Novel chromatic and structural biomarkers of diet in carotenoid-bearing plumage

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Previous attempts to establish a link between carotenoid-based plumage reflectance and diet have focused on spectral features within the human visible range (400–700 nm), particularly on the longer wavelengths (550–700 nm) that make these plumages appear yellow, orange or red. However, carotenoid reflectance spectra are intrinsically bimodal, with a less prominent but highly variable secondary reflectance peak at near-ultraviolet (UV; 320–400 nm) wavelengths visible to most birds but not to normal humans. Analysis of physical reflectance spectra of carotenoid-bearing plumages among trophically diverse tanagers (Thraupini, Emberizinae, Passeriformes) indicated that both the absolute and relative (to long visible wavelengths) amounts of short waveband (including UV) reflectance were lower in more frugivorous species. Striking modifications to the branched structure of feathers increased with frugivory. These associations were independent of phylogenetic relatedness, or other physical (specimen age, number of carotenoid-bearing patches) or ecological (body size, elevation) variables. By comparison, reflectance at longer visible wavelengths ('redness') was not consistently associated with diet. The reflectance patterns that distinguished frugivores should be more apparent to UV-sensitive birds than to UV-blind humans, but humans can perceive the higher plumage gloss produced by modified gross feather structure. Basic aspects of carotenoid chemistry suggest that increases in pigment concentration and feather dimensions reduce short waveband reflectance by the plumages of frugivores.

Keywords: feather; frugivory; pigment; tanager; ultraviolet; visual signal

1. INTRODUCTION

The extraordinary diversity of avian plumage colours challenges attempts to formulate unifying explanations. One generalization that has attracted considerable interest claims that the many yellow, orange and red colours based on carotenoid pigments are linked to what a bird eats (Slagsvold & Lifjeld 1985; Hill 1992; Hill *et al.* 2002; Fitze *et al.* 2003). The underlying premise of this 'diet scarcity' hypothesis is not controversial because a variety of studies indicate that birds, like other animals, must ingest carotenoids to use the characteristic long wavelength reflectance of these pigments for integumentary displays (Bjerkeng *et al.* 1990; Hill 1992, 1993; Putnam 1992; Linville & Breitwisch 1997; Inouye *et al.* 2001; McGraw *et al.* 2002). The diet scarcity hypothesis builds on this observation, however, in proposing that there is a direct connection between the kinds and amounts of carotenoids in the diet and those ultimately deposited in the plumage. The existence of such a link between diet and colour has potentially broad-reaching implications for theories of animal communication because any costs associated with the acquisition and deposition of carotenoid pigments could make them 'honest' signals of individual quality in relation to competition for limiting resources, including food and mates (Olson & Owens 1998; Hill 2002).

Despite the seemingly straightforward mechanism of dietary restriction on coloration, whether and how diet affects the expression of integumentary carotenoids remain contentious. It is well known that carotenoid-based colours in captive animals, fed artificial diets, can change dramatically, but the relevance of this phenomenon to wild

animals has been questioned (reviewed in Hill *et al.* 2002). Moreover, even when more natural diets are selected, quantitative effects of diet on carotenoid-based colour variation, within or between the sexes, appear to be relatively minor, possibly because a suite of physiological factors swamp the dietary ones (reviewed in McGraw *et al.* 2003). Conversely, sexual differences in carotenoid-based coloration occur even when males and females select the same diet (McGraw *et al.* 2003). It has also been claimed that plumage colour is shifted to longer 'redder' wavelengths (Hill 1996), or is more extensive (Mahler *et al.* 2003), or both, in species that ingest more carotenoids. Even a cursory survey reveals, however, that birds with redder and/or more extensive carotenoid-based colours are not limited to a particular trophic group.

Nevertheless, most tests of the diet scarcity hypothesis restrict the window of potentially informative variation to intraspecific (individual or intersexual) comparisons, and to the portion of the electromagnetic spectrum visible to humans. However, interspecific variation provides for greater distinctions in plumage and diet (Hill 1996; Mahler *et al.* 2003), and controls for sexual physiology, because comparisons can be restricted to one or the other sex. Moreover, carotenoid spectra exhibit a secondary reflectance peak in the near-ultraviolet (UV), wavelengths (Burkhardt 1989; Bleiweiss 2005) visible to most birds but not to normal humans (Cuthill *et al.* 2000; Hart *et al.* 2000). Both the visible (approximately 500–700 nm) and UV (approximately 300–400 nm) reflectance bands result from a strong absorption band over short visible wavelengths (approximately 400–500 nm), which creates (to

Figure 1. Reflectance spectra for carotenoid-based plumages of selected tanagers. Curves among panels are distinguished by location of cut-off reflectance (higher λR_{vis50} indicates redder hue), whereas curves within panels are distinguished by high (light line) or low (heavy line) UV (e.g. R_{UVpeak} and $R_{contrast}$ based on R_{min}). Taxa and patches are: (a) *Dubusia taeniata* (belly, light line, $R_{contrast} = 0.758$), *Tangara icterocephala* (rump, heavy line, $R_{contrast} = 0.925$); (b) *Rhodocichla rosea* (breast, light line, $R_{contrast} = 0.767$), *Ramphocelus flammigerus* (back, heavy line, $R_{contrast} = 0.945$); (c) different individuals of *Ramphocelus dimidiatus* (backs, distinguished by line weight, $R_{contrast} = 0.845$ – 0.860) collected in the same year. Spectral landmarks used for calculation of curve shape variables indicated on spectrum for *D. taeniata*. For all spectra, total reflectance relative to a $BaSO_4$ standard was measured in 1 nm intervals with a bandpass of 2 nm and a slit width of 4 mm. The background standard was measured, before acquisition of data for each specimen, with random selection of both specimen and then patch within specimen. A minimum of two scans was obtained for all yellow, orange or red plumage patches from among 13 standardized regions (table 1) large enough to be measured in the apparatus (excluding some narrow colour highlights on the eye, wing, thigh or vent). All patches comprised contour feathers. Shaded region indicates near-UV (320–400 nm), visible to most birds.

from the birds' ingestion and deposition of higher concentrations of carotenoids, augmented by modifications to feather dimensions.

2. MATERIAL AND METHODS

(a) Assessment of carotenoid characteristics

Reflectance spectra for human-visible (hereafter 'visible') yellow, orange and red plumage patches were recorded from single individuals of 53 species, and from two individuals of an additional three (*Ramphocelus* spp; see § 4) species (56 total species, comprising 27 or ca. 50% of recognized tanager genera; Isler & Isler 1999). Reflectance spectra over the range of wavelengths to which related birds are sensitive (320–700 nm; Cuthill *et al.* 2000; Hart *et al.* 2000) were generated with a Perkin-Elmer lambda-9 UV-VIS spectrophotometer equipped with a 60 mm integrating sphere (for details, see figure 1 and Bleiweiss 2005). Previous studies have demonstrated that carotenoids are largely responsible for yellow, orange and red plumages in tanagers (Brush 1967, 1970; Johnson & Brush 1972; Hudon 1989; Bleiweiss 2005). Excluded plumages combined carotenoids with other colour-generating mechanisms (blue structure or melanin pigment), which produce colours other than yellow, orange or red. Comparisons were limited to males to control for effects of various factors relating to sexual dichromatism (hormones, reproductive physiology, etc.).

(b) Variables of curve shape and feather structure

The spectral location of the reflectance band at long visible wavelengths (so-called 'cut-off' wavelength) was estimated as the value midway (λR_{vis50}) between that of minimum (λR_{min}) and maximum (at or near 700 nm) reflectance (figure 1a; Pryke *et al.* 2001). The absolute intensity of the UV peak was estimated as the maximum reflectance between 320–400 nm (R_{UVpeak} ; figure 1a). The relative intensity of the UV reflectance band ($R_{contrast}$) was estimated as the difference in reflectance between two spectral segments (non-overlapping wavebands; *sensu* Endler 1990), parti-

varying degrees) the bimodal profile (Ke *et al.* 1970; Bleiweiss 2005). Indeed, chemists use absorptive properties to analyse carotenoids because the atomic structure and concentration of the carotenoid molecules determine the shape and prominence of the absorption band in a very precise way. Thus, omitting UV wavelengths ignores the physical mechanism by which carotenoids produce colour, as well as how birds may perceive these pigments.

Herein, dietary correlates of carotenoid-based plumage reflectance are tested for trophically diverse species of tanager (Thraupini, Emberizinae, Passeriformes), a group of birds that are famous for their carotenoid-based plumage colours (Bleiweiss 2005). The emphasis on interspecific variation, and on UV reflectance, reveals potentially more general markers of diet based on properties of shorter waveband reflectance, and on modifications to the branched structure of feathers. The chemical properties of carotenoids suggest that reduced short waveband reflectance by plumages of frugivores results, in large part,

Table 1. Species-specific descriptive statistics for independent variables.

	minimum	maximum	average	s.d.
year ^a	1869	1982	1930.96	27.75
frugivory ^b	0.00	100.00	57.96	33.75
length ^c	11	21	16.01	2.62
elevational centre ^d	225	3200	1189.29	878.86
patch ^e	1	10	4.26	2.36

^a Year specimen collected; from museum tag.

^b Percentage of fruit in diet; from Isler & Isler (1999). Whenever possible, plumage of specimens was characterized from the geographic region, or subspecies for which diet data were available.

^c Total length (mm); from Isler & Isler (1999). Length, available for more taxa than mass, but significantly correlated with mass $r = 0.988$, $p < 0.00001$.

^d Average of minimum and maximum elevational occurrence (m), results similar with extremes; from Isler & Isler (1999).

^e Number carotenoid patches (see § 2a) from standard region: face, auriculars, forehead, crown, back, rump, throat, breast, belly, flank, thigh, wing, tail.

tioned by a segment divider (λd) and standardized to total reflectance ($R_{320-700}$), by the formula $[(R_{\lambda d-700}) - (R_{320-\lambda d}) / (R_{320-700})]$ (Pryke *et al.* 2001). Results for R_{contrast} calculated on the basis of either λR_{vis50} (Pryke *et al.* 2001) or λR_{min} as segment dividers, were compared because λR_{vis50} has been used in behavioural studies, but λR_{min} ($\bar{X} = 453$ nm, s.d. = 1.12) better partitions the reflectance curve between the long and short reflectance features ($\bar{X} = 553$ nm, s.d. = 2.12; see figure 1a). Results based on other measures of short-wavelength reflectance (e.g. $R_{320-400}$, R_{contrast} based on $\lambda d = 400$ nm) led to similar conclusions.

No specific psychometric interpretations of these variables of curve shape are necessary to determine their utility as biomarkers of diet. Pryke *et al.* (2001), however, determined that some preceding measures of curve shape explained significant variation in human colour perceptions of spectral location or hue (λR_{vis50} , 88%), and spectral purity or chroma (R_{contrast} based on λR_{vis50} , 75%), as estimated by the CIE system of human colour space (International Commission on Illumination 1971). Therefore, my measures of curve shape may approximate human or even avian (Endler 1990; Pryke *et al.* 2001) perceptions of colour, although the parameters I measured differ from those employed in psychology. In any case, these correlations validate use of λR_{vis50} , as a proxy for human perceptions, to test the association of diet with 'redness' (human-perceived hue variation, which excludes the UV).

In addition to their characteristic colours, the branching structure of pennaceous contour and flight feathers laden with carotenoids is often highly modified (Olson 1970; Brush 1990). These modifications (hereafter referred to as 'gross' structural changes, to distinguish them from internal nanoarchitectures that produce structural colours; Prum *et al.* 1999) can affect a feather's absorptive properties (Brush 1990). Modified feathers also have a strong surface gloss, which may be enhanced by the presence of a thick cuticle (Olson 1970). To determine if gross structural changes were associated with changes in diet, external feather structure was examined microscopically, and scored on an ordinal scale, based on distinctive changes to the multi-level branching structure of contour feathers, as follows: 1 = four-level branching including rachis, ramus, barbule and barbicules; 2 = loss of barbicules; 3 = distal fusion/loss of barbules; 4 = total fusion/loss of barbules; 5 = total fusion/loss of all branches (club-shaped; Innes 1979).

(c) Independent variables

Diet was quantified in terms of fruit consumption on the expectation that carotenoid content is broadly higher in fruits than in other dietary items such as insects (Goodwin 1984; Olson & Owens 1998). Trophic data were taken from the most recent monographic treatment of tanagers (Isler & Isler 1999). Each taxon received a diet index score, based on the proportion of fruit consumed. Diet data obtained by direct observations of foraging birds, although many diets were assessed from stomach contents alone. Diets based on stomach contents, and described only as having both fruit and animal material, were scored as 50% frugivorous. Sampled species range from entirely (100%) insectivorous to entirely frugivorous (table 1). This exceptional trophic divergence provides an excellent basis for evaluating links between diet and plumage reflectance. Literature values for several control variables (table 1) were entered into statistical analyses so that the effects of diet, independent of other potential affects on pigmentation, could be determined; control variables included collection year for each study skin (fading of pigmentation over time), body size (metabolism, life history relating to social behaviours), centre of elevational distribution (humidity, light environment) and number of carotenoid patches (see § 2a).

(d) Statistical analyses

All carotenoid-bearing patches were scanned at least twice and replicate curves were averaged for use in subsequent analyses. Differences in reflectance measurements, among replicate spectra for the same colour patch from the same or different individuals of the same taxon, were small (less than 2%, usually less than 1%; figure 1c), implying that inter-taxon variation far exceeds intra-taxon variation.

Multiple regressions, which contained all independent variables, were implemented, because the goal was to determine the association of spectral features with diet, while controlling for the potential effects of a shared set of additional independent variables (rather than implementing a stepwise procedure to determine the best predictive equation). Models that included all linear terms are reported (inspection of models with interactions failed to reveal any significant nonlinear responses). Data met assumptions of least-squares models following suitable transformations, as follows. Percentage of fruit in diet was arcsine-transformed because proportions form binomial rather than normal distributions (Sokal & Rohlf 1981). R_{contrast} values were squared to improve homogeneity of variance, which otherwise was significantly less among progressively more frugivorous species. The

square root of counts of carotenoid (red, orange, yellow) patches were taken, and the scores of feather structure were treated as ranks. All other continuous variables were natural log-transformed.

To incorporate phylogenetic information into statistical analyses, the method of independent contrasts was used (Felsenstein 1985), as implemented by software described in Garland *et al.* (1993, 1998). A working phylogenetic hypothesis for Thraupini (electronic Appendix A; see also Bleiweiss 2005) was constructed from the most recent molecular studies among genera (Burns (1997); see Banks *et al.* (2003) for qualifications on this phylogeny) and among species within genera (see Hackett 1996; Burns 1998), supplemented with hypotheses based on conventional taxonomy (Isler & Isler 1999). In the absence of branch-length estimates for most of the tree, speciation (unit branch lengths) change models were employed (Garland *et al.* 1992), which provided excellent standardization of contrasts (non-significant correlations between absolute values of standardized independent contrasts and their standard deviations; Garland *et al.* 1992) for all variables. Analyses presented here employed summary values representing the maximum, minimum and average values across all patches for each species. Other analyses, based on gradual (continuous branch lengths) models of change, and on distinguishing all carotenoid-based patches for each taxon (treated as soft polytomies in the phylogeny), led to similar conclusions.

Earlier studies lead to an expectation that increased redness (Hill 1996), reduced short-wavelength reflectance (Grether 2000; Saks *et al.* 2003) or even modification to feather structure (Brush 1990), should accompany ingestion of greater quantities of carotenoids (by more frugivorous species). Therefore, significance of directed (Rice & Gaines 1994), as well as two-tailed tests, are reported; general conclusions remain unchanged if only two-tailed probabilities are considered, however. The sequential Bonferroni-Holm method (Rice 1989) was used to adjust *p*-values within each multiple regression, as well as across summary values (average, minimum, maximum), when applicable. All statistical analyses were conducted in SAS for Windows (v. 8; SAS 2003).

3. RESULTS

(a) Independent variables

Considerable variation is present among values for all independent variables, which attests to a substantial diversification of tanagers with respect to diet, body size, elevational distribution and extent of carotenoid-based colours (table 1). No significant correlations were, however, observed among independent variables (table 2).

(b) Response of curve shape

All spectra had a major reflectance band that extended over longer (550–700 nm) visible wavelengths (figure 1). Spectra differed, however, in the prominence of a secondary reflectance band that extended from short visible (450 nm) through near-UV (320–400 nm) wavelengths (figure 1; Burkhardt 1989; Cuthill *et al.* 2000). Reflectance over the shorter waveband varied from less than 2% to *ca.* 20% of maximal visible reflectance; this nearly 10-fold difference is comparable to the variation observed at longer visible wavelengths (Bleiweiss 2005).

Four important results emerged from the multiple regression analyses (table 3; figure 2). First, both absolute (R_{UVpeak}) and relative ($R_{contrast}$ based on R_{min}) amounts of short-wavelength reflectance were significantly lower in

Table 2. Pearson correlation coefficients based on independent contrasts for independent variables. All $p > 0.15$.

	year	frugivory	length	elevational centre
year	—	—	—	—
frugivory	0.03825	—	—	—
length	0.00506	0.08092	—	—
elevational centre	0.03806	-0.01640	0.09102	—
patch ^a	-0.03648	0.04861	0.08908	-0.14161

^aSee table 1 for a description of the independent variables.

more frugivorous taxa (table 3; figure 2). Trends for absolute (R_{UVpeak}) and for relative ($R_{contrast}$ based on R_{min}) UV have opposite signs (figure 2) because a low value for the former corresponds to a high value for the latter. Notably, the relative measure of short wavelength reflectance that combines short with some long waveband reflectance ($R_{contrast}$ based on λR_{vis50}) was not significantly associated with diet. Second, gross modifications to branching feather structure (average and minimum) increased significantly in more frugivorous taxa. Third, measures of curve shape relating to total reflectance ($R_{320-700}$) and to 'redness' (λR_{vis50}), lacked strong and consistent associations with diet. Fourth, the control independent variables were not associated with absolute (R_{UVpeak}) or relative ($R_{contrast}$) levels of UV. These conclusions were strengthened when the outlier (contrast for yellow back feathers of *Chlorochrysa nitidissima*; see, especially, $R_{contrast}$ based on λR_{min} in figure 2) was removed (R. Bleiweiss, unpublished data).

4. DISCUSSION

The study indicates that frugivory is associated with a reduction in short-wavelength plumage reflectance, and with an increase in gross feather modifications that impart a higher plumage gloss. The stronger association of diet with shorter, compared with longer, wavelength reflectance seems paradoxical given the prominence of long wavelength reflectance by carotenoids (Bleiweiss 2005). Nevertheless, the weak associations observed between diet and longer wavelength ('redder') reflectance (figures 2 and 3) agree with results obtained by Hill *et al.* (2002) for individual wild house finches (*Carpodacus mexicanus*), suggesting a robust pattern. The large UV component to short wavelength reflectance should make visual distinctions between insectivores (high UV) and frugivores (low UV) more noticeable to UV sensitive birds (which include most oscine passerines like tanagers; Hart *et al.* 2000) than to UV-blind humans. Indeed, passerines respond behaviourally to differences in UV plumage reflectance far less (Andersson *et al.* 1998) than the variation documented among tanagers. The surface gloss associated with differences in gross feather structure should enhance the distinction between frugivores (high gloss) and insectivores (low gloss). Known chemical properties of carotenoids provide a strong basis for considering proximate and ecological causes of these novel biomarkers of diet.

Table 3. Multiple regressions on independent contrasts, using summary statistics for dependent variables.

	average		minimum		maximum	
	t^a	p^b	t	p	t	p
R_{UVpeak} (peak UV reflectance)						
year	0.910		1.200		0.660	
frugivory	-4.530	**	-5.290	**	-3.290	**
length	-0.570		-0.810		-0.160	
elevational						
centre	-1.610		-1.410		-1.830	
patch	0.290	**	-0.660	**	1.170	**
adjusted R^2	0.241		0.311		0.157	
$R_{contrast} (\lambda R_{min})^c$						
year	-0.780		-0.370		-1.190	
frugivory	4.370	**	4.010	**	4.350	**
length	-0.570		-0.800		-0.370	
elevational						
centre	1.940		1.960		1.600	
patch	-0.870		-2.130		0.070	
adjusted R^2	0.250		0.265		0.229	
$R_{contrast} (\lambda R_{vis50})$						
year	-2.100		-2.280		-1.710	
frugivory	0.360		-0.330		1.610	
length	-0.900		-0.910		-0.790	
elevational						
centre	0.750		0.450		0.910	
patch	-0.460		-1.160		0.180	
adjusted R^2	0.017		0.044		0.009	
$R_{320-700}$ (total reflectance)						
year	-0.150		-0.290		0.100	
frugivory	-2.610		-2.830		-1.930	
length	-0.850		-0.880		-0.700	
elevational						
centre	-1.000		-0.940		-0.950	
patch	-0.540		-1.690		0.530	
adjusted R^2	0.059		0.112		0.006	
λR_{vis50} (redness)						
year	1.280		0.950		1.620	
frugivory	2.310		1.620		2.910	*
length	1.970		1.750		2.030	
elevational						
centre	0.790		0.430		1.320	
patch	0.030		-0.280		0.380	
adjusted R^2	0.105		0.034		0.178	
feather structure ^d						
year	-0.780		-0.630		0.000	
frugivory	3.310	**	3.000	*	2.610	
length	0.340		-0.120		0.780	
elevational						
centre	2.450		2.040		2.880	
patch	-2.380		-2.870		-2.350	
adjusted R^2	0.244		0.232		0.233	

(a) Physico-chemical factors

Several empirical observations reinforce the chemical principle that bimodal reflectance spectra are an intrinsic property of carotenoids. First, pure extracts of carotenoids assayed *in vitro* produce similar bimodal spectra (Liebman & Granda 1975; Goldsmith *et al.* 1984), though chemical assays usually plot absorption spectra, which are inverted relative to reflectance (measure *in vitro*, as transmittance) spectra. Second, bimodal spectra are observed in such dif-

footnotes to table 3

^a A total of 59 patches (tip values). No significant collinearity among independent variables (see table 1). All regressions forced through the origin (Garland *et al.* 1992).

^b Bonferroni-Holm sequential method based on $k = 15$ [three indices (maximum, minimum, average) $\times$ five independent variables (year, diet, length, elevation, patch number)]: table-wide $\alpha = 0.05$, $p = 0.0033$ (two-tailed). Directed tests used $\gamma/\alpha = 0.8$ (see Rice & Gaines 1994) to set asymmetrical rejection regions, calculated from formula for results in the expected direction, which obtained in all cases. ** Significant for two-tailed tests at $p < 0.01$. * Significant for directed test at $p < 0.05$.

^c Term in parentheses after $R_{contrast}$ indicates segment divider used in calculation.

^d Analysis as ranks equivalent to Kruskal-Wallis k -sample test; F -test generated by parametric procedure applied to ranks better than χ^2 approximation used by Kruskal-Wallis (SAS 2003).

ferent carotenoid-laden structures as plumage (this study), flowers (Chittka *et al.* 1994) and retinal oil droplets (Goldsmith *et al.* 1984), suggesting that carotenoids underlie the bimodal reflectance in such different biological materials. Third, extraction of all carotenoids from (yellow) feathers changes the feathers' reflectance profile from bimodal to uniformly high ('white'; Mays *et al.* 2003). Internal nanostructures can also produce short-wavelength-specific reflectance (Prum *et al.* 1999). Removal of carotenoids, however, should not eliminate a distinct peak produced by nanostructures, which are formed from keratin bars and air spaces. Conversely, even amorphous media, such as *in vitro* solutions and oil droplets, can express the bimodal pattern, and a strong UV peak (measured as transmittance). Taken together, these patterns indicate that variation in carotenoid absorption dictates variation in bimodal reflectance observed in tanager feathers.

The data suggest that the reflectance qualities of plumage are also influenced by changes in gross feather structure, which parallel changes in degree of bimodality of spectra. A reduction in short-wavelength reflectance, owing to modifications in gross feather structure, is further implicated by exceptions to the general association between low UV reflectance and frugivory. Specifically, the yellow back feathers of *Chlorochrysa nitidissima* are as modified as those in frugivores, and reflect just as little UV, even though *C. nitidissima* is largely insectivorous (figure 2). Therefore, carotenoid pigments and gross feather structure may jointly alter reflectance among carotenoid-based plumages. The existence of chemical and physical determinants of reflectance is consistent with evidence that controls on gross feather structure are to some extent genetically distinct from those on carotenoid pigmentation (Brush 1990). The nature and independence of these effects, which may include pigment $\times$ feather protein interactions (Stradi *et al.* 1997), require further study.

In addition to these influences, differences in the overall reflectivity of the unpigmented feather matrix (whiteness; see Burkhardt 1989) may contribute to reflectance amplitude of visible and UV wavebands. Furthermore, concentrations of melanins, which absorb strongly in the UV, may alter reflectance amplitude, even in feathers that appear coloured entirely by carotenoids (Burkhardt 1989; Hudon 1989). However, deposition patterns for melanins depend, at least as much on endogenous metabolism, as on

Figure 2. Bivariate relationships (a–c) for independent contrasts of species-specific maximum values for R_{UVpeak} , $R_{contrast}$ (based on λR_{min}) and feather structure in relation to percent fruit in diet (not partial values that control for effects of other regressors). Term in parentheses after $R_{contrast}$ indicates segment divider used in calculation. For $R_{contrast}$ the datum representing the contrast between *Chlorochrysa nitidissima* and *C. calliparaea* (arrow) deviates markedly from the overall trend because short-wavelength reflectance by yellow back plumage in *C. nitidissima* is anomalously low for its largely insectivorous (87%) diet. Note significant correlations between variables of curve shape, or of feather structure with diet.

diet. Accounting for the effects of melanins on reflectance properties of feathers that also contain carotenoids should, therefore, improve associations between reflectance and diet.

(b) Dietary restrictions and plumage reflectance

According to the diet scarcity hypothesis, frugivores should ingest larger quantities of carotenoid-rich foods (fruits; Goodwin 1984), and, thus, deposit correspondingly more pigment in their feathers (Olson & Owens 1998). The reduction in short wavelength reflectance in frugivores (lower R_{UVpeak} or higher $R_{contrast}$) supports this prediction indirectly because increased carotenoid concentration

in vitro reduces short wavelength reflectance (usually measured as transmittance; Liebman & Granda 1975). For example, retinal oil droplets that contain higher carotenoid concentrations also have lower UV transmittance than droplets containing less pigment (Goldsmith *et al.* 1984). Ultraviolet transmittance also increases when droplets are diluted in mineral oil (Liebman & Granda 1975; Goldsmith *et al.* 1984). Similarly, carotenoids in *Ramphocelus* spp. plumage lack appreciable short-wavelength reflectance (figure 1b), but show high transmittance of short wavelengths when diluted *in vitro* (Brush 1970). Dilute carotenoids also generate more prominent local maxima and minima within the main absorbance band (400–500 nm), features also observed in plumage with more UV reflectance (figure 1a).

Higher concentrations of carotenoids in the plumages of frugivores can also be inferred from the positive association of $R_{contrast}$ with chroma (see §2) because a higher carotenoid concentration is strongly associated with a higher chroma (as calculated under a variety of analytical assumptions; compare results of Grether 2000 and Saks *et al.* 2003). An association between chroma, or $R_{contrast}$, and pigment concentration makes physical sense because both reflectance parameters estimate spectral contrast (deviations from uniformly high reflectance over all wavelengths, or ‘whiteness’). Given that carotenoids produce colour by the selective absorption of short wavelengths of light, increased carotenoid concentration should decrease uniformity of reflectance, and hence, increase values of chroma or $R_{contrast}$.

Changes in gross feather structure may contribute to associations between short-wavelength reflectance and diet because absorptive properties of pigments, including of carotenoids, depend on physical dimensions of the pigmented matrix (Liebman & Granda 1975). Notably, the modified feathers of frugivores are reduced to the thicker main branches (rachis and barb) of the feather. In similar feathers in other birds, the internal structure also changes such that the area laden with carotenoids (cortex) occupies the entire body of the feather barb (Olson 1970). These structural changes should lengthen the ‘optical’ path that light travels through the pigmented region, which should increase absorption (Liebman & Granda 1975). Although red shifts (higher λR_{vis50}) can result from changes in pigment concentration and optical path length, these shifts can also arise because of intrinsic differences in the absorptive properties of the pigment species, related to its atomic structure (Ke *et al.* 1970; Brush 1990). Therefore, the weaker association of redness with diet may arise, in part because pigment species are not associated with particular diets.

These considerations suggest that carotenoid-based plumage reflectance, particularly the short-wavelength component, can convey information about the physico-chemical composition, and hence, about the ‘honesty’ of the cost of producing a particular signal. The costs may relate both directly to the carotenoids and indirectly to associated changes in feather structure. For example, Dorst (1974) proposed that carotenoid-rich coloration in flight feathers is limited in occurrence because the accompanying structural modifications reduce aerodynamic efficiency. Moreover, loss of the branching structure of a pennaceous feather might compromise water-proofing (Elowson

1984). Indeed, a rich fruit diet may be necessary for both the pigmentary and structural characteristics of frugivore plumage because lipids and other fatty compounds, readily available in fruits, may be needed to dissolve the carotenoids before their deposition, and to form the thick outer cuticle often present on these feathers (Olson 1970), which may counteract wetting. On the other hand, several observations argue against a developmental response of gross feather structure to a frugivorous diet deficient in feather proteins, or their precursors. These observations include evidence for some genetic component to the gross structure of carotenoid-laden feathers (see Brush 1990), the presence of the same gross feather structure in some insectivores (yellow back plumage in *C. nitidissima*), and the absence of any trade-off between area of carotenoid-bearing plumage (structure) and diet (table 2). Therefore, modifications to gross feather structure seem to be associated with presence of carotenoids, not with absence of other potentially limiting dietary constituents (e.g. feather proteins). In any case, modified feather structures could serve as amplifiers (*sensu* Hasson 1989) of honest signal cost.

(c) *Additional ecological factors*

Carotenoid-rich epidermal displays have been suggested to arise through mate choice for signals that stimulate a pre-existing nervous system and/or sensory bias for foods rich in carotenoids (Ryan & Rand 1990; Endler 1992; Rodd *et al.* 2002). Based on the associations reported herein, such 'sensory exploitation' predicts that frugivorous tanagers forage preferentially at carotenoid-rich glossy fruits with disproportionately little UV reflectance. However, strong UV reflectance occurs in many bird-dispersed fruits (including ones whose skin or capsules appear coloured by carotenoids), and experimental manipulations indicate that the UV component is critical for attracting avian foragers (Altschuler 2001). UV reflectance by bird fruits may take the form of a distinct peak, or of an increase in reflectance over all wavelengths (decrease in R_{contrast}), owing to the presence of a waxy coating that produces an unglossed 'bloom' (Burkhardt 1982; Willson & Whelan 1989; Altschuler 2001). In either case, the reflectance patterns of UV-rich fruit signals resemble reflectance patterns of carotenoid-based plumages of insectivores (high UV) more than they do of frugivores (low UV). Conversely, some longer-wavelength reflectance patterns (human-visible yellows), which are widespread among frugivore plumages, are atypical for bird fruits in habitats where most tanagers live (Janson 1983; Wheelwright & Janson 1985).

The ambient light spectrum can also favour signals of certain wavelengths, based on what appears conspicuous or cryptic in a particular environment (Endler 1993). Contrary to expectations of such 'sensory drive', I found that UV reflectance by plumage did not increase at higher elevations, where there is more ambient UV (Hailman 1977). Indeed, the absence of obvious environmental correlates of pigmentation among tanagers suggests that diet affects plumage colour directly, rather than only indirectly through differences in availability of carotenoid-rich foods in different habitats (Slagsvold & Lifjeld 1985; Fitz *et al.* 2003). At a finer environmental scale, short-wavelength reflectance may camouflage insectivores against translucent foliage and, thereby, gain them an advantage in hunting for insect

prey, many of which can detect UV. Different insectivorous tanagers (high UV plumage reflectance), however, typically live and feed at different heights, and under very different ambient light conditions (Isler & Isler 1999), including in the canopy (*Hemithraupis* spp.), at mid-heights (*Thlypopsis* spp.), in thick underbrush (*Hemispingus* spp.) or even on the ground (*Rhodinocichla* sp.).

(d) *Evolution*

Residual variation in reflectance, not associated with diet, could result from the aforementioned, or other factors (e.g. habitat and vegetation cover), not considered herein. For example, frequent contact and contests between bird species over critical resources appear to be capable of driving divergence or convergence in appearance (Cody 1969; Moynihan 1979). Notably, many tanager species habitually associate among themselves and with other birds in mixed species feeding flocks (Isler & Isler 1999). These behavioural dynamics may interact with dietary preferences and other factors to determine plumage colour (e.g. via social mimicry). Although field studies are needed to address these possibilities, the diet-reflectance association implies that carotenoids code dietary niche evolution, including phenotypic divergence, and convergence in diet and plumage colour. As the dietary distinctions among tanagers apply to many levels of avian evolution, the patterns observed herein should apply broadly among birds.

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REFERENCES

- Altschuler, D. L. 2001 Ultraviolet reflectance in fruits, ambient light composition, and fruit removal in a tropical forest. *Evol. Ecol. Res.* **3**, 767–778.
- Andersson, S., Örnborg, J. & Andersson, M. 1998 Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proc. R. Soc. Lond. B* **265**, 445–450. (doi:10.1098/rspb.1998.0315)
- Banks, R. C., Cicero, C., Dunn, J. L., Kratter, A. W., Rasmussen, P. C., Remsen Jr, J. V., Rising, J. D. & Stotz, D. F. 2003 Fourty-fourth supplement to the American Ornithologists' Union. Check-list of North American birds. *Auk* **120**, 923–931.
- Bjerkeng, B., Storebakken, T. & Liaen-Jensen, S. 1990 Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin. *Aquaculture* **91**, 153–162.
- Bleiweiss, R. 2005 Variation in ultraviolet reflectance by carotenoid-bearing feathers of tanagers (Thraupini, Emberizinae, Passeriformes). *Biol. J. Linn. Soc.* (In the press).
- Brush, A. H. 1967 Pigmentation in the scarlet tanager, *Piranga olivacea*. *Condor* **69**, 549–559.
- Brush, A. H. 1970 Pigments in hybrid, variant and melanistic tanagers (birds). *Comp. Biochem. Physiol.* **36**, 785–793.
- Brush, A. H. 1990 Metabolism of carotenoid pigments in birds. *FASEB J.* **4**, 2969–2977.
- Burkhardt, D. 1982 Birds, berries and UV. A note on some consequences of UV vision in birds. *Naturwissenschaften* **69**, 153–157.

- Burkhardt, D. 1989 UV vision: a bird's eye view of feathers. *J. Comp. Physiol. A* **164**, 787–796.
- Burns, K. J. 1997 Molecular systematics of tanagers (Thraupinae): evolution and biogeography of a diverse radiation of neotropical birds. *Mol. Phylogenet. Evol.* **8**, 334–348.
- Burns, K. J. 1998 A phylogenetic perspective on the evolution of sexual dichromatism in tanagers (Thraupidae): the role of female versus male plumage. *Evolution* **52**, 1219–1224.
- Chittka, L., Shmida, A., Troje, N. & Menzel, R. 1994 Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Res.* **34**, 1489–1508.
- Cody, M. L. 1969 Convergent characteristics in sympatric species: a possible relation to interspecific competition and aggression. *Condor* **71**, 222–239.
- Cuthill, I. C., Partridge, J., Bennett, A. T. D., Church, S. C., Hart, N. S. & Hunt, S. 2000 Ultraviolet vision in birds. *Adv. Study Behav.* **29**, 159–214.
- Dorst, J. 1974 *The life of birds*, vol. 1. London: Weidenfeld and Nicholson.
- Elowson, A. M. 1984 Spread-wing postures and the water repellancy of feathers: a test of Rijke's hypothesis. *Auk* **10**, 371–383.
- Endler, J. A. 1990 On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**, 315–352.
- Endler, J. A. 1992 Signals, signal conditions, and the direction of evolution. *Am. Nat.* **139**, s125–s153.
- Endler, J. A. 1993 The color of light in forests and its implications. *Ecol. Monogr.* **63**, 1–27.
- Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15.
- Fitze, P. S., Kölliker, M. & Richner, H. 2003 Effects of common origin and common environment on nestling plumage coloration in the great tit (*Parus major*). *Evolution* **57**, 144–150.
- Garland Jr, T., Harvey, P. H. & Ives, A. R. 1992 Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**, 18–32.
- Garland Jr, T., Dickerman, A. W., Janis, C. M. & Jones, J. A. 1993 Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* **42**, 265–292.
- Garland Jr, T., Jones J. A., Dickermann A. W., Midford P. E. & Diaz-Uriarte R. 1998 Phenotypic diversity analysis programs, v. 5.0. Available from tgarland@citrus.ucr.edu.
- Goldsmith, T. H., Collins, J. S. & Licht, S. 1984 The cone oil droplets of avian retinas. *Vision Res.* **24**, 1661–1671.
- Goodwin, T. W. 1984 *The biochemistry of the carotenoids*, vol. II: Animals, 2nd edn. New York: Chapman and Hall.
- Grether, G. F. 2000 Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**, 1712–1724.
- Hackett, S. J. 1996 Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). *Mol. Phylogenet. Evol.* **5**, 368–382.
- Hailman, J. P. 1977 *Optical signals*. Bloomington, IN: Indiana University Press.
- Hart, N. S., Partridge, J. C., Bennett, A. T. D. & Cuthill, I. C. 2000 Visual pigments, cone oil droplets and ocular media in four species of estrildid finch. *J. Comp. Physiol. A* **86**, 681–694.
- Hasson, O. 1989 Amplifiers and the handicap principle in sexual selection: a different emphasis. *Proc. R. Soc. Lond. B* **235**, 383–406.
- Hill, G. E. 1992 Proximate basis of variation in carotenoid pigmentation in male house finches. *Auk* **109**, 1–12.
- Hill, G. E. 1993 Geographic variation in the carotenoid plumage pigmentation of male house finches. *Biol. J. Linn. Soc.* **49**, 63–86.
- Hill, G. E. 1996 Redness as a measure of the production costs of ornamental traits. *Ethol. Ecol. Evol.* **8**, 157–175.
- Hill, G. E. 2002 *A red bird in a brown bag*. Oxford University Press.
- Hill, G. E., Inouye, C. Y. & Montgomerie, R. 2002 Dietary carotenoids predict plumage coloration in wild house finches. *Proc. R. Soc. Lond. B* **269**, 1119–1124. (doi:10.1098/rspb.2002.1980)
- Hudon, J. 1989 Unusual carotenoid use by the western tanager (*Piranga ludoviciana*) and its evolutionary implications. *Can. J. Zool.* **69**, 2311–2320.
- Innes, R. C. 1979 Club-tipped feathers in some South American tanagers. *Auk* **96**, 808–809.
- Inouye, C. Y., Hill, G. E., Stradi, R. D. & Montgomerie, R. 2001 Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. *Auk* **118**, 900–915.
- International Commission on Illumination 1971 Colorimetry: official recommendation of the International Commission on Illumination (CIE). Paris: Bureau Central de la CIE.
- Isler, M. L. & Isler, P. R. 1999 *The tanagers. Natural history, distribution, and identification*. Washington, DC: Smithsonian Institution Press.
- Janson, C. H. 1983 Adaptation of fruit morphology to dispersal agents in a neotropical forest. *Science* **219**, 187–189.
- Johnson, N. K. & Brush, A. H. 1972 Analysis of polymorphism in the sooty-capped bush tanager. *Syst. Zool.* **21**, 245–262.
- Ke, B., Imsgard, F., Kjosen, H. & Liaaen-Jensen, S. 1970 Electronic spectra of carotenoids at 77 K. *Biochim. Biophys. Acta* **210**, 139–152.
- Liebman, P. A. & Granda, A. M. 1975 Super dense carotenoid spectra resolved in single cone oil droplets. *Nature* **253**, 370–372.
- Linville, S. U. & Breitwisch, R. 1997 Carotenoid availability and plumage coloration in a wild population of northern cardinals. *Auk* **114**, 796–800.
- McGraw, K. J., Hill, G. E., Stradi, R. & Parker, R. S. 2002 The effect of dietary carotenoid access on sexual dichromatism and plumage pigment composition in the American goldfinch. *Comp. Biochem. Physiol. B* **131**, 261–269.
- McGraw, K. J., Gregory, A. J., Parker, R. S. & Adkins-Regan, E. A. 2003 Diet, plasma carotenoids, and sexual coloration in the zebra finch (*Taeniopygia guttata*). *Auk* **120**, 400–410.
- Mahler, B., Araujo, L. S. & Tubaro, P. I. 2003 Dietary and sexual correlates of carotenoid pigment expression in dove plumage. *Condor* **105**, 258–267.
- Mays, H. L., McGraw, K. J., Ritchison, G., Cooper, S., Rush, V. & Parker, R. S. 2003 Sexual dichromatism in the yellow-breasted chat (*Icteria virens*): spectrophotometric analysis and biochemical basis. *J. Avian Biol.* **35**, 125–134.
- Moynihan, M. 1979 Geographic variation in social behavior and in adaptations to competition among Andean birds. *Publ. Nuttall Ornithol. Club* **18**, 1–162.
- Olson, S. L. 1970 Specializations of some carotenoid-bearing feathers. *Condor* **72**, 424–430.
- Olson, V. A. & Owens, I. P. F. 1998 Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 510–514.
- Prum, R. O., Torres, R., Williamson, S. & Dyck, J. 1999 Two-dimensional Fourier analysis of the spongy medullary keratin of structurally coloured feather barbs. *Proc. R. Soc. Lond. B* **266**, 13–22. (doi:10.1098/rspb.1999.0598)
- Pryke, S. R., Andersson, S. & Lawes, M. J. 2001 Sexual selection on multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. *Evolution* **55**, 1452–1463.
- Putnam, M. 1992 A review of the nature, function, variability, and supply of pigments in salmonid fish. In *Aquaculture and the environment* (ed. N. Depaauw & H. J. Joyce), pp. 245–263. Gent, Belgium: European Aquaculture Society.

- Rice, W. R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.
- Rice, W. R. & Gaines, S. D. 1994 'Heads I win, tails you lose': testing directional alternative hypotheses in ecological and evolutionary research. *Trends Ecol. Evol.* **9**, 235–237.
- Rodd, F. H., Hughes, K. A., Grether, G. F. & Barill, C. T. 2002 A possible non-sexual origin of mate preference: are male guppies mimicking fruit. *Proc. R. Soc. Lond. B* **269**, 475–481. (doi:10.1098/rspb.2001.1891)
- Ryan, M. J. & Rand, A. S. 1990 The sensory basis of sexual selection for complex calls in the tungura frog *Physalaemus pusulosus* (sexual selection for sensory exploitation). *Evolution* **44**, 305–314.
- Saks, L., McGraw, K. & Horak, P. 2003 How feather colour reflects its carotenoid content. *Funct. Ecol.* **17**, 555–561.
- SAS 2003 *SAS users' guide, v. 8. On line documentation*. Cary, NC: SAS Institute Inc.
- Slagsvold, T. & Lifjeld, J. T. 1985 Variation in plumage color of the great tit *Parus major* in relation to habitat, season and food. *J. Zool. Lond. A* **206**, 321–3328.
- Sokal, R. R. & Rohlf, J. F. 1981 *Biometry*. New York: W. H. Freeman.
- Stradi, R., Celentano, G., Boles, M. & Mercato, F. 1997 Carotenoids in bird plumage: the pattern in a series of red-pigmented Carduelinae. *Comp. Biochem. Physiol. B* **117**, 85–91.
- Wheelwright, N. T. & Janson, C. H. 1985 Colors of fruit displays of bird-dispersed plants in two tropical forests. *Am. Nat.* **126**, 777–799.
- Willson, M. F. & Whelan, C. J. 1989 Ultraviolet reflectance of fruits of vertebrate-dispersed plants. *Oikos* **55**, 341–348.

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