HUMAN VISION CAN PROVIDE A VALID PROXY FOR AVIAN PERCEPTION OF SEXUAL DICHROMATISM

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ABSTRACT.—The study of sexual dichromatism has played an important role in the development and testing of evolutionary theory. However, previous work has often relied on human vision to assess plumage color and contrast, an approach challenged by the finding that dichromatism is often visible to birds but invisible to humans. We explicitly tested whether the use of human vision undermines previous comparative analyses in antbirds (Thamnophilidae). Focusing on a sample of 71 species, we used (1) molecular sequencing of the SWS1 opsin gene to assess spectral sensitivity and (2) spectrophotometry and color discrimination models to compare human and avian perception of dichromatism. We show that antbirds, like the majority of avian families studied to date, are violet-sensitive (VS) rather than ultraviolet-sensitive (UVS). We also demonstrate that species perceived as monochromatic by humans may look dichromatic to antbirds, but that human and avian perceptions of dichromatism are nonetheless positively correlated. To assess whether this relationship validates the assumptions of published comparative analyses, we re-ran the analyses using avian-perceived dichromatism; the results remained qualitatively unchanged. Although it is clear that the use of spectrophotometry and visual models can improve measurements of plumage coloration, we conclude that scores generated from human perception provide a meaningful estimate of sexual dichromatism for the purposes of comparative analyses, at least in antbirds. Furthermore, our results suggest that discrepancies between human and avian perceptions of sexual color differences may be relatively minor in avian families with VS visual systems. *Received 7 July 2009, accepted 16 October 2009.*

Key words: antbirds, comparative analysis, plumage reflectance, sexual dichromatism, spectral sensitivity, Thamnophilidae, ultra-violet vision.

La Visión Humana puede Proveer una Aproximación Válida a la Percepción del Dicromatismo Sexual por parte de las Aves

RESUMEN.—El estudio del dicromatismo sexual ha jugado un papel importante en el desarrollo y puesta a prueba de la teoría evolutiva. Sin embargo, los estudios han dependido frecuentemente de la visión humana para evaluar el color y contraste del plumaje. Este enfoque ha sido desafiado por el hallazgo de que, con frecuencia, las aves pueden ver dicromatismo en situaciones en las que éste es invisible para los humanos. Evaluamos de forma explícita si el uso de la visión humana representa un problema para los estudios comparativos realizados previamente en los hormigueros (Thamnophilidae). Enfocándonos en una muestra de 71 especies, empleamos (1) secuenciación molecular del gen SWS1 de las opsinas para evaluar la sensibilidad espectral y (2) espectrofotometría y modelos de discriminación de colores para comparar la percepción del dicromatismo por parte de los humanos y de las aves. Nuestros resultados demuestran que especies de la familia de los hormigueros, como la mayoría de las familias de aves que han sido estudiadas, son sensibles al violeta y no son sensibles al ultravioleta. También demostramos que las especies que son percibidas como monocromáticas por los humanos podrían parecer dicromáticas para las aves, pero las percepciones del dicromatismo de los humanos y de las aves están correlacionadas positivamente. Para evaluar si esta relación valida las suposiciones de los análisis comparativos publicados, repetimos dichos análisis usando el dicromatismo percibido por las aves y obtuvimos resultados cualitativamente iguales. Aunque es claro que el uso de la espectrofotometría y los modelos visuales pueden mejorar las medidas de la coloración del plumaje, concluimos que los puntajes generados a partir de la percepción humana proveen un estimativo del dicromatismo sexual que resulta sensato para los propósitos de los análisis comparativos, al menos en los hormigueros. Además, nuestros resultados sugieren que las discrepancias entre los humanos y las aves en la percepción de las diferencias de colores entre sexos podrían ser pequeñas en las familias de aves que tienen sistemas visuales sensibles al violeta.

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Studies of avian sexual dichromatism have been central to the formulation of evolutionary theory from the outset (Darwin 1869, Wallace 1889) and remain prominent in reviews of sexual selection (Andersson 1994) and speciation (Price 2007). Indeed, the focus on sex differences in plumage color and pattern has intensified, partly because strong positive relationships have been reported between avian dichromatism and measures of sexual selection, such as the degree of polygyny, frequency of extrapair paternity, and relative testes size (Møller and Birkhead 1994, Owens and Bennett 1994, Dunn et al. 2001). These findings have led to widespread use of dichromatism as an index of sexual selection in comparative analyses. In recent decades, quantification of avian dichromatism for this purpose has been pervasive, involving numerous studies of speciation (Barraclough et al. 1995, Møller and Cuervo 1998, Owens et al. 1999, Morrow et al. 2003, Phillimore et al. 2006, Seddon et al. 2008) and extinction (McLain et al. 1995, 1999; Sorci et al. 1998; Doherty et al. 2003; Morrow and Pitcher 2003), as well as a wide range of other topics, including individual mortality (Promislow et al. 1992), immune defense (Møller 1997), signal evolution (Garamszegi et al. 2007), molecular evolution (Nadeau et al. 2007), and response to climate change (Spottiswoode et al. 2006).

Since the pioneering studies of Owens and Bennett (1994) and Owens and Hartley (1998), a common method for measuring dichromatism has been to score sex differences from painted illustrations or specimens. This technique assumes that human vision allows a reasonable approximation of dichromatism, or of bird coloration generally. Thus, a major objection to this approach stems from the fundamental differences between human and avian visual physiology (Cuthill et al. 2000a). In particular, humans are blind to the near ultraviolet (UV; 300–400 nm), to which many birds are sensitive, and humans have three retinal cone types, compared with at least four in birds (Bowmaker et al. 1997). On the basis of these differences, visual models predict that humans and birds see color very differently, particularly when reflectance spectra are more complex or UV-rich (Vorobyev et al. 1998). They also predict significant mismatches between human and avian perception of plumage color contrasts (Håstad and Ödeen 2008) and—crucially—that a high proportion of bird species classified as monochromatic by human observers are perceived as dichromatic by birds (Eaton 2005, 2007).

These findings call into question the central assumption of many previous studies that human and avian perceptions of dichromatism are correlated. Nonetheless, the human-vision method is so convenient that such analyses continue to be published, with their assumptions defended by logic rather than data (e.g., Seddon et al. 2008). The main supporting argument runs thus (Badyaev and Hill 2003):

> The fact that so many interesting patterns related to sexual dichromatism have been revealed in comparative studies that ignore the UV portion of the spectrum suggests that the visible portion of the spectrum must, in many cases, be a reasonable approximation of the overall coloration and dimorphism of a species.

This hypothesis received indirect support from a recent comparison of techniques, which again concluded that studies using human vision to assess avian sexual dichromatism "should be reliable" (Armenta et al. 2008b). As yet, the degree of mismatch between dichromatism as detected by humans and by birds has not been examined directly, and its influence on the conclusions of previous analyses remains to be tested.

Given the differences in visual physiology between humans and birds, it is inevitable that information is lost when plumage coloration is interpreted by human observers (Endler 1990, Bennett et al. 1994, Cuthill 2006, Håstad and Ödeen 2008). Evidence that the lost information is biologically significant stems from (1) research that documents the ubiquity of UV-reflecting plumage in birds (e.g., Eaton and Lanyon 2003); (2) the discovery that >90% of passerine species classified as monochromatic by human observers are probably perceived as sexually dichromatic by an avian visual system (Eaton 2007); and (3) numerous studies that show that spectral reflectance invisible to humans is used in a range of interspecific and intraspecific interactions, including species recognition and mate choice (e.g., Bennett et al. 1996, 1997; Andersson et al. 1998; Arnold et al. 2002; Bleiweiss 2004). The broad implication is that all studies that use human estimates of dichromatism are potentially invalid (Bennett et al. 1994, Vorobyev et al. 1998, Eaton 2005). However, this is not necessarily the case for comparative analyses, because most previous work has scored dichromatism on a scale, usually from 1 to 10, and the extent to which this approach is confounded depends on how well human-perceived dichromatism predicts avian-perceived dichromatism.

The overall aim of the present study is to assess the validity of human vision as a proxy for avian perception, specifically in color discrimination and perception of dichromatism. To achieve this, we provide the first direct assessment of sex-specific plumage reflectance in the antbirds (Thamnophilidae) and the first estimate of spectral sensitivity of the antbird eye. We use color spectrophotometry to measure plumage reflectance from museum specimens, molecular sequencing to determine spectral sensitivity (Ödeen and Håstad 2003, Ödeen et al. 2009), and color discrimination models to estimate avian perception of dichromatism (Vorobyev and Osorio 1998). These models generated values of perceived sex differences in color across the avian visual spectrum (300–700 nm) for each species (hereafter "dichromatism[avian]"), allowing comparison with scores generated by human observers (hereafter "dichromatism[human]"). Finally, to determine the extent to which dichromatism[human] is a valid surrogate for dichromatism[avian] in comparative analyses, we used both measures to reanalyze data from a recent study of antbirds (Tobias and Seddon 2009a). Our results reveal close concordance between human and avian perception, which suggests that, for the purposes of comparative analyses, human-generated scores can provide a meaningful proxy for avian-perceived dichromatism, at least when spectral complexity and UV reflectance are low.

Methods

Study system.—The antbird family contains >200 species of small to medium-sized (6.5–155 g) insectivorous passerines found in forest, woodland, and scrub throughout Central America and South America at elevations 0–3,000 m above sea level (Zimmer and Isler 2003). It provides an excellent system for this study because it is speciose, with much interspecific variation in plumage dichromatism, and it therefore gives us power to explore the

relationship between human and avian perceptions of this trait. A large collection of specimens is available for study, and males and females of all species have been illustrated in detail by the same artist (Zimmer and Isler 2003), facilitating estimation of dichromatism by human observers. Antbirds have also been subject to recent comparative analyses based on sexual dichromatism (Seddon et al. 2008, Tobias and Seddon 2009a), which allows us to test the validity of previous assumptions.

Plumage reflectance.—Spectral reflectance was measured from antbird specimens housed at the Natural History Museum, Tring, United Kingdom. We sampled 71 species, using three specimens of each sex where possible (male specimens per species [mean ± SE] = 3.2 ± 0.1; female specimens per species = 3.1 ± 0.1; $n = 450$ specimens). For each specimen, we recorded species, subspecies, sex, catalogue number, locality, collector, and date (see online Appendix). We selected the nominate subspecies where possible and ensured that all specimens were of the same subspecies. Specimens that showed signs of molt or juvenile plumage were not sampled.

Fresh specimens were unavailable and therefore we, like authors of other reflectance studies (e.g., Gomez and Théry 2007), used older specimens. Collection dates spanned more than a century (1845–1957), with the bulk of specimens (83.8%) collected in a 50-year period (1865–1915). Specimen age has been shown to influence plumage reflectance, particularly when specimens are >50 years old (Armenta et al. 2008a), and it is therefore likely that our measurements do not fully capture dichromatism in wild birds. However, we believe that specimen age is unlikely to affect our results, for three main reasons. First, the effects of age are generally minor, even in older specimens (Bleiweiss 2005, Armenta et al. 2008a, Doucet and Hill 2009). Second, we found no statistically significant effects of specimen age on mean levels of reflectance for any plumage patch in either the UV or human-visible parts of the spectrum (see below). Third, measurements of fresh plumage samples collected from mist-netted individuals suggest that the spectra of live antbirds are similar to those of old specimens, with similarly low levels of UV reflectance (Tobias and Seddon 2009b). Nonetheless, to minimize any potentially confounding effects of age on our calculations of dichromatism, we ensured that male and female specimens were collected at a similar time within each species (mean age difference [± SE] = 9.6 ± 1.1 years; correlation between mean age of males and females within species: Pearson's $r = 0.66$, $P < 0.001$, $n = 71$).

We measured spectral reflectance using an Ocean Optics USB4000 spectrometer (range: 200–1,100 nm), a DH2000-FHS pulsed xenon lamp (190–1,700 nm), and a bifurcated fiber-optic reflection probe (QR400-7-UV/BX) mounted in a matte black holder (RPH-1). The latter excluded external light and kept the probe perpendicular to the measurement surface at a fixed distance of 5 mm. Data were collected in SPECTRASUITE and expressed as the proportion of reflectance relative to an Ocean Optics WS-1 white standard, which reflects 97–98% of incident light. Following Doucet et al. (2007), we took five measurements from the center of each of five body regions: head (forecrown, crown, and nape), upperside (mantle and rump), underside (throat, breast, and belly), wings, and tail. In all cases, measurements were taken from the upperside of feathers and from parts of feathers visible without spreading or ruffling the plumage. When plumage regions were patterned with bands, spots, or stripes, we took three measures

in the dominant color and two measures in the alternate color. When regions contained more than one color (rare in antbirds), we took all measures from the dominant color. When regions contained a distinct color patch in at least one sex (i.e., black throats in males), we took measures from the center of the patch.

For the purpose of analysis, we averaged raw spectral data into 10-nm bins from across the entire avian-visible wavelength range (300–700 nm), thus producing a series of 41 reflectance values for each measure. Averaging reflectance for each plumage patch produced 5 reflectance curves per sex, 10 reflectance curves per species, and a total of 710 reflectance curves for analysis. Following Eaton and Lanyon (2003), each reflectance curve was classified as having a mean UV reflectance (300–400 nm) of <5%, 5–10%, 10–20%, or >20% of incident light, with any UV reflectance >5% treated as biologically meaningful. We then compared mean reflectance between the UV spectrum (300–400 nm) and the human-visible spectrum (400–700 nm) for each of the five body regions (sexes pooled) and by averaging across body regions to quantify reflectance for the plumage as a whole. Finally, we compared UV reflectance between males and females separately for all five body regions, and then by averaging across body regions to quantify UV dichromatism for the plumage overall.

The antbird visual system.—The spectral sensitivity of the avian retina varies widely between species. The wavelength of maximum absorbance (δ_{max}) of the short-wavelength-sensitive single cone (SWS1) falls into one of two classes (Cuthill et al. 2000b): ultraviolet sensitive (UVS; δ_{max}: 355–380 nm) and violet sensitive (VS; δ_{max}: 402–426 nm); for reviews, see Hart (2001, 2004) and Beason and Loew (2008). The sensitivity of avian single cones is tightly correlated with sequence variation in the SWS1 gene (Wilkie et al. 2000, Yokoyama et al. 2000, Carvalho et al. 2007), which makes it possible to estimate δ_{max} for the visual pigment by molecular sequencing of genomic DNA (Ödeen and Håstad 2003, Ödeen et al. 2009). We used this approach to identify the likely spectral sensitivity in antbirds.

Genomic DNA was extracted from blood samples taken from one individual each of three distantly related species of antbird: Peruvian Warbling Antbird (*Hypocnemis peruviana*), Chestnut-tailed Antbird (*Myrmeciza hemimelaena*), and Black-spotted Bare-eye (*Phlegopsis nigromaculatus*). Samples were taken from birds caught in 12 × 4 m mist nets at the Centro de Investigación y Conservación de Río Los Amigos (CICRA; 12°34′S, 70°06′W), Madre de Dios, Peru; 1.5 µL of blood was extracted from the brachial vein and stored in 90% ethanol. We amplified a key fragment of the SWS1 opsin gene containing the residues of amino acid positions 81–94 (following bovine rhodopsin numbering) and located in the second α-helical transmembrane region. Extractions, polymerase chain reaction (PCR; without explicit extension time), and cycle sequencing were carried out following Ödeen et al. (2009). We used primer SU200Ga in sequencing (5′-AYTACATCYTGGTGAACATCTCS-3′, modified for the present study from SU200Ca).

We identified the spectral tuning amino acid sites 86, 90, and 93 (Wilkie et al. 2000, Yokoyama et al. 2000), to estimate δ_{max} of the SWS1 cone pigment. Positions 86 and 90, in particular, are responsible for shifts in δ_{max}; C90S has a +35 nm effect in Budgerigar (*Melopsittacus undulatus*) wild-type pigment (Wilkie et al. 2000); +38 nm in Zebra Finch (*Taeniopygia guttata*; Yokoyama et al.

2000); and S86F can short-wave-shift pigeon and chicken SWS1 pigments by 31 and 47 nm, respectively (Carvalho et al. 2007). Minor shifts in δ_{max} of the budgerigar pigment have been achieved by A86S with 1 nm and T93V with +3 nm (Wilkie et al. 2000). Two other key vertebrate tuning sites, namely 49 (Shi and Yokoyama 2003) and 116 (Hunt et al. 2007), fell outside the range of our DNA amplifications. However, neither appears to be significantly involved in tuning the avian SWS1 opsin (Carvalho et al. 2007). We assumed that the effects of the key tuning sites were additive. This assumption disregards potential interactions between the tuning sites (see Shi et al. 2001) but still provides approximations of δ_{max} sufficient to distinguish between VS and UVS in the avian retina (Ödeen et al. 2009).

Measures of dichromatism.—Dichromatism was calculated from spectral reflectance data using two complementary methods. In the first, we simply calculated the maximum Euclidean difference across all 41 bins in reflectance between males and females within bins and then compared the mean maximum sex difference between the UV and human-visible spectra for each body region. In the second, we calculated dichromatism for each plumage region within each species using the Vorobyev-Osorio color discrimination model (Vorobyev and Osorio 1998). The model calculates a distance in avian perceptual color space (ΔS) between two colors, defined by the quantum catches and estimates of receptor noise of each photoreceptor type in the avian retina. Thus, ΔS for a given plumage region quantifies sexual dichromatism explicitly with respect to the avian visual system.

To calculate ΔS for each plumage-region comparison, we followed the calculations detailed in Eaton (2005). However, because amino acid sequencing suggests that antbirds may, in general, possess a violet-sensitive (VS) visual system (see below), ΔS values were calculated for each plumage region with spectral sensitivity data from Common Peafowl (*Pavo cristatus*; data provided by Hart 2002). These data likely provide a good estimate for other species with VS visual systems, because visual pigment characteristics (and, thus, cone-cell sensitivities) are highly conserved over much of the avian visual range (Hart 2001). However, because actual antbird spectral sensitivity is unknown, we also ran the models based on a UV-sensitive visual system, with cone-cell spectral-sensitivity data from Blue Tit (*Parus caeruleus*; Hart et al. 2000). Values of ΔS generated with a UVS system were strongly positively correlated with those generated with a VS system ($r = 0.62$; see online Appendix); thus, we present only the latter here.

Sexual dichromatism was calculated on a patch-by-patch basis, so we averaged ΔS values within each species to produce an overall estimate of the level of dichromatism perceived by birds (dichromatism[avian]). The units of ΔS are jnd (just noticeable differences), where 1.0 jnd is the threshold value for discrimination of two colors. Thus, ΔS values <1.0 jnd indicate differences in color that are most likely not visually discernible by birds, whereas values >1.0 jnd indicate a magnitude of color differentiation above the visual discriminatory abilities of birds (Vorobyev et al. 1998, Vorobyev 2003, Siddiqi et al. 2004, Eaton 2005). Visual performance can vary between species and viewing conditions (Vorobyev et al. 1998), but in general, at jnd = 1.0 for threshold, two colors are barely distinguishable under ideal conditions; as jnd becomes larger, two colors are more easily discernible under worsening viewing conditions (Siddiqi et al. 2004).

Using the same specimens from which spectral reflectance data were collected, two observers scored dichromatism on a scale of 0 (monomorphic) to 10 (maximum dichromatism). Following standard methodology (e.g., Owens and Bennett 1994, Owens and Hartley 1998), we allocated a score of 0 (= no difference between the sexes), 1 (= difference in shade or intensity), or 2 (= difference in color or pattern) to each of the five body regions described above. These were totaled to produce a composite dichromatism score for each species (dichromatism[human]), which we then directly compared with our estimates of dichromatism[avian], using both VS and UVS retinal models.

Using the same scoring system, we also quantified dichromatism from color illustrations (Zimmer and Isler 2003). This allowed a direct test of the validity of previous comparative studies in antbirds that have scored dichromatism in this way (Seddon et al. 2008, Tobias and Seddon 2009a). Note that dichromatism[human] scores generated by the two observers were strongly correlated (specimens: $r = 0.90$, $P < 0.001$, $n = 71$ species; illustrations: $r = 0.88$, $P < 0.001$, $n = 71$ species) and that the mean of their scores was used.

Testing the assumptions of comparative studies.—In a recent analysis, we found that dichromatism[human] (generated from color illustrations) was positively associated with ecological generalism in the antbird family (Tobias and Seddon 2009a). Specifically, we found that mean level of dichromatism was positively correlated with mean niche width, as measured by habitat range ($r = 0.33$, $n = 53$ genera). To investigate whether this finding was influenced by the use of human rather than avian perception of dichromatism, we re-ran the analysis and compared mean habitat range against (1) mean dichromatism[human] and (2) mean dichromatism[avian]. We used the same subset of 71 species from 41 antbird genera (i.e., those included in the present study) to calculate both. For methods used to calculate habitat range for each species and genus, see Tobias and Seddon (2009a).

Statistics.—Because common ancestry leads to non-independence of samples, classical statistics tend not to be applied in multispecies comparative analyses. However, previous work has revealed that dichromatism has a low phylogenetic signal within and between avian families (Phillimore et al. 2006), including antbirds (Seddon et al. 2008, Tobias and Seddon 2009a). Moreover, the key aim of our study was not to look for evidence of correlated evolution between traits (Price 1997) but to ascertain how well human scores of dichromatism reflect avian perception of dichromatism. We therefore treat species values as independent and compare traits using simple linear regression.

Reflectance and dichromatism were compared within plumage patches using paired *t*-tests, and dichromatism[human] and dichromatism[avian] were compared using Pearson correlation. All data were log transformed prior to analyses to meet the assumptions of parametric statistics. Results are presented as means ± SE. The complete data set is provided in the online Appendix.

RESULTS

Plumage reflectance.—Our analyses revealed that antbird plumage has a relatively low level of UV reflectance. Of the 710 plumage patches analyzed, 24.7% reflected >5% of incident UV light, 5.8% reflected >10%, and 0.7% reflected >20% (for examples of reflectance curves, see Fig. 1). Accordingly, we found that the mean percentage
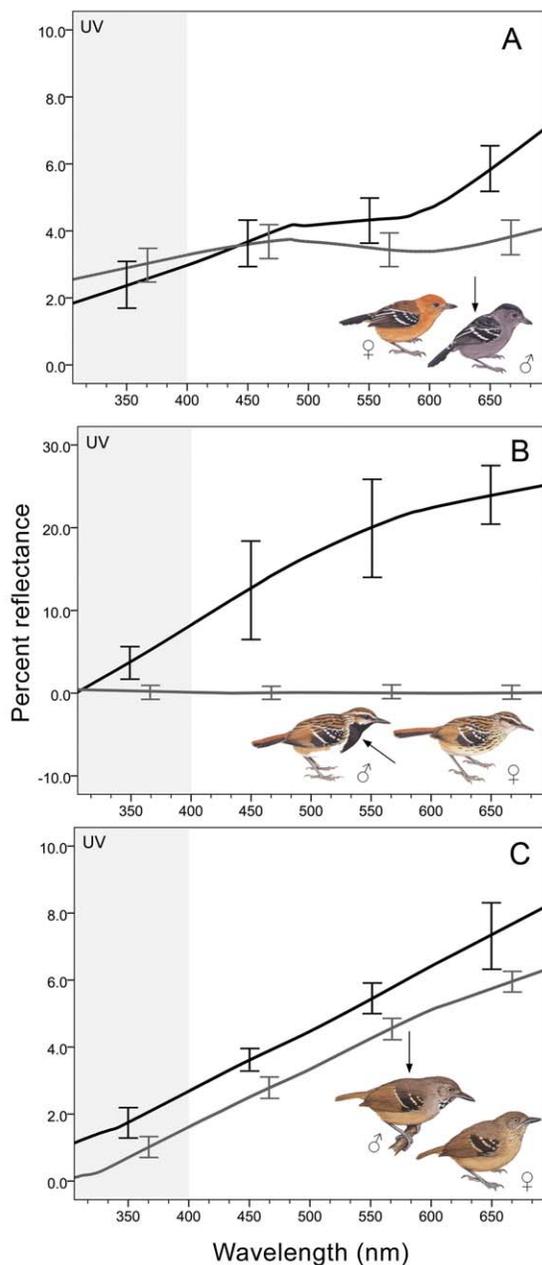
FIG. 1. Mean reflectance curves across avian-visible wavelengths, including the ultraviolet (UV; shaded, 300–400 nm), for selected plumage patches in three typical antbird species: (A) Amazonian Antshrike (*Thamnophilus amazonicus*), (B) Stripe-backed Antbird (*Myrmorchilus strigilatus*), and (C) Checker-throated Antwren (*Epinecrophylla fulviventris*). Data are means derived from 5 measures per patch taken from 2–3 males (gray line) and 3 females (black line) of each species; arrows mark the plumage patch from which measures were taken. The vertical distance between these lines is a measure of dichromatism. Error bars show the 95% confidence intervals for reflectance values every 100 nm. Mantle feathers in A and throat feathers in B look strongly dichromatic to humans; mantle feathers in C are brighter in the male but essentially monochromatic. Illustrations are reproduced and adapted from *Handbook of the Birds of the World* with the permission of Lynx Edicions.

reflectance per 10-nm bin of UV wavelengths averaged across all body regions was low ($3.7 \pm 0.2\%$, range: 0.7–6.4%), with very little variation from one region to the next (Fig. 2A). Only the front (i.e., chest and belly) of antbirds reflected >5% UV light ($5.8 \pm 0.5\%$).

Most reflectance detected in antbird plumage involved wavelengths in the human-visible part of the spectrum. Specifically, mean percentage reflectance per 10-nm bin of visible wavelengths was significantly greater in the human-visible than in the UV parts of the spectrum, both across the plumage as a whole ($5.2 \pm 0.4\%$ vs. $3.0 \pm 0.3\%$; $t = -7.0$, $P < 0.001$, $n = 71$ species) as well as in each of the five body regions (Fig. 2A). Analyzing the reflectance data by sex, we found that male plumage reflected significantly more UV than female plumage in three of the five body regions (Fig. 2B) and across the plumage as a whole ($4.0 \pm 0.3\%$ vs. $3.4 \pm 0.3\%$; $t = -3.47$, $P = 0.001$). Note that, controlling for the effects of sex and body region, there was no overall effect of specimen age on plumage reflectance in either the UV or the human-visible part of the spectrum (GLMM: $0.40 < F < 0.75$, $0.926 < P < 1.0$).

Antbird visual system.—Using the PCR forward primer SU193a, we amplified 50 base pairs (bp) of the SWS1 DNA sequence in *Hypocnemis peruviana*, and using SU149a we amplified 81 bp in *Myrmeciza hemimelaena* and 82 bp in *Phlegopsis nigromaculatus*. The gene sequences were virtually base-pair identical. The sole difference was in the phenylalanine present in amino acid position 95 (outside the second α-helical transmembrane region but still amplified), which showed a synonymous substitution: cytosine in the third position in *P. nigromaculatus* but thymine in the other two species. This indicates that all three species bear identical amino acid sequences for the section of the second α-helical transmembrane region in the SWS1 opsin (amino acid positions 73–94). The sequence FSGFL**C**CIF**S**VF**T**V (with the key tuning sites 86, 90, and 93 marked in bold) predicts that $\delta_{max} = 406$ nm and, hence, that there is a violet-sensitive (VS) type of photoreceptor in the retinas of these three species. The sequences have been deposited in GenBank (accession numbers GQ924590–GQ924592).

Extent of dichromatism.—We found that the maximum Euclidean distance in spectral reflectance between males and females was significantly greater in the human-visible spectrum than in the UV spectrum for all body regions (Fig. 2C). In other words, a high proportion of measured dichromatism in antbirds is visible to humans. However, using retinal models, we found some evidence of cryptic dichromatism. Values of ΔS ranged from 0.219 to 54.1 jnd (just noticeable difference) among all plumage patches sampled, and 92.2% (333 of 361) had $\Delta S > 1.0$ jnd and, thereby, met the criteria of being dichromatic to birds (Fig. 2D). With the threshold discrimination set at 1.0 jnd, we found that all species sampled are likely to be perceived as dichromatic by avian vision, including the four species in our sample that are classified as monochromatic by humans: Bicolored Antbird (*Gymnopithys leucaspis*), Russet Antshrike (*Thamnistes anabatinus*), Ocellated Antbird (*Phaenostictus mcleannani*), and White-plumed Antbird (*Pithys albifrons*). Only the Ocellated Antbird was classified as monochromatic using a more conservative threshold of 2.0 jnd (following Eaton 2005). Further, we found strong positive relationships between dichromatism[avian] and dichromatism[human] using both the VS (Pearson's $r = 0.62$, $P < 0.001$, $n = 71$ species; Fig. 3) and UVS retinal model ($r = 0.47$, $P < 0.001$). Re-running these
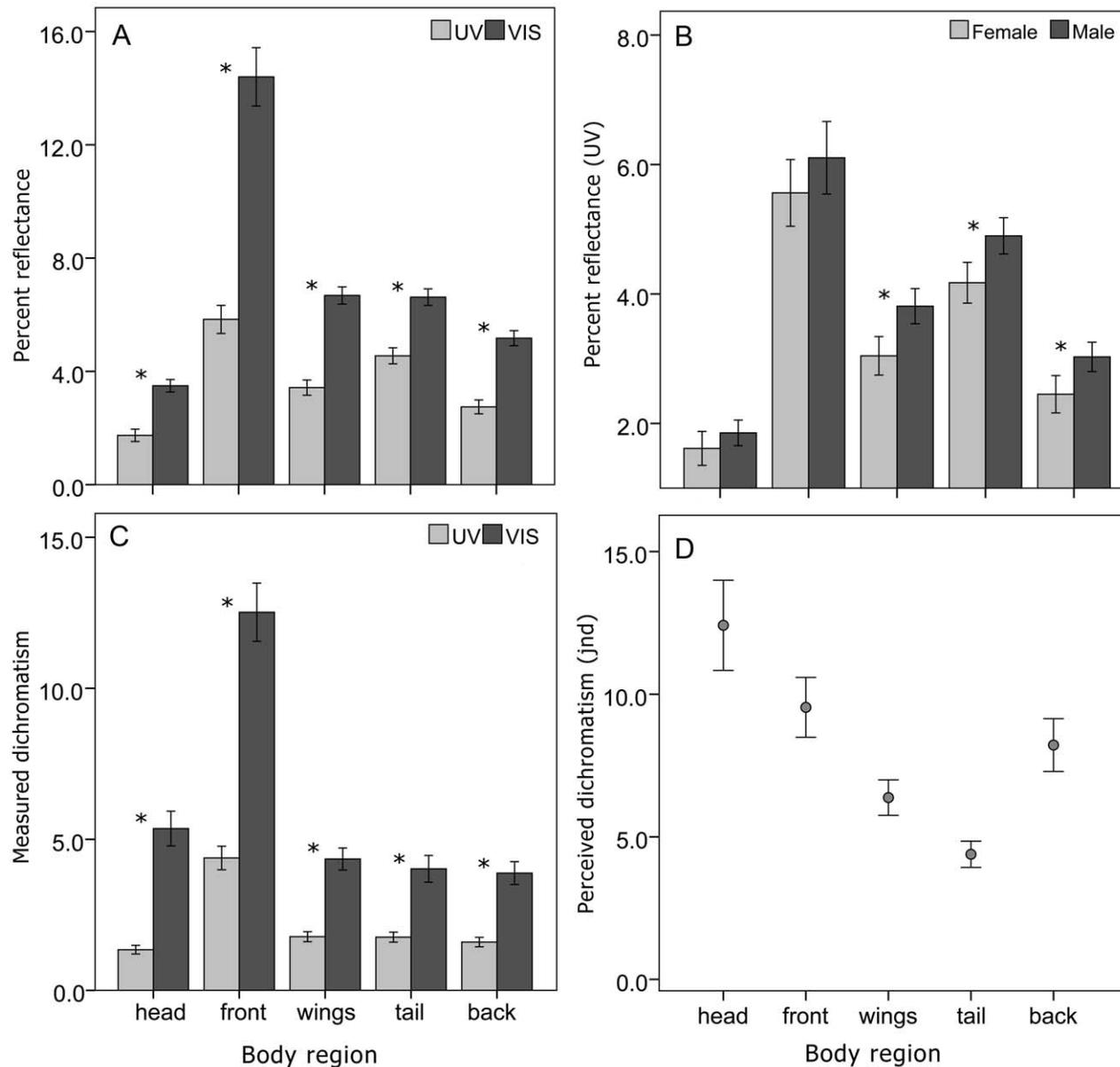
FIG. 2. Variation among body regions in (A) mean reflectance across the full spectrum (sexes pooled, averaged by 10-nm bin), (B) reflectance in the ultraviolet (UV) in each sex (averaged by 10-nm bin), (C) maximum Euclidean sex differences in reflectance (across all 10-nm bins), and (D) dichromatism[avian] (ΔS). In A and C, reflectance and dichromatism are compared between the UV spectrum (300–400 nm) and the human-visible spectrum (VIS; 400–700 nm), respectively, and show how both are greatest in the visible part of the spectrum. Panel B reveals that male antbirds reflect more UV light from all plumage patches than females. Panel D shows that peak dichromatism[avian] occurs in the head region (i.e., the area of plumage with the lowest levels of UV reflectance). Bars show means ± SE; $n = 71$ species; $P < 0.001$ for all paired comparisons (t-tests) denoted with an asterisk (A–C).

analyses using scores generated from color illustrations produced near-identical results (VS: $r = 0.61$, $P < 0.001$; UVS: $r = 0.45$, $P < 0.001$). Hence, dichromatism scores estimated using human vision captured a high proportion of interspecific variation in dichromatism perceived by the birds themselves, regardless of whether the scores were generated from specimens or color illustrations. It is possible that a better fit between human and avian assessments of plumage dichromatism in antbirds could be achieved by including measures of brightness or achromatic contrast in our visual

models; further work could usefully investigate this possibility. However, including brightness would not change the overall conclusion of the present study.

Comparative study.—We found a strong positive relationship between dichromatism and habitat range across antbird genera using both dichromatism[human] (slope ± SE, specimens: 0.291 ± 0.073, $F = 15.9$, df = 1 and 39, $P < 0.001$; illustrations: 0.314 ± 0.076, $F = 17.1$, df = 1 and 39, $P < 0.001$) and dichromatism[avian] (0.386 ± 0.110, $F = 12.2$, df = 1 and 39, $P = 0.001$). Although dichromatism[human]
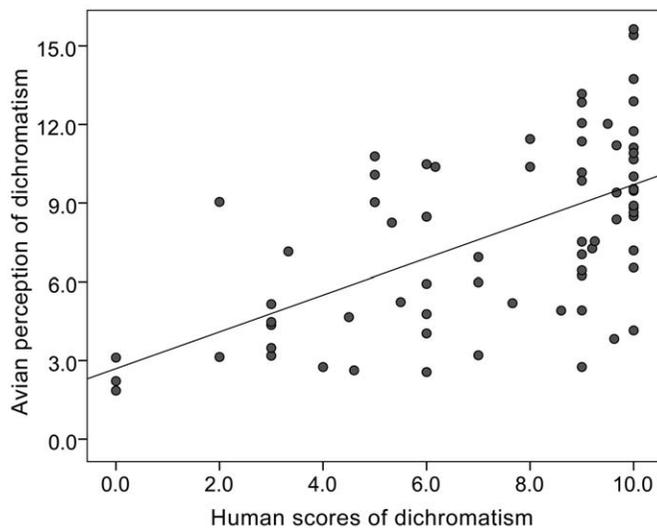
FIG. 3. Relationship between human estimates of dichromatism in antbirds scored from museum specimens (dichromatism[human]) and levels of dichromatism perceived by antbirds (dichromatism[avian]). The units on the *y*-axis are values of ΔS calculated from color discrimination models, assuming a violet-sensitive (VS) visual system ($r^2 = 0.34$, $n = 71$ species).

explained slightly more intergeneric variation in habitat range ($r^2 = 0.29$) than dichromatism[avian] ($r^2 = 0.24$), the slopes were very similar (Fig. 4). Hence, the use of dichromatism[avian] did not qualitatively change the results of this comparative analysis.

DISCUSSION

Visual physiology and plumage reflectance.—Molecular analyses of the SWS1 opsin gene revealed that, for all three antbird species sampled, the wavelength of maximum absorption was ~406 nm. This compares with 416 nm for the equivalent cone in humans (Dartnall et al. 1983). It is also similar to the values reported for the only other suboscine passerines previously analyzed: White-bearded Manakin (*Manacus manacus*; 405 nm) and Brown-crested Flycatcher (*Myiarchus tyrannulus*; 406 nm) (Ödeen and Håstad 2003). These estimates should be considered approximate because they were based on three key amino acid positions, and such calculations can misjudge extremes in spectral tuning by ≤16 nm (Ödeen et al. 2009). However, even if our estimates are out by ±16 nm, our findings strongly indicate that antbirds have VS rather than UVS photoreceptors.

It is often assumed that most birds possess a UVS short-wave photoreceptor, but there is increasing evidence that the VS visual system is widespread. Using microspectrophotometry (e.g., Hart et al. 2000) and molecular analyses (e.g., Ödeen and Håstad 2003), UVS has been directly demonstrated in 25 bird species from 12 families within only four orders (Ödeen and Håstad 2003; Håstad et al. 2005a, 2009). By contrast, violet-sensitivity (VS) has been demonstrated throughout the avian phylogenetic tree and appears to be ancestral: it occurs in one family of oscine passerines (Corvidae) and in all suboscine passerines and nonpasserines analyzed so far, with the exception of gulls (Laridae), parrots (Psittacidae), and rheas (Rheidae) (i.e., 49 species from 28 families within
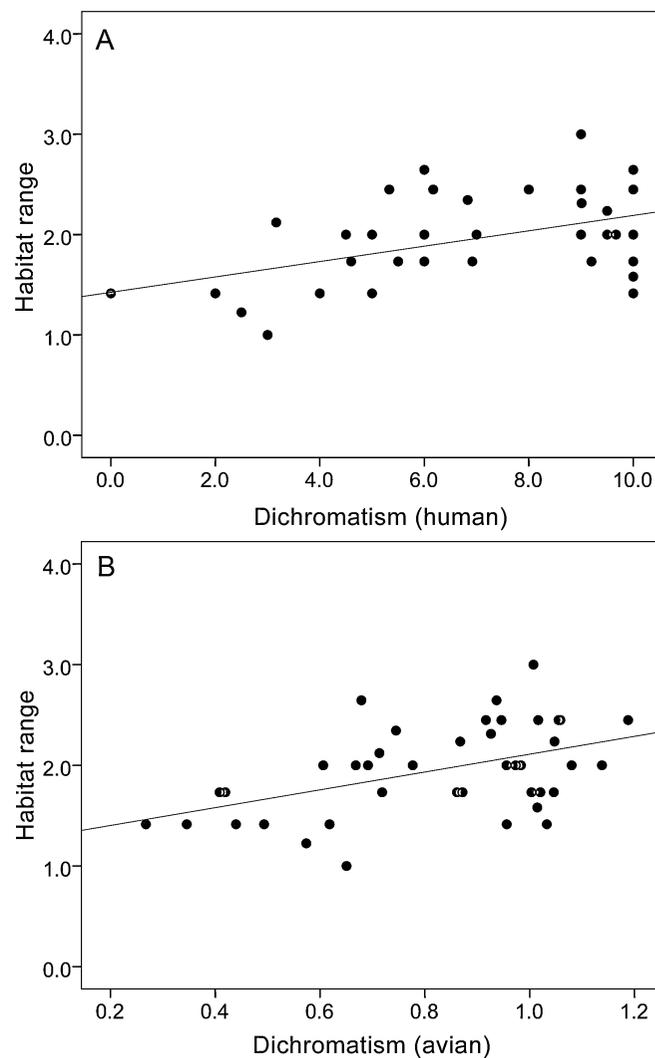

FIG. 4. Relationship between dichromatism and habitat range across 71 antbird species in 41 genera, using (A) human estimates of dichromatism (dichromatism[human], scored from museum specimens) and (B) levels of dichromatism (dichromatism[avian]; LOG10ΔS; perceived by antbirds), as calculated with color discrimination models that assume a violet-sensitive (VS) visual system. Slopes were similar in both cases; $r^2 = 0.29$ in A and $r^2 = 0.24$ in B. Data on habitat range are from Tobias and Seddon (2009a).

12 orders; Wright and Bowmaker 2001, Ödeen and Håstad 2003, Håstad et al. 2005b, Ödeen et al. 2009). One recent analysis of reflectance data from ~1,000 species concluded that UVS might be more prevalent in birds than molecular data suggest (Mullen and Pohland 2008), but this was mainly based on the idea that plumage reflectance spectra can predict photoreceptor physiology, an assumption that has yet to be tested.

The UV contribution to plumage color is thought to be the main source of disparity in human and avian perceptions of bird coloration, and of sexual differences in particular (e.g., Hunt et al. 1998). This may hold true even in birds with VS vision, because their sensitivity to UV wavelengths remains greater than that of humans. Nonetheless, we found that UV dichromatism in antbirds

was relatively minor. A comparison between the sexes revealed that male antbirds reflected more incident UV wavelengths than females (Fig. 2B), but levels of UV reflectance were generally low across all plumage patches in all 71 species. Overall, dichromatism was heavily biased toward the human-visible part of the spectrum: in each body region, mean reflectance in the UV was greatly exceeded by mean reflectance in human-visible wavelengths (Figs. 1 and 2A).

Low UV reflectance makes sense because antbird plumage is dominated by black, brown, and rufous (Zimmer and Isler 2003, Tobias and Seddon 2009a), colors that in other taxa have been shown to reflect only modest amounts of UV (Eaton and Lanyon 2003). Reflectance spectra may, in turn, be linked to ecology. For example, many antbirds occur in dense vegetation, including the understory of tropical forest (Zimmer and Isler 2003), a habitat penetrated by low levels of UV light (Endler 1993) and generally associated with low or moderate UV reflectance in avian plumage (Gomez and Théry 2004, 2007). It is tempting to conclude that UV wavelengths are unlikely to play an important functional role in antbird plumage signals. However, absolute levels of UV reflectance convey little about biological meaning, given that several studies have shown that intraspecific interactions can be mediated by very minor (<5%) differences in UV reflectance (e.g., Bennett et al. 1997, Andersson et al. 1998, Siitari et al. 2002). Further behavioral experiments would be required to ascertain whether UV reflectance has any important signaling function in antbirds.

Use of human vision as a proxy for avian perception of dichromatism.—Most evidence of discordance between human and bird perception of coloration stems from species in families well known to possess UVS (Andersson and Amundsen 1997, Bennett et al. 1997, Hunt et al. 1998, Arnold et al. 2002, Eaton 2006). Consequently, the incidence of hidden sexual dichromatism in birds with VS visual physiology remains largely unknown. It is of interest, therefore, that all four species in our sample that look monochromatic to humans had jnd scores >1.0, the standard threshold for avian color discrimination (Vorobyev and Osorio 1998), which suggests that they look sexually dichromatic to birds. In other words, our results confirm that cryptic dichromatism is as prevalent in antbirds as it is in other families (Eaton 2005, 2007).

The validity of human-generated measures of dichromatism is not undermined if differences between the sexes are sometimes cryptic, as long as human and avian perceptions of those differences are sufficiently correlated. A direct assessment of this relationship across 71 antbird species showed that scores generated by human observers explained a reasonable proportion (34%) of variation in avian-perceived dichromatism. The strength of this relationship suggests that human-generated scores may give a fair approximation of dichromatism perceived by antbirds, at least for the purposes of comparative analyses. This was confirmed when we re-ran an earlier analysis and found that replacing dichromatism[human] with dichromatism[avian] did not alter the conclusions (Fig. 4).

There is little doubt that estimates of perceived levels of dichromatism can always be improved by using spectral data and visual models. For this reason, we strongly advocate the use of spectrophotometry and color-discrimination models, particularly for studies of intraspecific variation in plumage color (Eaton 2005, 2007; Håstad and Ödeen 2008). When appropriate data are unavailable, however, our results indicate that the positive relationship between human and avian perception of dichromatism may be sufficiently strong that the former serves as a valid proxy for the latter in interspecific comparative analyses. This is clearly the case in antbirds, and potentially in other groups with VS visual physiology and variable levels of dichromatism. We are less able to extend our conclusions to avian families in which plumage reflectance spectra are more complex or UV-rich, because in these cases human vision performs less well in relation to bird vision (Vorobyev et al. 1998). Nonetheless, given that human-visible dichromatism and VS photoreceptors are widespread, our findings provide broad support for the conclusions of previous comparative studies of the causes and consequences of sexual dichromatism.

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