female (white bars) Blue-fronted Amazons (*Amazona aestiva xanthopteryx*) captured in northern Argentina applying DF₁ discriminant function.

We found one discriminant function with a combination of morphological measurements that was a more accurate and reliable predictor of sex than each single measurement. Considering the accurate results obtained by sexing Blue-fronted Amazons using a discriminant function based on morphological characters, this method could be a safer, faster and less expensive way of sexing individuals than laparoscopy or genetic DNA-based techniques. However, the latter techniques should still be used for those cases where discriminant scores are close to cut-off values. Further studies should evaluate the observer error and the repeatability of the measurements. Because our study was conducted with individuals of *A. a. xanthopteryx*, which are slightly larger than individuals of *A. a. aestiva* (Darrieu 1983), we cannot be sure that our discriminant function can accurately sex individuals of the latter subspecies. Therefore, an additional study would be

required to assess morphological differences between both subspecies and the accuracy of applying our discriminant function in *A. a. aestiva*.

Sexual size-dimorphism in the Psittaciformes has been described in a few species (Bond *et al.* 1991; Moorhouse *et al.* 1999; Masello and Quillfeldt 2003). The results of these studies indicate that sexual differences were more noticeable in bill-size rather than in overall body size (Bond *et al.* 1991). Similarly, we also found that bill-depth was the most accurate single-measurement for sexing this species, classifying correctly 80% of the individuals. The dimorphism in bill-size is likely to be related to foraging. This could be important, as for most of the year these birds move and travel together as a pair, so if they can exploit resources slightly differently they would not compete for the same food.

We also found differences in head colouration, with males showing larger yellow patches on the head than females. This result supports the common thinking that male Blue-fronted Amazons have a greater extent of non-green colouration (usually red, yellow, white or blue) over the forehead (Low 2005). However, the high coefficient of variation among males (49%) and females (85%) suggests that other factors besides sexual differences are acting on the extent of yellow colour on the head. For example, Forshaw (1989) mentioned that immature individuals have reduced blue and yellow plumage of the head, which may imply that the age of individuals could explain some of the within-sex variation in yellow plumage that we observed. A previous study of Blue-fronted Amazons (Santos *et al.* 2006) also found evidences of sexual dichromatism in the UV spectrum in three areas of the body (forehead, wing-tip and alula). However, these authors did not find sexual differences in plumage colour in the human visible spectrum (400–700 nm). However, whereas the patch of yellow plumage on the head probably shows similar reflectance spectra between males and females to the human eye, people can easily perceive the variation in size.

Studies of sexual dimorphism can serve as an initial assessment of the strength of sexual selection and may help to identify characters likely to be the subject of selection (Badyaev and Hill 2000). It has been proposed that ornaments in birds are a signal for direct (e.g. good parents) or indirect (e.g. good genes) benefits to prospective partners (Andersson 1994). Patches of coloured plumage have been shown to signal individual quality independently of how the colouration is achieved (see review in Hill and McGraw 2006). Plumage patches with colours based on structural composition (e.g. Griffith *et al.* 1999; Keyser and Hill 2000; Siefferman and Hill 2005) or on pigments such as carotenoids (e.g. Hill 1991; Senar *et al.* 2002), melanins (e.g. Siefferman and Hill 2003; Safran and McGraw 2004) and even psittacofulvins (Masello and Quillfeldt 2003), only present in parrots, have been found to correlate with individual quality. We found that the yellow facial patch in Blue-fronted Amazons is proportionally larger in males and, therefore, this plumage patch could act as a potential sexual ornament. Female preferences for larger mates with a more conspicuous yellow facial patch could also be a possible explanation of some of the sexual differences we documented.

To summarise, our study shows that Blue-fronted Amazons are sexually dimorphic for most measurements of body size and

thus can be sexed by morphological variables easily measurable in the field. We lack evidence that can confirm whether or not size of the body or the yellow patch, or both, are used by conspecifics to recognise the sex or quality of an individual. More information concerning variation in reproductive success and general breeding ecology is needed for Blue-fronted Amazons to elucidate better how selection shapes the morphology and plumage colour of this species of parrot. Considering that populations of Blue-fronted Amazon are declining (Bucher *et al.* 1992; Collar 1997; Fernandes Seixas and Mourao 2002) the ability to sex this species in the hand will be valuable for conservation studies as well as other studies addressing intersexual and intrasexual differences in ecology and behaviour, like foraging strategies, dominance and aggressive behaviours, and vocalisations, among others.

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Sexual dimorphism and determination of sex by morphometrics in Blue-fronted Amazons (*Amazona aestiva*)

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Fig. A1. Low (left), intermediate (centre) and high (right) values of percentage of yellow on the head of female (upper line) and male (lower line) Blue-fronted Amazons (*Amazona aestiva xanthopteryx*) captured in Northern Argentina.

