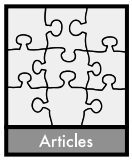

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Do museum specimens accurately represent wild birds? A case study of carotenoid, melanin, and structural colours in long-tailed manakins *Chiroxiphia linearis*

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Museum specimens continue to be an invaluable resource for taxonomic, systematic, and comparative studies, and are increasingly relied upon for novel research purposes. Evaluating variation in the colour of avian study skins forms the basis for a broad range of research questions, yet few studies have investigated whether the plumage colouration of museum specimens accurately reflects colouration in wild birds. In this study, we use reflectance spectrometry to compare the plumage reflectance of avian museum skins and wild birds. We use long-tailed manakins *Chiroxiphia linearis*, to investigate these potential differences in colour. Long-tailed manakins are ideal for this type of study as their colourful plumage patches result from three primary plumage colouration mechanisms found in birds: melanin pigmentation, carotenoid pigmentation, and structural colouration. These features of their plumage allowed us to independently assess variation in each plumage colouration mechanism. Reflectance spectra obtained from museum specimens were very similar to those obtained from wild birds, and the colouration of specimens was usually well within the range of variation observed in wild birds. As such, museum specimens can accurately represent the colouration of wild birds. Nevertheless, we found significant differences in colouration between museum skins and wild birds. We documented differences in brightness, hue, saturation, and chroma, although the direction and magnitude of these differences varied by mechanism of colouration. Multivariate analyses revealed that the age of museum specimens and the time of year at which they were collected contributed to some of these differences. We discuss potential proximate causes of these changes in colour, many of which apply to both museum specimens and wild birds, and identify the types of studies that are likely to be most sensitive to these changes.

Studies based on museum specimens form the foundation of our understanding of the diversity of life on earth (Winker 2004). Museum specimens continue to be an invaluable resource for taxonomic, systematic, and comparative studies and are increasingly being used for previously unanticipated purposes (Winker 2004). Many contemporary studies use data from museum specimens as windows into the past, for example, to investigate the effects of nuclear disasters (Ellegren et al. 1997) or habitat degradation (Lens et al. 2002) on populations, to document historical changes in diet (Norris et al. 2007), and to assess ecosystem-level changes in the environment (Schell 2000). With museum specimens being put to such diverse and important uses, it seems reasonable to question whether specimens are an accurate representation of wild animals. Clearly, for certain relatively stable features such as morphology and genetic or isotopic signature, the answer is yes. However, some features, such as the colouration of bare parts, fur, or feathers of museum study skins, might be expected to change over time (e.g., Test 1940, Gabrielson and Lincoln 1951, McNett and Marchetti 2005), or be influenced by the specimen preparation or preservation process (e.g., Montgomerie 2006b, Pohland and Mullen 2006).

Evaluating variation in the colour of avian museum skins forms the basis for a broad range of research questions. The colour of study skins can be used as a taxonomic marker (e.g., Brumfield and Remsen 1996, Prum 1997, Johnson et al. 1998), to sex or age birds (e.g., Jeffrey et al. 1993, Doucet et al. 2007a), or to document plumage reflectance patterns of a particular species or group of species (e.g., Brumfield et al. 2001). Moreover, colour itself is often the primary target of museum-based studies. Researchers have measured the colour of study skins, for example, to reveal unusual reflectance patterns or patterns invisible to humans (Finger and Burkhardt 1994, Andersson 1996, Eaton and Lanyon 2003), to document historical changes in colour (Zahn and Rothstein 1999, but see Hill 2001), to examine patterns of sexual dichromatism (Mahler and Kempenaers 2002, Eaton 2005, Hofmann et al. 2007), to investigate patterns of geographic variation (Chui and Doucet 2009), or to test broad–scale predictions about the evolution of particular types of colour signals (Endler and Théry 1996, Owens and Hartley 1998, Hausmann et al. 2003, Heindl

and Winkler 2003, Gomez and Théry 2004, Doucet et al. 2007b). These studies highlight the importance of ascertaining that the plumage colours of study skins are representative of the colouration of wild birds.

Until recently, studies of plumage colouration relied on human visual assessments of colour. It has become increasingly clear, however, that the avian visual system differs considerably from that of humans (Cuthill 2006). Among other differences, many bird species can see ultraviolet wavelengths (Cuthill 2006). Such fundamental differences between human and avian vision have led to the development of improved methods for quantifying plumage colouration, such as full–spectrum reflectance spectrometry (Bennett et al. 1994, Cuthill et al. 1999, Andersson and Prager 2006). Our goal in this study was to use reflectance spectrometry to determine whether the plumage colouration of museum skins accurately represents the plumage colouration of wild birds. A number of studies have published qualitative observations of dulling, fading, and foxing of museum specimens (Gabrielson and Lincoln 1951, Endler and Théry 1996, Eaton and Lanyon 2003, Hausmann et al. 2003). A few recent studies also present reflectance spectra for both museum specimens and wild birds (Mahler and Kempenaers 2002, Parker et al. 2003, Jouventin et al. 2005). Because the assessment of museum specimens was not the primary aim of these studies, however, the authors did not usually include statistical analyses, account for other sources of variation in colour, or use identical techniques for measuring specimens and wild birds (Mahler and Kempenaers 2002, Parker et al. 2003, Jouventin et al. 2005). Recently, McNett and Marchetti (2005) published a comprehensive examination of the effects of feather wear in wood warbler specimens. They found that museum specimens were substantially duller than wild birds, and that this pattern was more pronounced for the UV part of the spectrum (McNett and Marchetti 2005). Another recent study found that across five passerine species, fading was minimal for specimens collected in the past 50 years (Armenta et al. 2008).

Here, we use full–spectrum reflectance spectrometry to compare the plumage reflectance of wild–caught long–tailed manakins *Chiroxiphia linearis*, with museum skins of this species. Long–tailed manakins are ideal for this type of investigation, as their colourful plumage patches are produced by three primary colouration mechanisms in birds: carotenoid pigmentation, melanin pigmentation, and structural colouration. Definitive–plumaged adult males have bright red carotenoid–based crown patches, structural sky–blue mantles, and melanin–based black body plumage and flight feathers (Doucet et al. 2007a). These features of manakin plumage ornamentation allowed us to independently assess the potential effects of wear or fading on each of these mechanisms. We also evaluate the effects of specimen age, sampling location, and seasonal variation in plumage colour.

Methods

Study system

Long–tailed manakins are distributed along the Pacific coast of North and Central America from southeastern Mexico to northwestern Costa Rica. The sexes are strongly sexually dichromatic and males progress through a series of age–specific predefinitive plumages before attaining their definitive adult plumage in their fifth year (Doucet et al. 2007a). We used biochemical and microscopic techniques to confirm the mechanisms of colour production in feathers collected from definitive-plumaged male manakins. Most red plumage in passerines birds results from the deposition of carotenoids into growing feathers (McGraw 2006a). To confirm that this mechanism was also responsible for the red crown colouration of adult male long-tailed manakins, we used a thermochemical extraction procedure and high performance liquid chromatography to identify specific carotenoids in the red crowns of adult males (Doucet and McGraw, unpubl. data, McGraw et al. 2005). Most black plumage in birds results from the deposition of melanin pigments into growing feathers (McGraw 2006b). To confirm that this mechanism was also responsible for the black colouration of body and flight feathers in adult male long–tailed manakins, we compared the reflectance spectra of black body regions in long-tailed manakins to known melanin-based spectra (Mennill et al. 2003, McGraw 2006b) and confirmed the presence of pigment granules of the appropriate shape, size, and density in transmission electron micrographs (Doucet et al. 2004, S. M. Doucet, unpubl. data). All non–iridescent blue plumage colours in birds investigated to date result from the constructive interference of light scattered by materials of different refractive indices (air and keratin) within the medullary keratin matrix of feather barbs (Prum 2006). To confirm that this mechanism was also responsible for blue colouration in long-tailed manakins, we immersed blue feathers in liquid of the same refractive index as keratin and found that the blue colour disappeared, demonstrating that the colour is produced structurally (Mason 1923, Shawkey and Hill 2006). We also used transmission electron microscopy to identify the presence of a medullary keratin matrix similar to that known to produce blue and ultraviolet colouration in several passerine birds (Prum et al. 1999, Prum et al. 2003, Shawkey et al. 2003a, Doucet et al. 2004).

Field methods

From March to July 2003 and 2004, we studied long–tailed manakins at Santa Rosa National Park, Guanacaste conservation area, Costa Rica (10° 40′N, 85° 30′W). We used mist nets to capture 58 definitive-plumaged male manakins at or near lek sites (all birds captured between 05.00 and 12.00 CST). We fitted each bird with a numbered aluminum leg band and a unique combination of three coloured leg bands. We measured the spectral reflectance of wild birds using an Ocean Optics USB 2000 spectrometer and PX–2 flash lamp (Ocean Optics, Dunedin, FL); see Andersson and Prager (2006) for a review of colour measurement techniques. Our reflectance probe was mounted in a black rubber probe holder, which excluded all external light and maintained the probe at a fixed distance (5 mm) from, and perpendicular to, the feather surface. We measured the reflectance of five body regions, namely the crown, mantle, tail, wing, and breast of each bird. We recorded five measurements per body region, each

of which comprised an average of 10 readings collected in rapid succession by the operating software. Because the breast, wing, and tail were of similar colouration within birds, we averaged these together and refer to them as 'body colour' throughout. All reflectance data are expressed as the percentage of reflectance from a white standard (WS–1, Ocean Optics). We collected 5–10 crown and mantle feathers and the right outer rectrix of each bird for biochemical and structural analysis of colour production mechanisms (described above).

Museum data

We visited the collections of the Louisiana State University Museum of Natural Science in March of 2003 and the American Museum of Natural History in January of 2004. We measured the plumage reflectance of the study skins of 55 definitive-plumaged male long–tailed manakins as described above for wild birds. At both museums, we measured all definitive-plumaged male specimens that were not grossly damaged. We recorded the collection date of the specimens indicated on specimen tags when available; these ranged from 1871 to 1962. We also recorded the location of collection indicated on specimen tags when available. We then used an online database to obtain the latitude and longitude coordinates of all specimen collection locations and recorded these to the nearest degree. If only a broad region such as a state or province was identified on specimen tags, we recorded the coordinates of the approximate center of the distribution of long–tailed manakins within that region. These data allowed us to control for sampling location in our analyses, an important factor to consider since subspecific distinctions in this species are based in part on colour (Snow 2004).

Colourimetric variables

For each body region, we calculated three colour variables to approximate the three principal dimensions of colour: brightness, hue, and saturation (Hailman 1977, Andersson and Prager 2006, Montgomerie 2006a). For all body regions, we calculated brightness as the total reflectance across the bird visible spectrum from 300 nm to 700 nm. We calculated saturation, a measure of spectral purity, as the maximum reflectance minus the minimum reflectance divided by the total reflectance. Because of large differences in the shape of reflectance spectra between different body regions (Fig. 1), we used different methods for calculating the hue of each region in order to ensure that this variable captured relevant information. For the blue mantle and black body, we calculated hue as the wavelength of maximum reflectance. It should be noted that it is rather difficult to measure hue accurately for black colours, since their reflectance spectra are nearly flat. For the red crown, we calculated hue as the wavelength at which reflectance reached 50% of its maximum. To examine whether variation in reflectance between wild birds and study skins was evenly distributed across the visual spectrum of birds, we calculated four measures of chroma. To do this, we divided the visual spectrum into four 100–nm segments and

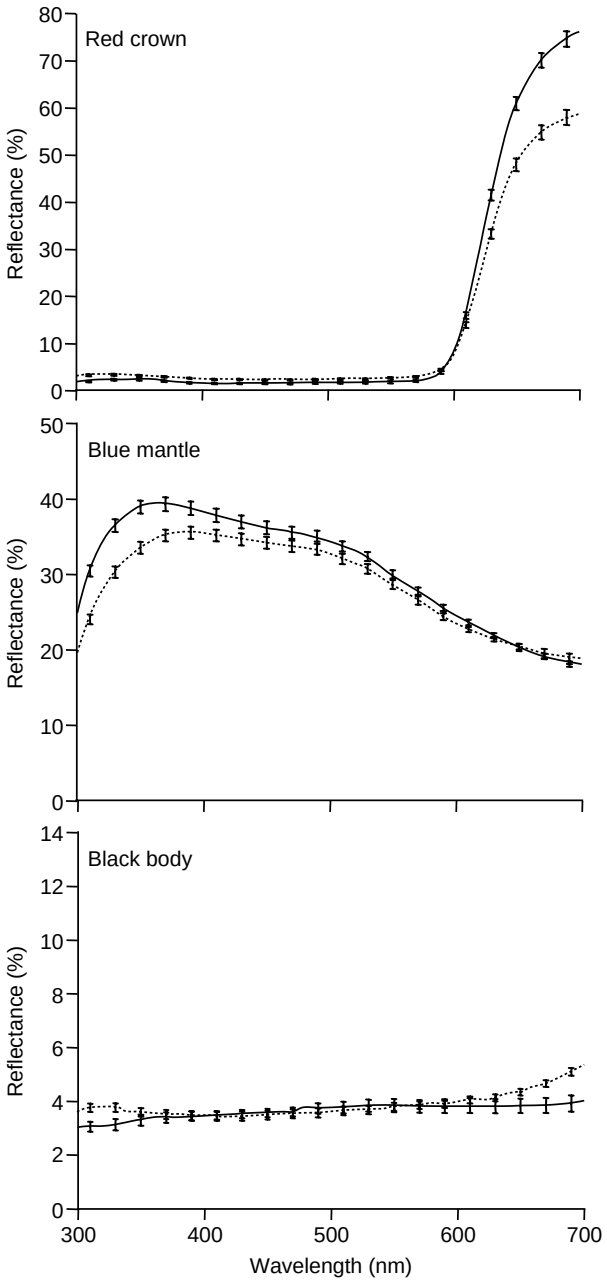

Figure 1. Mean reflectance spectra for the red crown, blue mantle, and black body among male long–tailed manakins in definitive adult plumage. Solid lines represent live–caught males (n = 58), and dashed lines represent study skins (n = 55).

divided the reflectance in each segment by the total reflectance. We refer to these as UV chroma (300–400 nm), blue chroma (400–500 nm), green chroma (500–600 nm), and red chroma (600–700 nm).

Statistical analyses

Because the distribution of long–tailed manakins follows a roughly northwest to southeast axis (Snow 2004), latitude and longitude coordinates of sampling locations were highly correlated ($r_s = 0.87$, $n = 111$, $P < 0.001$). We used

principal components analysis to combine these two variables into one index of location. Both latitude and longitude loaded highly and positively (component loadings = 0.71) on the first principal component (PC1), which explained 98% of the variation in these coordinates. We therefore used PC1 as a measure of geographic location in our analyses. We used Shapiro–Wilk tests to determine whether the distribution of the original variables, or of their residuals, met normality assumptions as required by t-tests or regression analyses. Where standard transformations did not improve the fit to normality, we used non–parametric tests. Slight variations in sample size arose because not all information was available from all individuals/specimens.

Results

Plumage reflectance of long–tailed manakins

Adult male long–tailed manakins have highly saturated red crown patches that reflect very little at short and medium wavelengths, but increase steeply in reflectance at long wavelengths (Fig. 1). By contrast, their blue mantles reflect maximally at ultraviolet wavelengths and exhibit lower reflectance at long wavelengths (Fig. 1). The black body plumage of adult male manakins exhibits low, uniform reflectance across the bird–visible spectrum (Fig. 1). These reflectance spectra show that in comparison with wild birds, the red crowns of museum specimens reflect more at UV wavelengths and less at longer wavelengths, their blue mantles reflect less at shorter wavelengths, and their black bodies reflect more at very short and very long wavelengths.

Overall differences between museum specimens and wild birds

There were significant differences in plumage colouration between museum skins and wild male long–tailed manakins in definitive plumage. In particular, wild males had significantly brighter and more highly saturated red crown colouration than study skins (Fig. 1 and 2; brightness: $T = 4.31$, $n_1 = 56$, $n_2 = 54$, $P < 0.001$; saturation: $T = -6.39$, $n_1 = 56$, $n_2 = 54$, $P < 0.001$). Wild males also had significantly brighter blue mantles that peaked at shorter (more UV) wavelengths than study skins (Fig. 1 and 2; brightness: $T = 4.07$, $n_1 = 56$, $n_2 = 55$, $P < 0.001$; hue: $Z = 7.54$, $n_1 = 56$, $n_2 = 55$, $P < 0.001$). The black body colouration of live–caught males was also significantly darker and less saturated and peaked at significantly shorter wavelengths than that of museum skins (Fig. 1 and 2; brightness: $Z = -3.43$, $n_1 = 56$, $n_2 = 54$, $P = 0.001$; hue: $Z = 5.46$, $n_1 = 56$, $n_2 = 54$, $P < 0.001$; saturation: $Z = 5.04$, $n_1 = 56$, $n_2 = 54$, $P < 0.001$).

Effects of specimen age

To determine whether differences in plumage colouration between wild males and study skins might have resulted from degradation in colour over time, we investigated the relationship between plumage colour variables and the age of museum specimens. Because of the significant differences between wild birds and study skins documented above, we restricted this analysis to museum specimens. We found that the brightness of the red crown increased with specimen age (Fig. 3; $r = 0.60$, $n = 54$, $P < 0.001$). Similarly, the hue of the blue mantle increased with specimen

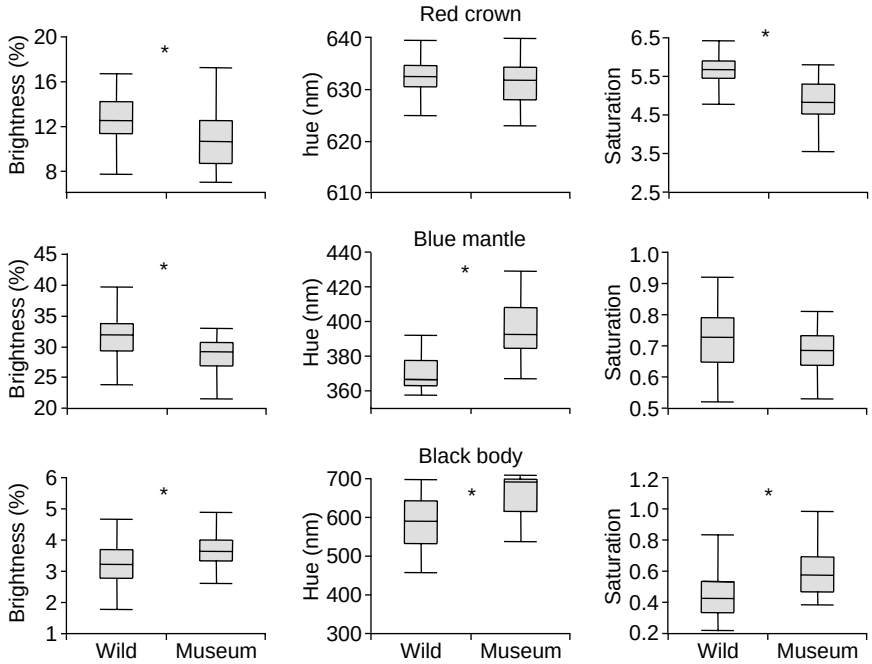

Figure 2. Box plots showing differences in plumage colour variables between live–caught male long–tailed manakins in definitive adult plumage and study skins. Horizontal lines in box plots show 10th, 25th, 50th, 75th, and 90th percentiles. Asterisks identify differences that are statistically significant (see text for details).

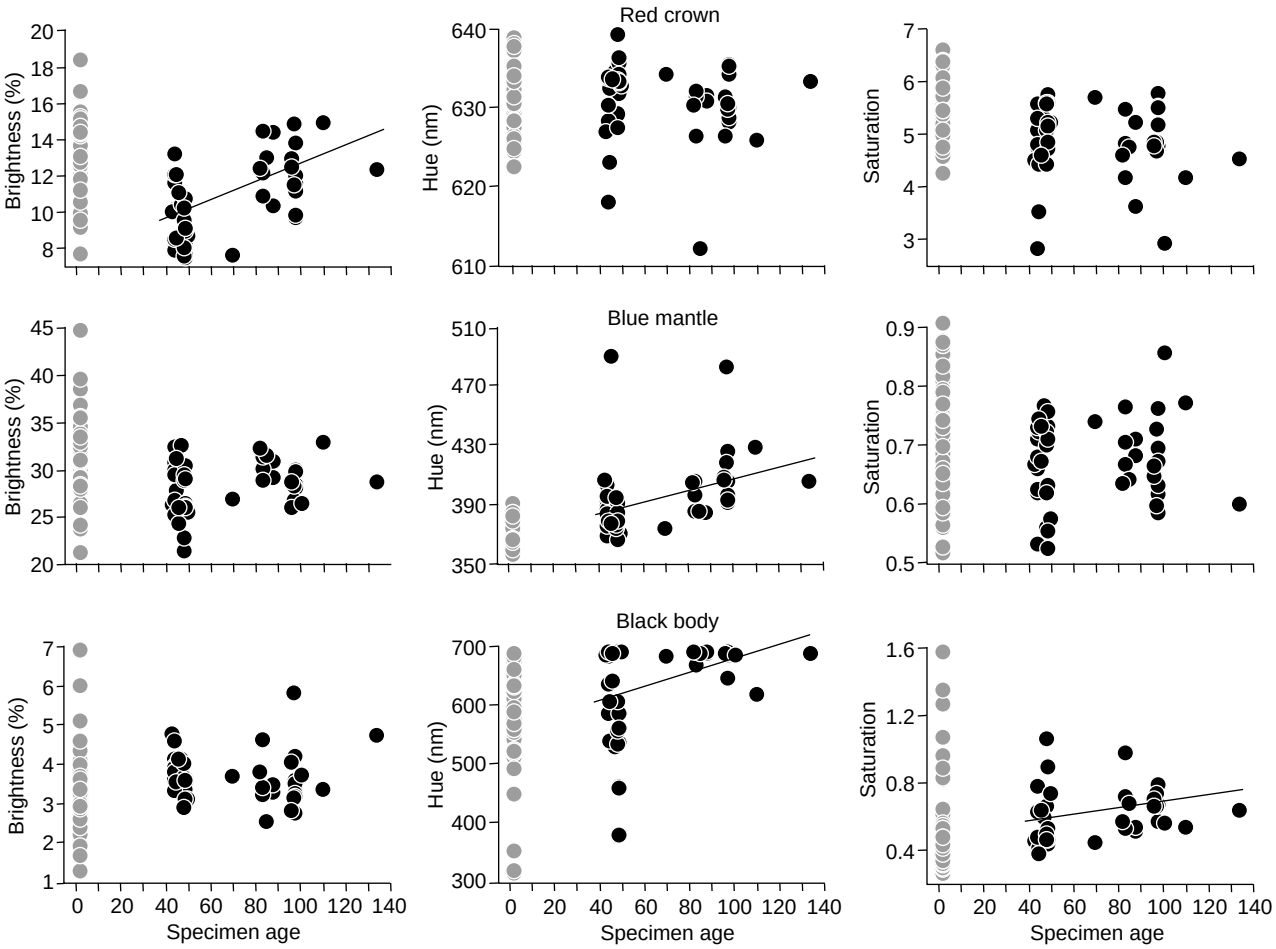

Figure 3. Scatterplots showing relationship between plumage colour variables and specimen age among male long–tailed manakins in definitive adult plumage. Wild birds are represented by grey circles, whereas museum specimens are represented by black circles. Because there are significant colour differences between museum specimens and wild birds (see Fig. 2 and text), we restricted our correlation analyses to museum specimens, and include wild birds as a reference only. Regression lines are included in plots that show statistically significant correlations between colouration and specimen age (see text for details).

age, such that newer specimens peaked at shorter (more UV) wavelengths than older specimens (Fig. 3; $r = 0.39$, $n = 46$, $P = 0.008$). Finally, both the hue and the saturation of the black body increased with specimen age (Fig. 3; hue: $r_s = 0.33$, $n = 45$, $P = 0.02$; saturation: $r_s = 0.37$, $n = 45$, $P = 0.005$). None of the other colourimetric variables were significantly related to the age of museum specimens (all $P > 0.15$).

Multivariate analyses

The analyses above show that there are significant differences between museum specimens and wild birds, and that the age of the study skins might also affect colouration. Univariate tests also revealed effects of sampling location and the time of year at which specimens were collected (unpubl. data). Because univariate relationships could be confounded by any of these variables, we used multivariate analyses to assess their relative influence on plumage colouration. We found that red crown colouration was significantly brighter and more saturated in live birds than in museum specimens, and older specimens had brighter red crowns than younger specimens (Table 1). Additionally, the hue of the blue mantle was significantly affected by specimen age, with older specimens peaking at longer wavelengths, away from the UV (Table 1). Finally, the hue of the black body plumage was significantly affected by the timing of collection, with birds collected during the breeding season peaking at significantly longer wavelengths than birds collected during the non-breeding season (Table 1).

To determine whether the relative influence of specimen age on plumage colour varied across the bird-visible spectrum, we used similar multivariate analyses to assess variation in four measures of chroma for each body region. The red crown colouration of museum specimens reflected proportionally more at UV and blue wavelengths, and proportionally less at red wavelengths, than wild birds (Table 2). By contrast, the blue mantles of museum specimens reflected proportionally less at UV wavelengths, and proportionally more at green wavelengths than wild birds (Table 2). Finally, the black body plumage of museum specimens reflected proportionally more at red wavelengths than wild birds; red chroma also increased with the age of museum specimens and was higher in birds captured during

Table 1. Multivariate analyses of hue, brightness and saturation in adult male long-tailed manakins. Model effects include type (museum specimen vs. wild bird), age (the number of years since a specimen was collected), location (PC score of collection location), and season (breeding vs. non-breeding). For all analyses, degrees of freedom are Model: 4, 94, Type: 1, 98, Age: 1, 98, Location: 1, 98, Season: 1, 98.

Body region		Brightness (%)			Hue (nm)			Saturation		
		F	R^2/ß	P	F	R^2/ß	P	F	R^2/ß	P
Red crown	Model	13.8	0.37	<0.001*	1.39	0.06	0.24	10.6	0.31	<0.001*
	Type	9.98	0.92	0.002*	2.48	0.56	0.12	6.96	0.80	0.01*
	Age	8.53	0.66	0.004*	0.77	0.24	0.38	0.32	0.13	0.57
	Location	1.62	−0.16	0.21	4.35	0.33	0.04	0.91	0.13	0.34
	Season	0.40	0.06	0.52	0.48	−0.08	0.48	1.34	−0.12	0.25
Blue mantle	Model	4.88	0.17	0.001*	31.1	0.57	<0.001*	2.64	0.10	0.04
	Type	0.80	0.30	0.37	0.83	−0.21	0.36	0.11	0.12	0.73
	Age	0.00	0.01	0.96	11.8	0.65	0.001*	0.15	−0.10	0.70
	Location	1.58	−0.18	0.21	2.09	−0.17	0.15	0.88	−0.14	0.35
	Season	0.15	−0.04	0.70	4.82	0.17	0.03	0.00	0.00	0.97
Black body	Model	1.67	0.07	0.16	10.4	0.31	<0.001*	4.22	0.15	0.003*
	Type	2.08	−0.51	0.15	2.32	−0.46	0.13	0.00	0.01	0.95
	Age	0.35	−0.16	0.56	0.91	0.23	0.34	2.17	0.30	0.14
	Location	0.58	−0.12	0.45	2.68	−0.22	0.10	0.04	−0.03	0.84
	Season	1.70	0.15	0.19	13.3	0.37	0.001*	0.32	−0.06	0.57

*Significant after sequential Bonferroni correction within each plumage patch.

the non-breeding season than birds captured during the breeding season (Table 2).

Discussion

In this study, we used long-tailed manakins to evaluate whether the colouration of museum specimens can be used to accurately represent wild birds. We found that reflectance spectra collected from museum specimens were very similar to those measured from wild birds for carotenoid, melanin, and structural plumage (Fig. 1). Interestingly, variation in reflectance in a single population of wild birds often matched or exceeded variation in museum specimens spanning the entire geographic distribution of long-tailed manakins over a period of more than one hundred years (Fig. 3). These observations suggest that in general, museum specimens reliably represent the colouration of wild birds. Nevertheless, we documented significant plumage colour differences between museum specimens and wild birds. Moreover, colours produced by different mechanisms showed spectrally different patterns of degradation. These differences, however, were subtler than those documented in other studies (Mahler and Kempenaers 2002, Parker et al. 2003, McNett and Marchetti 2005). Below, we review potential sources of differences in plumage colouration between study skins and wild birds and discuss the implications of our findings.

Differences between museum specimens and wild birds

We found significant differences in plumage reflectance between museum specimens and wild bids, some of which persisted even when we controlled for other influential variables (Table 1 and 2). Some of these differences may result from the specimen preparation process *per se*.

Table 2. Multivariate analyses of chroma variables in adult male long-tailed manakins. Model effects include type (museum specimen vs. wild bird), age (the number of years since a specimen was collected), location (PC score of collection location), and season (breeding vs. non-breeding). For all analyses, degrees of freedom are model: 4, 94, type: 1, 98, age: 1, 98, location: 1, 98, and season: 1, 98.

Body region		UV chroma			Blue chroma			Green chroma			Red chroma		
		F	R^2/ß	P	F	R^2/ß	P	F	R^2/ß	P	F	R^2/ß	P
Red crown	Model	17.4	0.43	<0.001*	16.7	0.42	<0.001*	11.5	0.33	<0.001*	16.2	0.41	<0.001*
	Type	6.78	−0.72	0.01*	6.22	−0.69	0.01*	4.15	−0.61	0.04	6.23	0.70	0.01*
	Age	0.42	−0.14	0.51	0.01	−0.02	0.91	0.03	0.04	0.87	0.05	0.05	0.83
	Location	0.62	0.09	0.43	0.08	−0.04	0.77	0.84	−0.12	0.36	0.02	0.02	0.90
	Season	0.14	0.04	0.71	0.05	0.02	0.83	0.01	0.01	0.92	0.06	−0.02	0.81
Blue mantle	Model	20.1	0.46	<0.001*	2.66	0.10	0.04	12.6	0.35	<0.001*	4.11	0.15	0.004*
	Type	0.07	0.07	0.78	0.45	−0.23	0.50	0.04	0.06	0.84	0.00	0.00	0.99
	Age	10.1	−0.67	0.002*	0.37	0.16	0.55	8.41	0.67	0.005	2.17	0.39	0.14
	Location	0.51	0.09	0.48	1.03	−0.16	0.31	0.09	−0.04	0.75	0.00	0.00	0.99
	Season	2.82	−0.15	0.10	2.94	0.20	0.08	0.8	0.09	0.37	0.07	0.03	0.79
Black body	Model	8.08	0.26	<0.001*	22.3	0.49	<0.001*	13.7	0.37	<0.001*	69.7	0.74	<0.001*
	Type	1.24	−0.35	0.27	4.72	0.57	0.03	3.65	0.56	0.05	8.44	−0.51	0.004*
	Age	2.96	−0.43	0.09	0.11	−0.07	0.74	1.18	0.25	0.28	11.4	0.47	0.001*
	Location	5.18	0.32	0.02	1.02	−0.12	0.31	3.13	−0.23	0.08	1.83	−0.11	0.18
	Season	2.93	−0.18	0.09	0.08	−0.03	0.77	1.60	0.12	0.21	6.35	0.15	0.01*

*Significant after sequential Bonferroni correction within each plumage patch.

Chemicals used as preservative agents are one possible source of colour change in museum specimens. Historically, a variety of chemicals were used, some of which were applied to the underside of skins. Fumigation is currently a common pest-deterrent strategy. Unfortunately, it is nearly impossible to determine whether or how preservative agents might have influenced our results, since preservation techniques change over the years, differ between various collectors and museums, and often remain undocumented. Accidental staining by some preservation agents has been shown to affect a small minority of specimens, although stains from preservation agents are generally uncommon, small, spectrally distinct, and often distinguishable by visual inspection (Pohland and Mullen 2006); none of the specimens we measured showed visual or spectral evidence of this type of contamination. Another possibility is that the feathers of study skins have been stripped of their uropygial oil or powder down secretions. Uropygial oils, which are secreted by the preen gland, create a protective sheen on the feathers that could potentially affect plumage brightness or saturation (Piersma et al. 1999, Reneerkens and Korsten 2004, Montgomerie 2006b). Several taxonomic groups (mostly non-passerines) also have powder down patches that secrete a greasy powdery substance thought to have similar functions to uropygial secretions (Wetmore 1920, Brown and Toft 1999). These secretions could be destroyed during the specimen preparation process through absorption by the corn meal or potato flour used to prepare specimens or by chemical breakdown when detergents are used to clean the skins. Alternatively, the oils might simply degrade over time. The potential effects of uropygial and powder down secretions on plumage reflectance need to be investigated in more detail. It should be noted, however, that washing museum skins during the specimen preparation process is often essential to remove blood, fat, or dirt from feathers, and that, in most cases, any plumage colour differences caused by the lack of preen or powder down secretions are likely to be much smaller than differences caused by heavily soiled plumage (Eaton and Lanyon 2003, Montgomerie 2006b). Indeed, Montgomerie (2006b) documented substantial differences in colour between visibly soiled specimens that had been washed during the preparation process and similar specimens which had been left unwashed; these differences persisted even after several years of storage.

General patterns of colour degradation

When controlling for other sources variation in colour, we found significant relationships between various aspects of plumage colour and the age of study skins. These data suggest that the plumage colours fade or degrade over time. Some of the general differences we documented between museum specimens and wild birds are also likely to have been caused by degradation. We attribute most sources of specimen degradation to one of three main categories: soiling, physical damage, and biochemical breakdown. Soiling may rank among the most important sources of colour degradation in museum specimens. For example, the long-term accumulation of dust is likely to affect the colouration of specimens. Similarly, the accumulation of oils through handling of specimens by researchers or through leaching from the specimens themselves may affect their colouration. Dust should decrease the brightness of most colours, although it could increase the brightness of very dark colours. Both dust and oils transferred by handling could also change the spectral shape of reflectance spectra, since dirt, fat, and proteins have absorption peaks in the UV (Andersson and Prager 2006). Mahler and Kempenaers (2002) noted that the Picui dove *Columbina picui*, specimens they measured were visibly dusty, and that they could approximate changes in reflectance spectra by adding dust to fresh feathers. Similarly, unpublished observations by Eaton and Lanyon (2003) suggest that human visual assessments of dirtiness can explain decreased UV reflectance in museum specimens. An interesting consequence of variation in soiling, as revealed by our data, is that newer specimens are not necessarily more similar to live birds. Sometimes, specimens that originated from private collections appeared visibly dusty, even if they were collected relatively recently, whereas some well-preserved older specimens were visually indistinguishable from newer specimens. We therefore suspect that the colouration of specimens is strongly affected by how well they were cared for.

Physical damage constitutes a second important source of specimen degradation. In particular, the abrasion or breakage of feather barbs or barbules could have an important influence on colouration. For example, Endler and Théry (1996) noted that dull specimens of the cock-of the rock, *Rupicola rupicola*, were covered in minute, broken, colourful barbules. Physical damage might also result from feather-degrading bacteria (Shawkey et al. 2007). Uropygial oil can suppress the growth of feather-degrading bacteria and fungus (Pugh 1972, Shawkey et al. 2003b). Thus, the removal or degradation of uropygial secretions on museum skins could effectively eliminate this protective mechanism. No study has investigated the direct effects of feather chewing lice or mites on plumage colouration; however, correlational and experimental studies suggest an association between louse or mite abundance and plumage colour (Thompson et al. 1997, Harper 1999, Figuerola et al. 2003). Although the effects of these ectoparasites on colour could be indirect, they might also affect plumage colouration directly in a manner similar to feather-degrading bacteria. Bactericides and insecticides are sometimes used in specimen preservation and likely reduce the potential effects of keratinolytic bacteria, fungi, and feather-chewing ectoparasites. Physical damage and biochemical breakdown are discussed in more detail in relation to specific colouration mechanisms below.

Mechanism-specific colour degradation

Interestingly, feathers from preserved specimens did not simply become duller or change in colour evenly across the spectrum, thereby suggesting that the cause of colour degradation varied, at least in part, according to the different colour production mechanisms. Carotenoid-based colours are produced by the deposition of carotenoid pigments into growing feathers (Fox and Vevers 1960, McGraw 2006a). The resulting colour depends on the

absorptive properties of the carotenoids in question (McGraw 2006a), and the underlying (usually white) structure of the feather (Shawkey and Hill 2006). Carotenoids can degrade by oxidization or isomerization (Test 1940, Britton 1995). Oxidization breaks down carotenoid pigments, resulting in pigment bleaching (Britton 1995). Since carotenoid breakdown will allow more of the underlying white microstructure to show through, it should result in increased brightness and UV reflectance (Shawkey and Hill 2006). Not all carotenoids are equally reactive to oxidization (Britton 1995), however, and this form of degradation will be more pronounced in species where more reactive carotenoids are used as pigments. Isomerization refers to changes in the stereochemistry (geometry) of carotenoid pigments (Britton 1995). Carotenoid pigments in nature, including those in bird feathers, are most often found in the all-*trans* form (Britton 1995, McGraw 2006a). Exposure to light, heat, oxygen, humidity, or acidity can promote the isomerization of carotenoids, resulting in less stable *cis*-isomers (Shi and Le Maguer 2000). The absorption maxima of carotenoid *cis*-isomers are usually located at shorter wavelengths than those of *trans* isomers, which may result in reduced UV reflectance (Lyan et al. 2001, Updike and Schwartz 2003). Additionally, the instability of *cis*-isomers often results in their eventual degradation. In our study, the reduced saturation and red chroma of the crown feathers of long-tailed manakin specimens, together with the increase in UV chroma and blue chroma, is more consistent with pigment degradation. Our findings contrast with those of McNett and Marchetti (2005), who found reduced UV reflectance for carotenoid colours in warbler specimens, a pattern they primarily attributed to pigment isomerization. Perhaps the types of carotenoids found in warblers are more susceptible to isomerization. Alternatively, the patterns they documented could have been caused by physical damage to the underlying microstructure or soiling.

Non-iridescent structural colours are produced by the constructive interference of light in the medullary keratin matrix of feather barbs (Prum et al. 1999, Prum 2006). In at least one species, variation in structural blue colour results from variation in the dimensions of components of the keratin matrix as well as variation in larger-scale factors such as the thickness of the feather cortex and the density of barbules (Shawkey et al. 2003a, Shawkey et al. 2005). Moreover, experimentally-induced degradation of the barb cortex and keratin matrix from keratinolytic bacteria increases brightness, hue, and saturation, and decreases chroma of structural blue feathers (Shawkey et al. 2007). Thus, structural blue colours may be particularly sensitive to damage caused by bacteria and lice. The age-related changes in hue and chroma of the blue mantle that we documented are consistent with either physical damage, parasitic degradation, or soiling.

Melanin-based colours are produced by the deposition of melanin pigments into growing feathers (McGraw 2006b). Eumelanins generally absorb light evenly across the bird-visible spectrum, resulting in flat reflectance spectra of low amplitude (Mennill et al. 2003, McGraw 2006b). As large polymers with strong cross-links to proteins, melanins are thought to confer structural stability to bird feathers (Burtt 1986, Bonser 1995). Melanized feathers are more resistant to abrasion (Bonser 1995, McGraw 2006b), and are less susceptible to feather chewing lice (Kose et al. 1999), and feather degrading bacteria (Goldstein et al. 2004). Moreover, melanin pigments are highly biochemically stable (Riley 1997). We therefore expected that, of the three types of colour we investigated, melanins would be the least susceptible to long-term degradation (Test 1940). However, we found significant, albeit subtle, variation in the black body plumage of long-tailed manakins. In particular, museum specimens had higher red chroma than live birds; this effect was especially pronounced in older specimens. One possible explanation is that these changes in colour result from physical or biochemical changes to other parts of the feather rather than the melanin granules. Alternatively, eumelanin pigments, which are black, may be more susceptible to degradation than phaeomelanin pigments, which are reddish-brown. Since many melanin-based colours contain both eumelanin and phaeomelanin (McGraw et al. 2004), a reduced proportion of eumelanin might result in higher reflectance at longer wavelengths (Test 1940). Yet another possibility is that biochemical degradation of eumelanin pigments alters their colour.

Seasonal and geographic variation

Differences between basic and alternate plumages aside, overall colour differences between study skins and wild birds could result from the different times of year at which specimens were collected. Seasonal variation only affected one of the variables in our study, although the majority of our birds were sampled within a four-month period during the breeding season. Generally speaking, however, seasonal variation is known to influence plumage colour (Örnborg et al. 2002, McGraw and Hill 2004, Delhey et al. 2006), and within-year variation in colour caused by wear is expected to be even greater in species that attain a bright nuptial plumage by wearing down the tips of their feathers (e.g., Lyon and Montgomerie 1995, Willoughby et al. 2002). These effects underscore the importance of considering annual variation in plumage colour when measuring museum specimens. When controlling for other variables, sampling location did not significantly affect colour in our sample of long-tailed manakins. However, univariate tests revealed clinal variation in colour across the range of this species (unpubl. data). Since geographic variation in long-tailed manakin plumage is subtle compared to that documented in other species, this potentially confounding variable should also be considered in museum-based studies.

Conclusions and implications

Interestingly, studies published to date reveal surprisingly different patterns of specimen degradation. McNett and Marchetti (2005), and Parker et al. (2003) found that parulid specimens had substantially lower UV reflectance than wild birds. Armenta et al. (2008) discovered that specimens collected within about 50 years showed little evidence of fading, and that susceptibility to fading varied across different types of colour. Endler and Théry (1996) noted that cock-of the rock specimens had lower chroma relative to live birds. Eaton and Lanyon (2003) present

(unpublished) evidence of a negative correlation between UV reflectance and specimen age. Hausmann et al. (2003) stated that brightness and chroma, but not hue, decreased with specimen age. Lastly, reflectance spectra published by Mahler and Kempenaers (2002) suggest that specimens can be brighter or duller than live birds, depending on the colour of the region measured. We suggest that general conclusions can be drawn from these seemingly discordant observations. First, different sources of degradation, such as soiling, physical damage, and biochemical breakdown, will have different effects on specimen colouration. Second, different mechanisms of colouration will vary in their susceptibility to different sources of degradation. Third, older specimens may be more degraded than newer specimens. Finally, other factors, such as seasonal variation and sampling location, can confound the effects of specimen age and should also be taken into account when investigating specimen degradation.

Together with other studies, our findings have important implications for colouration studies based on museum specimens. Importantly, differences between study skins and wild birds in our study were quite subtle (Fig. 1). As such, study skins can serve as a generally accurate representation of the plumage reflectance patterns of wild birds, particularly for studies that involve characterizing the reflectance of a particular species or testing hypotheses that rely on quantifying interspecific differences in colour. Other types of studies, such as studies of sexual dichromatism or geographic variation, will be more vulnerable to the effects of specimen degradation. In addition, because the magnitude and direction of changes in colour varied according to colour production mechanisms, studies focusing on particular spectral regions, such as the UV spectrum, may be confounded by divergent patterns of specimen degradation. To maximize the quality of specimen-based reflectance data, we suggest that researchers avoid specimens that appear visibly dirty and account for variation caused by specimen age, sampling location, and annual variation in colour by using these variables as covariates in multivariate analyses or by restricting samples to a particular time period, geographic location, and time of year. Our findings also highlight the importance of museum practices in maintaining not only the structural integrity of museum skins, but also the quality of their plumage colouration. Minimizing exposure to light, handling, bacteria, fungi, insects, and lice will help to preserve the quality and colour of study skins, which, in this era of reduced emphasis on collecting (Remsen 1995, Winker 2004), will allow researchers to benefit from their use well into the future.

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