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Sexual Dichromatism in the Blue-fronted Amazon Parrot (*Amazona aestiva*) Revealed by Multiple-angle Spectrometry

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Abstract: Seventy-five percent of psittacine species, including the blue-fronted Amazon parrot (*Amazona aestiva*), are classified as sexually monomorphic. However, this classification is based on the inability of the trichromatic human eye to perceive light in the near-ultraviolet spectrum. Spectrometry is a technique that enables humans to recognize the increased range of color perceived by the tetrachromatic avian eye. By using this technique, researchers have reclassified many avian species as sexually dimorphic. In this exploratory study, several body regions of 30 blue-fronted Amazon parrots (males and females) were investigated by multiple-angle spectrometry. A model was developed that enabled gender prediction with 100% accuracy based on plumage color characteristics. However, the areas that were most promising in our model (forehead and wing tip) need to be confirmed independently to exclude the possibility of type I error attributed to multiple testing.

Key words: sexual dimorphism, spectrometry, ultraviolet reflectance, plumage color, avian, blue-fronted Amazon parrot, *Amazona aestiva*

Introduction

Birds have traditionally been categorized as sexually monomorphic or dimorphic based on their anatomy and plumage color as judged by the human eye.[1,2] Approximately 70%–80% of birds are sexually dimorphic by this system, but the males and females of many species of birds, including 75% of parrot species, remain indistinguishable to the human eye.[3,4] Knowledge of a bird's gender is important for the veterinary practitioner, the owner, and the breeder. Accurate gender determination is essential for proper pairing of birds, and knowing the gender of a bird will allow the veterinarian to rule in or out gender-specific diseases.

Several means of gender determination have been developed for sexually monomorphic avian species. In poultry, cloacal sexing of day-old chicks is widely used, but this method of sexing birds is limited to only a few species. Gonad visualization by laparoscopy is a direct and accurate method for sexing a bird.[5] Laparoscopic sexing, however, requires training and expensive instruments and carries with it the risk of anesthesia and injury from an invasive procedure. It is also not 100% accurate, especially when immature birds are examined.[6,7] More recently, laboratory-based assays for gender determination have been developed, including hormone assays of feces; examination of cells in metaphase for Z and W chromosomes; and molecular techniques such as restriction fragment length polymorphism, analysis of polymerase chain reaction amplification products, and random amplified polymorphic DNA markers.[5,8–14] Although laboratory-based tests, particularly molecular assays, have proven to be affordable and accurate for many avian species, each has its limitations, and none can be applied to all species of birds.

Recently, it has been shown that birds have the ability to see the near-ultraviolet (UV) spectrum and that most birds have plumage that reflects UV light. Some psittacine species also have fluorescent plumage, which absorbs short wavelengths and re-emits them at longer wavelengths, resulting in a color that

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is a combination of UV reflectance and fluorescence. Ultraviolet reflectance may play a role in sexual communication, mate choice, and courtship displays.[15–21]

Reflectance spectrometry in the avian visible range (320–700 nm) has been shown to be an important tool for plumage color assessment in various studies.[2,17–27] Many studies have shown that birds, initially classified as sexually monochromatic, are actually dichromatic when the UV part of the spectrum is considered.[18,28–32] Most of these studies used only 1 illumination and 1 observation angle. More recent work shows that by varying these angles, additional information can be obtained, resulting in the detection of gender-specific plumage differences.[33] In the study reported here, multiple-angle reflectance spectrometry was used to investigate gender differences in plumage color in the blue-fronted Amazon parrot (*Amazona aestiva*).

Materials and Methods

Spectroscopy

A total of 30 adult blue-fronted Amazon parrots (17 live, 13 dead) were used in the study. All procedures were performed with permission from the animal experimentation committee from the Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands (DEC 0404.0703).

Five body regions were examined in each bird based on their importance in sexual displays, UV photography, and previous findings from other species of birds[33]: the frons (blue forehead), the occiput (green nape), the tetriceces alulae dorsales (yellow alula), the pars pennacea of the first remiges primariae (dark-blue wing tip), and the tectrices caudales (green tail, middle portion). Live birds were anesthetized with isoflurane for all measurements, and each carcass was examined within 2 days of a bird's death. Ten reflectance measurements were taken from each body region. After each measurement, the probe was removed from and reapplied to the same area. Feathers were illuminated with a DH-2000 deuterium-halogen light source (Avantes BV, Eerbeek, The Netherlands), and measurements were made with an AVS-USB2000 spectrometer (Avantes BV) connected to a computer notebook with software (Avasoft-basic, Avantes BV, Eerbeek, The Netherlands) that stored data and calculated all color parameters.

Five different illumination/observation angles were used for spectra measurements: 45°/45°, 45°/90°, 45°/135°, 90°/75°, and 90°/90°. The illumination/observation angles of 45°/45° and 90°/90° were made with a bifurcated fiber-optic probe (FCR-7UV400–2, Avantes BV) that had a black plastic sheath fixed to the fiber end. The sheath kept the probe the same distance from the feathers for all body regions measured. All other illumination/observation angles were achieved with an AFH-15 angled fiber holder (Avantes BV) (Fig 1A). The fiber holder was lightly pressed on the feathers perpendicular to the long axis of the bird's body (Fig 1B). Reflectance spectra with wavelengths of 320–700 nm were measured over an approximately 2-mm area. Reflectance was defined as the percentage of reflected light compared with the reflective light from a polytetrafluorethylene white standard tile (WS-2, Avantes BV, Eerbeek, The Netherlands) and a dark standard (light source off). White and dark references were taken before each different illumination/observation angle was investigated and at the beginning of each measurement session. The gender of each bird was unknown at the time spectra measurements were recorded. After measurements were obtained, each bird's gender was determined by DNA analysis[13] or, in the cases of deceased birds, by dissection.

To determine if fluorescence was present in the body regions examined and to prevent influence of

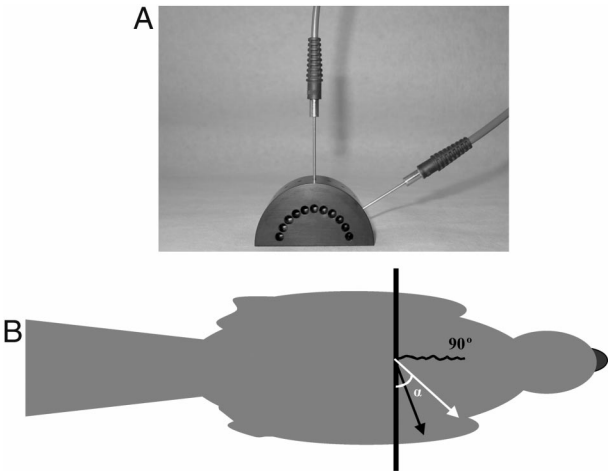

Figure 1. Multiple-angle spectrometric measuring technique showing the angle fiber holder (A) and positioning of the holder on the plumage of a blue-fronted Amazon parrot (B). The angle fiber holder is a mechanical device with 15°-angle steps that holds illumination and observation fiber-optic probes in position at a fixed distance (2 mm) from the measuring surface, preventing exposure to any external reflectance and illumination. The holder was pressed lightly on the bird's plumage surface while oriented perpendicular to the bird's longitudinal axis (B) for every measurement. The incident illumination beam is shown 90° to the plumage surface, and the 2 arrows (black and white) represent 2 examples of observation positions ($\gamma = 45°$).

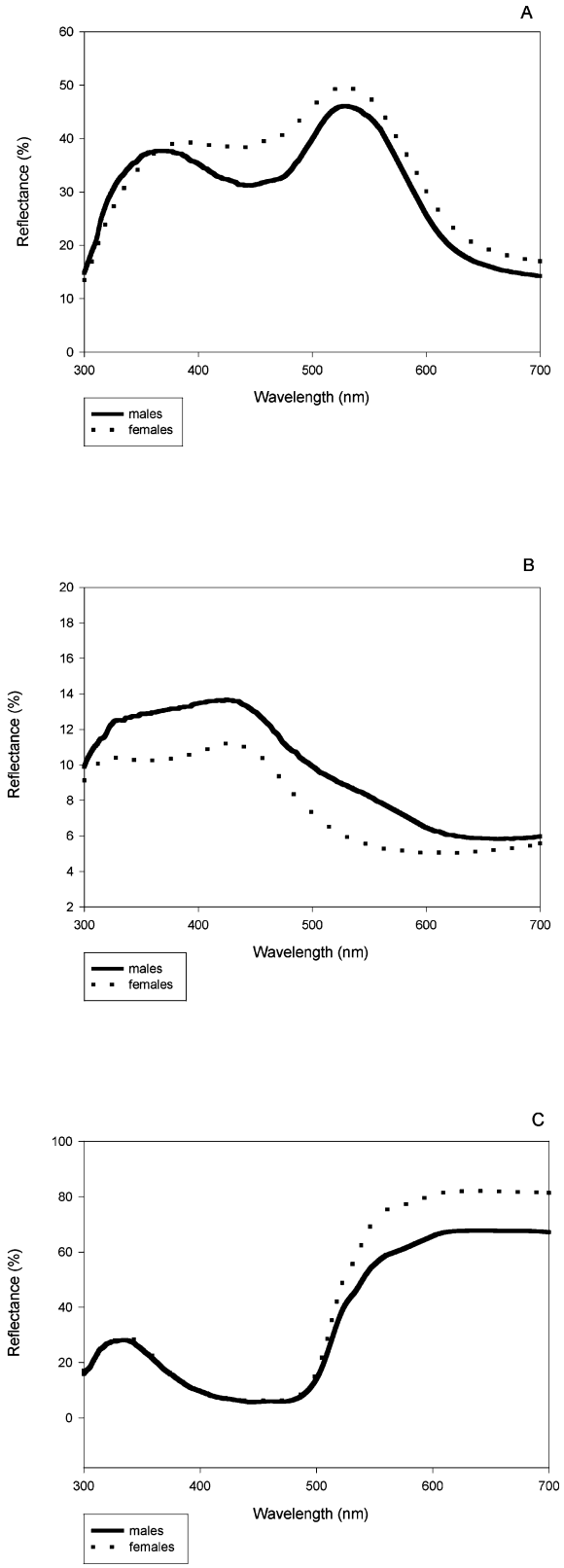

Figure 2. Examples of angle geometries demonstrating sexual dichromatism in 3 body regions of male and female blue-fronted Amazon parrots. (A) Forehead measured with 90°/75° illumination/observation angles. (B) Wing tip measured with 90°/90° illumination/observation angles. (C) Alula measured with 90°/75° illumination/observation angles. Males (n = 12) are represented with solid lines and females (n = 18) are represented with dotted lines. Each line represents the average of the medians of 10 measurements.

this trait on reflectance results, an additional survey was made in the plumage of 6 birds with a UV light.

Data analysis

Each reflectance spectrum contained 1206 data points taken at 0.31-nm intervals. The original spectra were compressed to 102 data points to facilitate calculations. Reflectance data were summarized in several parameters, both in the UV spectrum (wavelength = 320–400 nm) and in the total spectrum (wavelength = 320–700 nm). Definition of parameters was as follows: lightness (L), the light reflected by the plumage surface, was calculated as the sum of percent reflectance values from the considered range; color intensity (R_{max}) was the maximum reflectance reached in the considered range; hue (H) was the wavelength at peak reflectance in the considered range (λR_{max}); contrast (C) was the difference between the maximum and minimum reflectance in the considered range ($R_{max} - R_{min}$); and UV Chroma (UV Ch) was the reflectance sum over the UV range divided by the total reflectance ($R_{320-400}/R_{320-700}$).[35] Medians of the values of the 10 parameters (for each bird and each body region) were used in a logistic regression analysis to test for sexual plumage color dichromatism in each body region and each angle geometry, and significance was tested with the Likelihood ratio test.[35] To establish even more accurate models to predict the gender of blue-fronted Amazon parrots, new logistic regression models were established by a combination of parameters from different angle geometries and body regions. These parameters were the selected variables (relevant for the model) for the first logistic regression. Results were evaluated for *P* values of .05 and .001. The latter value was used to reduce the possibility of type I error attributed to multiple testing. Statistical analysis was conducted by SPSS 11.0 (SPSS Inc, Chicago, IL, USA).

Results

According to DNA analysis or direct visualization of the gonads at necropsy, 18 of the blue-fronted Amazon parrots were identified as females and 12 were identified as males. Fluorescence was not found in any body region of the birds; therefore, all

Table 1. Results from logistic regression analysis for each angle geometry and body region of the blue-fronted Amazon parrot.[a]

Angle geometry (illumination/ observation)	Body region	Model variables	Mean ± SD		OR[b]	*P*	Overall % correct gender
			Females (n = 18)	Males (n = 12)			
45°/45°	Forehead	C_{uv}	18.65 ± 6.69	13.33 ± 2.86	0.82	.0151	65.4
45°/90°	Forehead	C_{uv}	10.64 ± 3.00	7.42 ± 3.10	0.69	.0098	76.9
	Wing tip	H_{uv}	389.01 ± 16.90	372.56 ± 19.20	0.96	.0435	71.4
45°/135°	Forehead	UV Ch	0.20 ± 0.03	0.23 ± 0.01	6.10E + 26	.0091	84.6
		C_{uv}	6.99 ± 2.29	4.82 ± 1.91	0.54	.0122	
90°/90°	Forehead	H_{uv}	380.63 ± 12.14	370.24 ± 7.01	0.09	.0135	69.2
	Wing tip	H_{tot}	433.91 ± 38.49	396.28 ± 48.47	0.98	.0265	71.4
90°/75°	Forehead	UV Ch	0.21 ± 0.02	0.23 ± 0.01	2.98E + 70	.0008	84.6
		H_{uv}	386.55 ± 13.7	371.17 ± 11.11	0.88	.0112	
	Alula	C_{tot}	76.78 ± 10.13	63.40 ± 14.21	0.91	.0067	74.1
	Wing tip	H_{tot}	414.77 ± 37.64	360.50 ± 44.05	0.97	.0023	78.6

[a] OR indicates odds ratio; C, contrast ($R_{max} - R_{min}$); uv, ultraviolet range (320–400 nm); H, hue (λR_{max}); UV Ch, UV Chroma ($R_{320-400}/R_{320-700}$); and tot, total range (320–700 nm).

[b] Confidence intervals of the OR were omitted because of inaccurate standard errors.

reflectance captured by the spectrometer was attributed to light reflectance. Reflectance spectra from all body regions were characterized by 2 spectral peaks, 1 in the UV and 1 in the ''visible'' portion of the spectrum. These 2 peaks were less clearly defined in the wing tips. Reflectance spectra reached a maximum of 102.5% in the alula; the next highest value was 84.2% in the forehead. The forehead was the body region that showed highest brightness in both the UV and visible spectra.

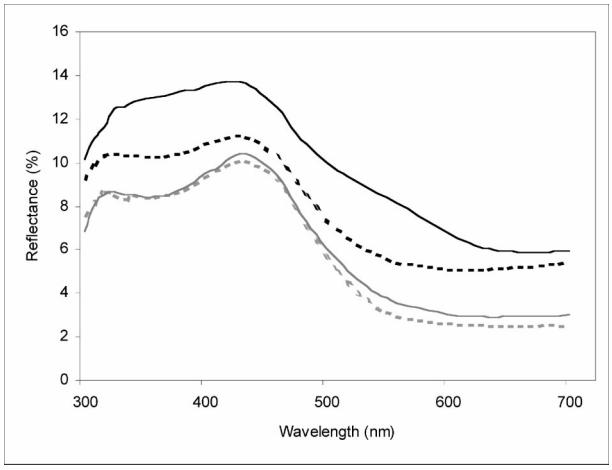

Figure 3. Example of different angle geometries showing or not showing gender differences in the wing tips of blue-fronted Amazon parrots. Solid lines correspond to males (n = 12) and dotted lines correspond to females (n = 18). Gray lines correspond to 45°/45° illumination/observation angles and black lines correspond to 90°/90° illumination/observation angles. Each line is the average of the medians of 10 measurements.

Significantly different ($.001 < P < .05$) mean values for brightness, color, or hue were demonstrated on the forehead, wing tip, and alula between the male and female groups (Table 1; Fig 2). Differences in the means for the males and females were seen in all angle geometries of the forehead. Logistic regression analysis showed that males could be separated from females with 84.6% certainty in 2 angle geometries, 45°/135° and 90°/75°, each based on the calculation of 2 parameters in the UV part of the spectrum, that is, UV Ch and C_{uv} or UV Ch and H_{uv}, respectively (Table 1). The 90°/75° angle geometry appeared to show gender differences most clearly. In all body regions, the reflectance spectra, and consequently most calculated parameters, changed significantly when the angle geometry was changed (Fig 3). The H_{uv} was an exception to this in the forehead, alula, and nape.

Several models combining different parameters, body regions, and angle geometries resulted in a 100% accurate gender determination of all the birds. Table 2 illustrates some of the possible combinations.

Discussion

The results of this study demonstrate that mean values of several spectra parameters measured were significantly different ($P \leq .05$) between male and female blue-fronted Amazon parrots. These data

Table 2. Examples of combined multiple logistic regression models for gender prediction in the blue-fronted Amazon parrot.[a]

Body regions (parameters)	Angle geometry (illumination/ observation)	OR[b]	*P*	Overall % correct gender
Wing tip (H_{tot})	90°/75°	0.56	.0058	
Forehead (UV Ch)	90°/75°	—	.0005	100
Alula (L_{tot})	90°/75°	0.99	.0146	
Wing tip (H_{tot})	90°/75°	0.02	.0000	
Wing tip (L_{tot})	90°/90°	1.11	.0081	100
Wing tip (C_{tot})	90°/90°	7.12E + 135	.0000	
Wing tip (UV Ch)	45°/135°	—	.0000	
Wing tip (H_{tot})	90°/75°	0.86	.0006	96
Forehead (UV Ch)	90°/75°	1.52E + 219	.0002	
Forehead (UV Ch)	45°/45°	1.96E + 78	.0021	82.6
Alula (H_{tot})	45°/45°	1.10	.0015	

[a] OR indicates odds ratio; H, hue (λR_{max}); tot, total range (320–700 nm); UV Ch, ultraviolet Chroma; L, lightness; C, contrast ($R_{max} - R_{min}$).

[b] Confidence intervals of the OR, and some OR values (indicated by empty cells), were omitted because of inaccurate standard errors.

suggest that there is sexual dimorphism in this species that cannot be recognized by the human eye. The use of a single measurement in an individual bird, however, did not provide sufficient accuracy for applied use of this technique. When groups of 3 variables were used in the logistic regression, 2 combinations of variables were found that could distinguish gender in 100% of the birds examined. Additional studies are needed to determine whether these values will be equally predictive in other populations of blue-fronted Amazon parrots. Examination of other areas of feathering may also reveal gender-linked variations.

Psittaciforms are some of the most colorful birds; however, few studies have been performed on parrot coloration,[22,36–40] and even fewer have been performed on the ecological and evolutionary significance of their plumage color.[37,40] Recent studies have suggested that avian plumage colors have the potential to indicate male quality,[30,37,41–44] immunocompetence,[44,45] body condition,[34,47,48] health,[46,49–53] and the ability to provide parental care.[32,54,55] The range of values observed in the population of birds examined with the overlap of male and female values may reflect the variations in the underlying health of the birds. Therefore, it is possible that this technique may have some value as a diagnostic tool in addition to its potential use for gender determination.

This exploratory study suggests that multiple-angle spectroscopy may provide a quick, readily applied, and noninvasive method for gender determination in the blue-fronted Amazon parrot. The use of multiple-angle geometries in the forehead and calculation of contrast in the UV range seems the most promising model for gender determination in this species. However, further independent work is needed to confirm the usefulness of this model.

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