doi: 10.1111/j.1420-9101.2005.00940.x

Interspecific variation in the use of carotenoid-based coloration in birds: diet, life history and phylogeny

V. A. OLSON* & I. P. F. OWENS†

*Department of Zoology and Entomology, University of Queensland, Queensland, Australia
†Division of Biology and NERC Centre for Population Biology, Imperial College London, Ascot, Berkshire, UK

Keywords:

birds;
carotenoids;
coloration;
diet;
phylogenetic analysis;
signals.

Abstract

Birds show striking interspecific variation in their use of carotenoid-based coloration. Theory predicts that the use of carotenoids for coloration is closely associated with the availability of carotenoids in the diet but, although this prediction has been supported in single-species studies and those using small numbers of closely related species, there have been no broad-scale quantitative tests of the link between carotenoid coloration and diet. Here we test for such a link using modern comparative methods, a database on 140 families of birds and two alternative avian phylogenies. We show that carotenoid pigmentation is more common in the bare parts (legs, bill and skin) than in plumage, and that yellow coloration is more common than red. We also show that there is no simple, general association between the availability of carotenoids in the diet and the overall use of carotenoid-based coloration. However, when we look at plumage coloration separately from bare part coloration, we find there is a robust and significant association between diet and plumage coloration, but not between diet and bare part coloration. Similarly, when we look at yellow and red plumage colours separately, we find that the association between diet and coloration is typically stronger for red coloration than it is for yellow coloration. Finally, when we build multivariate models to explain variation in each type of carotenoid-based coloration we find that a variety of life history and ecological factors are associated with different aspects of coloration, with dietary carotenoids only being a consistent significant factor in the case of variation in plumage. All of these results remain qualitatively unchanged irrespective of the phylogeny used in the analyses, although in some cases the precise life history and ecological variables included in the multivariate models do vary. Taken together, these results indicate that the predicted link between carotenoid coloration and diet is idiosyncratic rather than general, being strongest with respect to plumage colours and weakest for bare part coloration. We therefore suggest that, although the carotenoid-based bird plumage may a good model for diet-mediated signalling, the use of carotenoids in bare part pigmentation may have a very different functional basis and may be more strongly influenced by genetic and physiological mechanisms, which currently remain relatively understudied.

Introduction

Carotenoid pigments are responsible for many of the red, orange and yellow colours of birds (Brush, 1981,1990). In addition to their use as pigments, carotenoids are of great importance in animal bodies, and are used in

Correspondence: Valérie A. Olson, Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK.
Tel.: +44 020 7449 6650; fax: +44 020 7586 2870;
e-mail: valerie.olson@ioz.ac.uk

numerous physiological pathways (Latscha, 1990; Bendich, 1993; Scheidt, 1998). Combined with the fact that carotenoids must be obtained from the diet (Goodwin, 1984; Latscha, 1990), this multiplicity of functions has led to numerous proposed links between carotenoid-based signals and parasites and/or immunity (Hamilton & Zuk, 1982; Folstad & Karter, 1992; Lozano, 1994), foraging ability and/or resource abundance (Hill, 1994a,b,1996), and general physiological value (Olson & Owens, 1998). Indeed, many of the classic examples of honest signalling in animals are founded on carotenoid-based coloration (e.g. Hill, 1991; Burley *et al.*, 1992; Metz & Weatherhead, 1992).

In addition to being of great topical interest in the fields of animal signalling (e.g. Johnson & Lanyon, 2000; Hill, 2002) and ecological immunology (e.g. Blount *et al.*, 2003; Faivre *et al.*, 2003), carotenoid-based coloration is also extremely variable across animal taxa. Among birds, for instance, carotenoids can be present in both the plumage and the bare parts (i.e. bills, legs, wattles and bare skin). Although pure carotenoid-based colour typically varies in hue from yellow to red according to the specific carotenoids present, carotenoids can also occur in combination with melanin pigments to produce, for example, olive greens, or with structural colours to produce purples or bright greens. In a few species, carotenoproteins in feathers produce unusual pastel blues and mauves (Goodwin, 1984).

The overall aim of this study is to ask why different avian taxa should make such very different use of carotenoid-based coloration. Because animals cannot synthesize carotenoids *de novo* and must instead obtain them from their diet (Goodwin, 1984; Latscha, 1990), the traditional explanation for differences in the expression of carotenoid-based coloration is dietary limitation (Hill, 1994a). That is, carotenoid-based coloration will be most common in those animals that have access to a plentiful supply of carotenoids in their diet. This prediction has received abundant support from single-species studies showing that variation between individuals is closely associated with variation in their access to dietary carotenoids (e.g. Hill *et al.*, 2002; McGraw *et al.*, 2003). However, such single-species examples do not satisfactorily show that variation between species in carotenoid-based coloration is also due to dietary limitation (see Bennett & Owens, 2002; Owens, 2005). It is equally plausible, for instance, that differences between species in the use of carotenoid-based coloration may be due to differences between species in their genetic or physiological ability to absorb or synthesize carotenoids (Olson & Owens, 1998; Tella *et al.*, 2004). Alternatively, differences between species in the use of carotenoid-based coloration may be due to differences in the ecological or behavioural advantages and disadvantages of having bright coloration, such as the risk of predation (e.g. Martin & Badyaev, 1996; McNaught & Owens, 2002), the potential for increasing reproductive success (e.g. McGraw *et al.*, 2001b) or the light environment provided by the habitat (Endler, 2000). Previous comparative studies of bird coloration have shown that many of these factors can play a role (reviewed in Owens, 2005) but have typically been limited in not distinguishing between carotenoid-based coloration and other forms of coloration (e.g. Baker & Parker, 1979; Butcher & Rohwer, 1989, but see Mahler *et al.*, 2003) and in failing to test for an association between coloration and dietary carotenoids *per se*.

In this study we overcome these shortfalls by compiling a new database on coloration and diet in birds and test explicitly for an association between interspecific variation in carotenoid-based coloration and interspecific variation in dietary carotenoids. The traditional, diet-mediated hypothesis predicts that there should be a positive association between these factors and we therefore test whether taxa that use carotenoid-based colours have a consistently more carotenoid-rich diet than species that do not use carotenoid-based colours. However, it has also been suggested that there may be important functional differences between different sorts of coloration. For instance, birds can show coloration with respect to either their plumage or their bare parts, and it is possible that these may differ in terms of both their physiological control and signalling function (Owens & Short, 1995; Shykoff & Widmer, 1996). In this study we therefore tested separately for correlations between diet and both plumage coloration and bare part coloration and predicted that, if there is a difference between these two types of coloration, they should show different patterns of correlation with diet. Similarly, Hill (1996) has presented comparative evidence that red coloured carotenoids may be more costly than yellow coloured ones because red carotenoids are more rare in the diet. We also therefore tested separately for associations between diet and both yellow carotenoid-based coloration and red carotenoid-based coloration. Again, if red and yellow carotenoids differ in cost, then we would predict that these two colours should differ in the relative strengths of their relationships with diet.

In addition to testing for associations between various aspects of carotenoid-based coloration and diet, we also build multiple regression models to test the relative importance of variation in other aspects of life history and ecology. Here the traditional, diet-mediated hypothesis predicts that interspecific variation in dietary carotenoids will be the key predictor of interspecific variation in colour, with other factors being relatively less important. On the other hand, if variation in coloration is more closely linked to variation in other factors, such as habitat openness, nest conspicuousness and mating system, then dietary availability of carotenoids may not be the prime factor in explaining differences between species in the expression of carotenoid-based coloration.

Methods

Collection of pigmentation data

We compiled data on coloration for 140 avian families using information from field guides, regional and taxonomic monographs, encyclopaedic references, and tertiary sources of images (e.g. internet, general interest books). We did not include the Psittacidae (parrots), Musophagidae (turacos) or Spheniscidae (penguins) in this analysis, as these families are known to use pigments other than carotenoids to produce red, orange and yellow colours (Fox, 1979; Stradi *et al.*, 2001; McGraw *et al.*, 2004).

For all remaining avian families we used a combination of methods to assign coloration as either carotenoid-based or noncarotenoid-based. For some of these families, there is direct biochemical evidence for the presence of carotenoids. For the remainder, we examined museum skins of representative species where possible to look for colours and feather structures typically associated with carotenoid pigmentation (Olson, 1970). Where this was not possible, and for bare parts, we followed the procedure of Gray (1996) and Owens & Hartley (1998), which is based on *a priori* knowledge of the colours that are typically carotenoid-based (see http://www.blackwellpublishing.com/products/journals/suppmat/jeb/jeb940/jeb940sm.htm for the level of documentation available for each family). For each family we obtained images of as many species as possible, and assessed each species using Gray's (1996) criteria that bright reds, oranges, yellows and some greens and purples, likely result from carotenoids (see also Owens & Hartley, 1998). We did not include brick reds, dark reddish-browns, tawny yellows, or olive browns as being indicative of carotenoids, as many of these colours can be produced by melanins alone (Fox, 1979). Similarly, we excluded structural blues and purples, iridescent colours, and also pigmentary greens in certain pheasants, a jacana and an eider that may be caused by porphyrin-based pigments similar to those occurring in turacos (Dyck, 1992). As we discuss in more detail in the Discussion, this method is likely to be an imperfect means of assigning the source of coloration, and some families may be mis-assigned. However, because we have specifically excluded those families where it has been established that carotenoids are not used in coloration (see above), any mistakes should be nonbiased with respect to the hypotheses under test and therefore simply contribute error variance. Our tests should therefore be conservative with respect to the predicted relationships between diet and coloration.

Using the combination of approaches described above to identify cases of carotenoid-based coloration, we separated presence/absence data based on whether the pigment was present in the plumage or bare parts of the species. Where carotenoids were present, we also recorded the presence or absence of red, orange and yellow coloration, based on the verbal species descriptions from our literature sources. We considered both sexes in this assessment–for example, if the male of a species used red and the female yellow, then both red and yellow were considered to be present for that species. Colours that were described as, or appeared to be, intermediate between red and orange or yellow and orange, were recorded in both relevant colour categories. In the final analysis, we omitted the orange category to achieve a clear separation between theoretically less costly yellow, and more costly red carotenoids (Hill, 1996). Again, using human-oriented scales for distinguishing between 'yellow' and 'red' coloration is an imperfect method but, given the scope of our study, we were unable to collect accurate data on reflectance spectra for all species. Also, any mistakes should again be random with respect to the hypotheses being tested and should therefore lead to conservative tests.

Using these data on the occurrence of carotenoid based coloration, we calculated the proportion of species surveyed within each family that used any form of carotenoid-based coloration, the proportions of species that expressed carotenoid-based coloration in each of the two types of tissue, and the proportions of species that expressed red and/or yellow carotenoid-based colours in each of the two types of tissue. We also collapsed these proportional values to binary indices for each family, with zero indicating the absence, and one indicating the presence, of a given form of pigmentation in a family.

Collection of diet data

To assess the typical dietary carotenoid content at the family level, we used a coarse-scale index of carotenoid intake, in which a series of diet categories, ranked in order of carotenoid content, were assigned relative levels of importance in the diets of members of each family present in this study (http://www.blackwellpublishing.com/products/journals/suppmat/jeb/jeb940/jeb940sm.htm), with three representing foods of high importance (i.e. numerically/proportionally dominant), two for intermediate importance, one for low importance (i.e. numerically/proportionally least abundant), and zero indicating absence from the diet. Once we had collected diet data for as many species as possible, we calculated a family level diet score based on the relative proportional contribution of each diet category to the overall diet. We did this by calculating the total score for each diet category for all surveyed species within the family, determining the proportional contribution of each diet category to the sum of all categories combined, multiplying each proportion by its category, and summing these to create a weighted mean diet score for the family. Once again we recognize that this is an imperfect method for quantifying the availability of carotenoids in the diet but it has the great advantage of being readily applied to a

large number of relatively poorly studies species. Also, we anticipate that any errors should be unbiased with respect to the hypotheses under test and therefore again contribute to the conservative nature of our tests.

Collection of other ecological and life history data

We also collected data on a number of other factors that have previously been shown to be associated with coloration in birds (e.g. Baker & Parker, 1979; Butcher & Rohwer, 1989; Savalli, 1995; Martin & Badyaev, 1996; Andersson, 2000; Endler, 2000; Johnson & Lanyon, 2000; Bennett & Owens, 2002). Female body sizes (g), egg masses (g), clutch sizes, and number of broods per year for members of a given family were as reported in Bennett & Owens (2002). Body size data were log-transformed prior to inclusion in statistical analyses. Mean total mass of eggs produced per year was calculated by multiplying mean clutch size by mean number of broods per year by mean egg mass. The values resulting from this calculation were log-transformed. Adult survival rates for families were as reported in Bennett & Owens (2002). These were the means of all available annual adult survival rates for members of a given family.

Social mating system for a family was ranked based on the proportion of family members that are polygamous. We did not distinguish between polyandry and polygyny in this study because we were interested in estimating the strength, rather than the direction, of sexual selection. Families were therefore classified as being monogamous, with less than 5% of species being recorded as regularly polygamous (1); partially polygamous, with up to 50% of members being regularly polygamous (2); or polygamous, with over 50% of its members regularly polygamous (3).

We used descriptions of the typical habitats used by a family, as presented in Bennett & Owens (2002). We assigned scores to these habitats based on more fine-scale rankings, with one being the most closed and eight the most open (http://www.blackwellpublishing.com/products/journals/suppmat/jeb/jeb940/jeb940sm.htm). For each family, we selected all of the habitats used by its species, and used the mean of their ranks as an index of habitat openness. For nest locality, we ranked nest types by their level of conspicuousness and accessibility to typical ground dwelling but agile predators. We assigned scores based on the initial assumption of a concealed nest, with low values indicating lower nest visibility and accessibility, and then provided a 'penalty' addition to these scores for nests located in open locations (see *Supplementary material*). For each family, we selected all applicable nest categories, and used the mean as a family level index. For degree of coloniality, families were assigned to one of three categories as follows: no species in the family colonial (1.0); some species in the family colonial, or all species in the family found in very loose associations or small colonies of up to 50 pairs (1.5); or most or all species in the family colonial (2.0).

Statistical analysis

Before conducting our main comparative tests, we examined the frequency distributions of our carotenoid characters across the families used in our test. We did this by constructing frequency histograms and calculating skewness across families using the g_1 moment statistic (i.e. μ_3; Sokal & Rohlf, 1981). We calculated these indices separately for each type of tissue and for each colour of carotenoid-based coloration (yellow vs. red).

Because of the ongoing debate concerning the most appropriate methods for comparative analyses (e.g. Freckleton *et al.*, 2002; Martins & Housworth, 2002; Blomberg *et al.*, 2003), we performed our comparative tests in two stages–first based on raw family level data, and second on phylogenetically corrected contrasts (see Bennett & Owens, 2002). In the first set of tests we performed ANOVAS to test for differences in dietary carotenoid content between families expressing carotenoid pigmentation and families not expressing such pigmentation. We tested each tissue and carotenoid-based colour separately, using the binary indices for each type of pigmentation as categories, and the mean dietary carotenoid content as the dependent variable. We also performed Spearman rank correlations to test for associations between mean dietary carotenoid intake and the proportion of each family using any carotenoid pigments, red carotenoids or yellow carotenoids, respectively. We performed these tests for plumage and bare parts separately.

Our second set of analyses was based on phylogenetically independent contrasts (Felsenstein, 1985). To calculate contrasts, we used the phylogenetic analysis program CAIC (Purvis & Rambaut, 1995). Using these contrasts, we first performed simple regressions using dietary carotenoid intake as the independent variable, and each of the proportional pigmentation indices as dependent variables. As a phylogenetically controlled version of the ANOVAS on raw data, we used the BRUNCH algorithm in CAIC to calculate contrasts in the binary indices of carotenoid pigmentation. We then used Wilcoxon's tests to see if the associated changes in diet were significantly different from zero (e.g. Bennett & Owens, 1997).

To ensure that the results of our phylogenetic analyses were not dependent on the topography of a single phylogenetic hypothesis, we performed all phylogenetic analyses twice using different phylogenies. Initially we used the family level avian phylogeny of Sibley & Ahlquist (1990), which is based on DNA–DNA hybridization and until recently was the only class-wide phylogeny for the birds. Subsequently, we combined two recently published avian phylogenies (Barker *et al.*,

2004; Cracraft *et al.*, 2004), which together provide an updated evolutionary hypothesis for the relationships between avian families.

To test for correlations between all nine life history and ecological variables and the six indices of carotenoid use, we used contrasts in the life history/ecological traits as independent variables and contrasts in the carotenoid indices as dependent variables in multiple regression analyses. We used the CRUNCH algorithm in CAIC to calculate contrasts for all variables. We then used these contrasts to build minimum adequate models to explain variation in each of the dependent variables. We followed the technique of Purvis *et al.* (2000) to identify minimum adequate models. All regression models were forced through the origin, as required when analysing phylogenetic contrast data (Purvis & Rambaut, 1995).

Results

Frequency distribution of carotenoid-based coloration

We were able to gather carotenoid pigmentation data for 8126 species representing 140 families, (87.8% of all species listed within the families studied; Monroe & Sibley, 1993). On average, 94% of each family was surveyed (see *Supplementary material*). Additionally, we found data on diets for 5772 species, representing 62% of all species within the 140 families. For each family, we were able to survey an average of 83% of species. The distribution of within-family prevalence of carotenoid pigmentation was highly skewed to low values for plumage, but this skew was either absent or less pronounced for bare parts (Fig. 1a, b). That is, for most families, only a small proportion of species had plumage pigmentation, but a much larger proportion had bare part pigmentation. Within each of these tissues, for most families, only a small proportion of species had red coloration, a somewhat larger proportion had yellow coloration, and a larger proportion again had carotenoid pigmentation of any colour (Fig. 1a, b).

Relationship between diet and carotenoid pigmentation

Our ANOVA models based on raw family typical data revealed no significant overall differences in dietary carotenoid content between families expressing carotenoids and families not expressing carotenoids, even when different colours and tissues were considered separately (Fig. 2). In the case of the tests of bare part coloration (Fig. 2b) there was no strong indication of biologically relevant differences in diet with respect to colour. However, for plumage coloration there was a general tendency for families with carotenoid coloration to have more carotenoid-rich diets, but this trend was marginally nonsignificant in all cases ($0.07 > P > 0.08$). This nonsignificant trend is hard to interpret biologically because our tests are relatively conservative and the lack of significance may therefore be due either to a lack of a relationship or a lack of statistical power. However, the confidence limits around the mean differences in these tests all only just covered zero (all plumage: −0.391, 0.017; yellow plumage: −0.407, 0.015; red plumage: −0.425, 0.010), and so it is likely that there is no simple effect of diet on plumage carotenoid expression (Colegrave & Ruxton, 2003).

When we used Sibley & Ahlquist (1990) to control for phylogeny we found that, in the case of plumage coloration, increases in the use of carotenoid-based coloration were associated with increases in dietary carotenoid intake (Wilcoxon signed rank–all carotenoids: $Z = -2.00$, $P < 0.05$, mean contrast: 0.022 ± 0.009; yellow: $Z = -2.25$, $P < 0.05$, mean contrast 0.019 ± 0.007; red: $Z = -1.80$, $P = 0.07$, mean contrast 0.025 ± 0.013). In the case of bare part coloration, there was a significant association between increases in the overall use of carotenoid-based coloration and increases in dietary carotenoids ($Z = -2.25$, $P < 0.05$, mean contrast 0.021 ± 0.011), but this relationship did not hold when we treated red and yellow bare part coloration separately (yellow: $Z = -1.23$, n.s., mean contrast 0.012 ± 0.010; red: $Z = -0.55$, n.s., mean contrast 0.001 ± 0.008). These results remained qualitatively unchanged when we used the alternative phylogeny (Barker *et al.*, 2004; Cracraft *et al.*, 2004).

The Spearman rank correlations of carotenoid use and diet across families also showed that there was a significant increase in the prevalence of plumage carotenoid pigmentation as mean dietary intake of carotenoids increased (Fig. 3a). This increase was also significant for both red (Fig. 3b) and yellow (Fig. 3c) plumage carotenoids. For bare parts, there were no significant associations with dietary carotenoid intake (Fig. 3d–f). Phylogenetic analysis of these continuous data, based on the Sibley & Ahlquist (1990) phylogeny, provided qualitatively similar results. In particular, the correlation between plumage carotenoid pigmentation and dietary carotenoid intake was again significant, while that between bare part pigmentation and diet was not significant (Table 1), regardless of which type of pigmentation was considered. Again, these results remained qualitatively unchanged when we used the Cracraft & Barker phylogeny (Barker *et al.*, 2004; Cracraft *et al.*, 2004), apart from the fact that the correlation between red bare parts and dietary carotenoid intake was then significant (Table 1).

Multiple regression models including other ecological factors

When we performed multiple regression analyses including dietary carotenoid intake plus eight other variables,

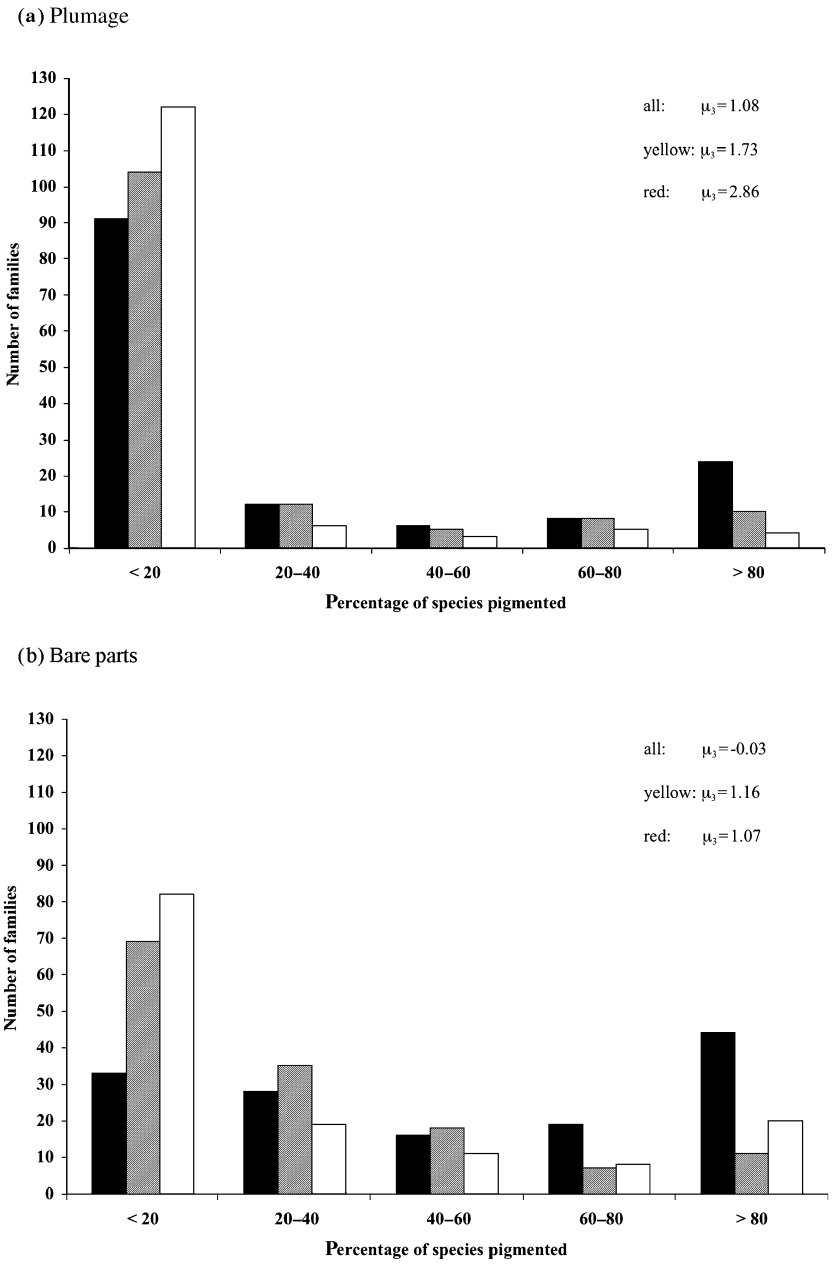

Fig. 1 Frequency histograms of the percentages of all species within families expressing any carotenoids (black bars), yellow carotenoids (grey bars) or red carotenoids (white bars) in (a) plumage and (b) bare parts. Values shown are skewness (μ_3) of each distribution.

we found that other life history and ecological characteristics were often more strongly correlated with carotenoid pigmentation than was diet (Table 2). The only dependent variables for which diet explained a significant portion of the variance were the three measures of plumage coloration and red bare part coloration (Table 2). The complement of variables entering models varied depending on the tissue deposition site and specific colour in question, and in general the preferred models for plumage carotenoids explained more of the total variation in pigmentation prevalence than did the preferred models for bare part carotenoids. All of these major results remained the same whether we used the Sibley & Ahlquist (1990) phylogeny or the new Cracraft-Barker amalgamated phylogeny (Barker *et al.*, 2004; Cracraft *et al.*, 2004).

Models for the three forms of plumage carotenoid pigmentation studied were reasonably consistent in terms of the independent factors included in the models. Irrespective of which phylogeny was used to calculate contrasts, for both red and yellow plumage coloration, increases in the prevalence of pigmentation were

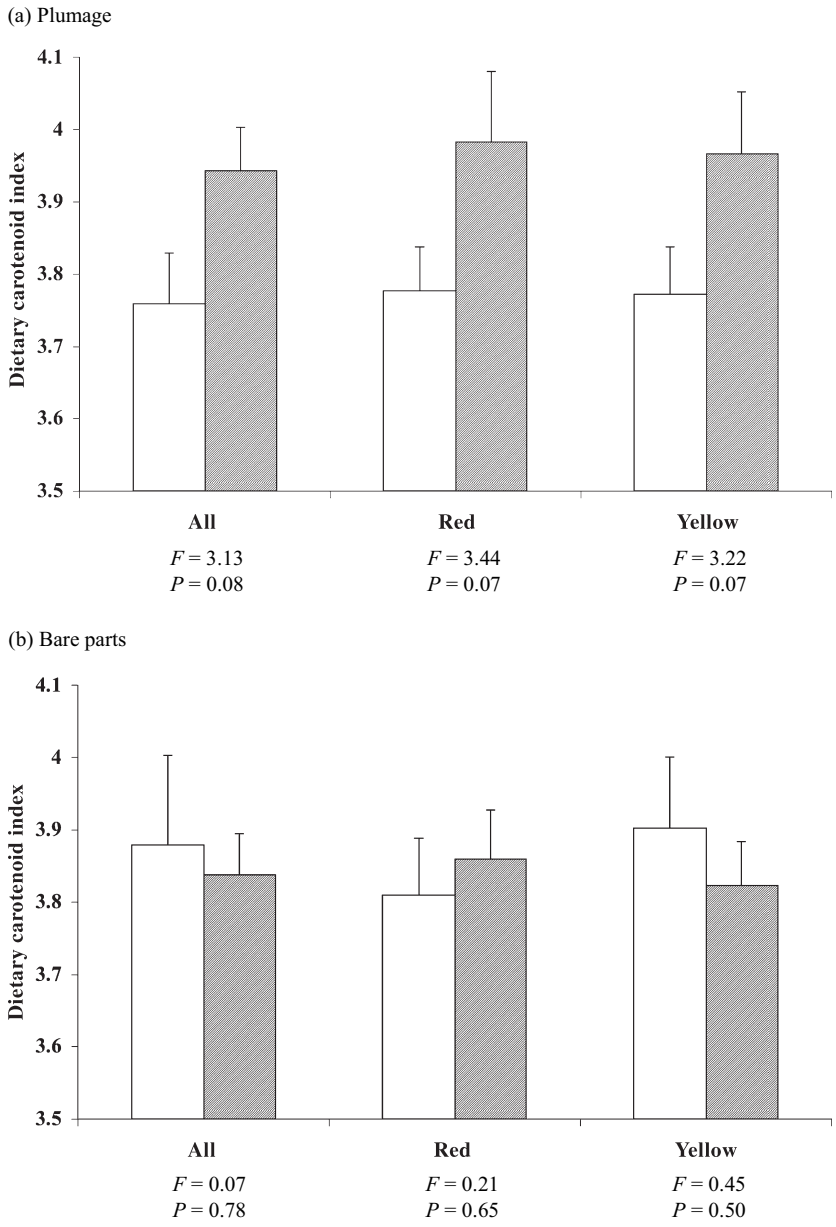

Fig. 2 Mean dietary carotenoid content (±SE) of families not expressing carotenoid pigmentation (open bars) and those expressing carotenoid pigmentation (filled bars). Plots are presented for (a) plumage and (b) bare parts. Pigmented and unpigmented families did not differ significantly in any form of carotenoid pigmentation. Values shown are *F* and *P*-values from ANOVAS.

associated with decreases in the total mass of eggs produced per year, with the correlation being strongest for red plumage, and weaker for yellow plumage carotenoids. Also regardless of the phylogeny used to generate contrasts, increases in these three forms of plumage pigmentation were associated with increases in dietary carotenoid intake, again with the influence of diet being stronger for red than for yellow coloration. There were, however, differences in the additional variables entering models for each plumage colour and for each phylogeny. Increases in the prevalence of all plumage carotenoids were consistently correlated with increases in social polygamy, while increases in the prevalence of yellow and red carotenoids were consistently correlated with increases in total mass of eggs produced per year. No other patterns were consistent across both phylogenies for the models of plumage coloration.

The multiple regression models of bare part coloration provided less evidence of a role for dietary carotenoid intake (Table 2). Red bare part coloration was the only aspect of bare part coloration that showed a consistent correlation with dietary carotenoids for both phylogenies, but even in that case the extent of coloniality was a stronger correlate (Table 2). In the case of overall bare part coloration, nest conspicuousness was the only consistent correlate, and for yellow bare part coloration

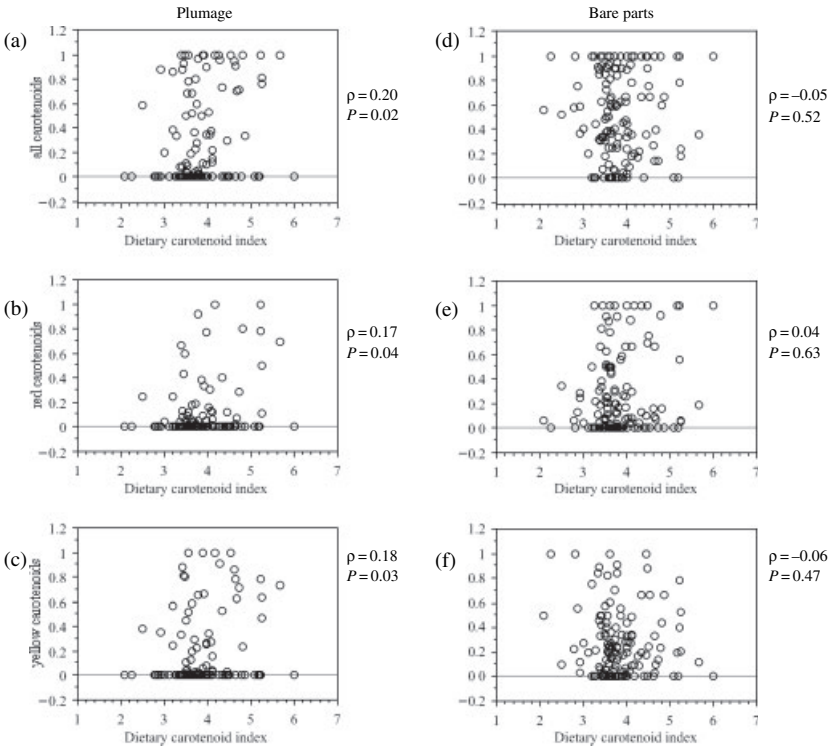

Fig. 3 The relationship between dietary carotenoid intake, and the use of carotenoid pigments in avian plumage and bare parts. Values shown are ρ and *P*-values from Spearman rank correlations.

Table 1. Single regression models of various aspects of plumage and bare part carotenoid expression vs. dietary carotenoid intake, controlling for phylogeny (see text for details).

Dependent variable	*n*	*F*	*P*	Slope ± SE	r^2
(a) Models based on Sibley-Ahlquist phylogeny					
Plumage carotenoids					
All	134	17.98	<0.0001	0.19 ± 0.04	0.120
Red	135	7.61	<0.01	0.08 ± 0.03	0.054
Yellow	135	11.76	<0.005	0.13 ± 0.04	0.081
Bare part carotenoids					
All	133	0.39	n.s.	0.03 ± 0.05	0.003
Red	133	3.74	n.s.	0.09 ± 0.04	0.028
Yellow	134	0.76	n.s.	0.03 ± 0.04	0.006
(b) Models based on amalgamated Cracraft-Barker phylogeny					
Plumage carotenoids					
All	106	13.24	<0.001	0.19 ± 0.05	0.110
Red	106	7.37	<0.01	0.10 ± 0.04	0.066
Yellow	106	7.21	<0.01	0.12 ± 0.05	0.064
Bare part carotenoids					
All	106	3.28	n.s.	0.09 ± 0.05	0.030
Red	106	13.72	<0.001	0.20 ± 0.05	0.120
Yellow	106	0.09	n.s.	–0.01 ± 0.05	0.001

only mating system was consistent across phylogenies. Other correlates were idiosyncratic to one or other phylogenetic hypothesis.

Discussion

The main objective of this study was to test whether, among birds, there is an association between interspecific variation in the use of carotenoid pigmentation and interspecific variation in the carotenoid content of the diet. The results of our tests are complex and suggest that the relationship between these factors is context dependent. In our most general tests we found that there was at best a weak association between carotenoid pigmentation and dietary carotenoids. However, when we considered different aspects of carotenoid pigmentation separately we found that, in some cases, there was a significant association with frequent use of carotenoid pigmentation

Table 2. Results of multiple regression analysis, controlling for phylogeny, of various aspects of coloration versus diet, life history and ecology.

Dependent	Independent	*t*	*β*	*F*	r^2
(a) Models based on Sibley-Ahlquist phylogeny					
All plumage	Total mass of eggs/year	−2.73**	−0.300	$6.19_{4,83}$***	0.241
	Nest conspicuousness	2.17*	0.240		
	Dietary carotenoid intake	3.44***	0.239		
	Mating system	1.54	0.164		
Red plumage	Total mass of eggs/year	−3.52***	−0.433	$7.43_{4,83}$****	0.274
	Body mass	3.14**	0.414		
	Dietary carotenoid intake	3.46***	0.231		
	Mating system	1.34	0.143		
Yellow plumage	Total mass of eggs/year	−2.71**	−0.292	$5.42_{3,83}$**	0.169
	Mating system	2.63**	0.246		
	Dietary carotenoid intake	2.23*	0.188		
All bare	Clutch size	2.28*	0.235	$2.96_{5,88}$*	0.152
	Nest conspicuousness	2.07*	0.231		
	Coloniality	2.06*	0.230		
	Habitat openness	−1.96*	−0.219		
	Mating system	0.23	0.024		
Red bare	Coloniality	2.87**	0.250	$6.08_{2,127}$**	0.089
	Dietary carotenoid intake	2.17*	0.130		
Yellow bare	Total mass of eggs/year	2.60**	0.282	$4.10_{3,83}$**	0.133
	Habitat openness	−2.19*	−0.221		
	Mating system	0.852	0.092		
(b) Models based on amalgamated Cracraft-Barker phylogeny					
All plumage	Dietary carotenoid intake	4.60****	0.608	$10.70_{2,73}$****	0.205
	Mating system	0.74	0.106		
Red plumage	Coloniality	2.65**	0.327	$6.81_{3,79}$***	0.175
	Total mass of eggs/year	−2.61**	−0.315		
	Dietary carotenoid intake	2.29*	0.264		
Yellow plumage	Dietary carotenoid intake	4.10†	0.571	$5.83_{4,73}$***	0.200
	Total mass of eggs/year	−2.97**	−0.433		
	Habitat openness	1.97*	0.285		
	Clutch size	1.86	0.276		
All bare	Nest conspicuousness	2.04*	0.332	$3.68_{2,90}$*	0.055
	Body size	1.60	0.262		
Red bare	Coloniality	2.66**	0.335	$9.80_{3,90}$****	0.221
	Body size	2.41*	0.305		
	Dietary carotenoid intake	2.32*	0.296		
Yellow bare	Mating system	2.46*	0.399	$5.40_{2,72}$**	0.106
	Coloniality	−2.17*	−0.355		

Traits entering models are listed from strongest to weakest standard coefficients (i.e. β-values). Preferred models were characterized by a significant ($P \leq 0.05$) overall *F*-statistic, significant individual *t*-values for the majority of independents, and optimized r^2.
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$. (See text for further details.)

being linked to a carotenoid rich diet. These major conclusions remained qualitatively unchanged whether or not we controlled for phylogeny, and irrespective of which of two major phylogenies we used in our analyses.

Implications for carotenoid-based signalling

Some of our most striking findings highlighted the importance of considering tissue type when testing for ecological correlates of pigmentation. Here we used two categories of tissue type–plumage and bare parts–and found different biological patterns for each. In terms of frequency of use, carotenoid pigmentation of the bare parts is far more common than that of the plumage–the frequency distribution of bare part pigmentation was not markedly skewed, while that for plumage pigmentation was highly skewed to low prevalence values. Less than a quarter of the families surveyed were in our bottom prevalence category (i.e. <20% of species pigmented) with respect to overall expression of carotenoids in bare parts, while nearly two-third of families were in the bottom prevalence category with respect to plumage carotenoid expression. While gains of bare part pigmentation among families appeared to be in some way linked

to diet, we were unable to identify any robust association between within-family prevalence of carotenoid pigmentation of bare parts and dietary carotenoids. In contrast, we found several strong and robust associations between dietary carotenoids and the use of carotenoids in plumage pigmentation, with high prevalence of pigmentation being associated with carotenoid-rich diets. This finding is in agreement with the large number of single-species studies that have shown that carotenoid-based plumage pigmentation is closely linked to the dietary availability of carotenoids (e.g. Slagsvold & Lifjeld, 1985; Hill, 1992; Hill *et al.*, 1994; Hudon *et al.*, 1996; Linville & Breitwisch, 1997; McGraw *et al.*, 2001a, 2003), but the lack of association with bare part coloration suggests that the same mechanisms may not apply to all aspects of carotenoid-based pigmentation.

In addition to highlighting the importance of considering plumage and bare parts separately, our results also suggest that there may be important differences between different hues of carotenoid pigment. In our analyses we conducted separate tests for yellow and red pigmentation and found that these often showed different patterns of association, especially in the case of plumage pigmentation. Our first step again was to look at the overall frequency of each type of coloration in the bare parts and plumage of avian families, and here we found that red coloration is far less common than yellow coloration in plumage, but not necessarily in bare parts. In plumage, both red and yellow pigmentation were skewed to low prevalence values, but the skewness for red was twice that for yellow. For bare parts, the skewness values for red and yellow were similar. Subsequently, when we tested for associations between different aspects of pigmentation and dietary carotenoids we found that such associations were particularly apparent for red plumage pigmentation, with red coloration being typically restricted to those taxa with the most carotenoid-rich diets. Both of these findings therefore agree with Hill's (1996) suggestion that red carotenoid pigments may be rarer in nature than are comparable yellow pigments and that red carotenoid pigments may play a special role in animal signalling. We would add that our results suggest that this is especially likely to be the case for plumage pigmentation, as opposed to bare part pigmentation.

The final aspect of our analyses was to use multiple regression models to test whether interspecific variation in carotenoid pigmentation is associated with variation in ecological factors other than the availability of dietary carotenoids, such as mating systems, life histories and light environments. In agreement with previous analyses, we found that the results of our models differed with respect to tissue type and hue of the carotenoid pigment under consideration. In general we found that, as predicted by various theories of biological coloration (see Baker & Parker, 1979; Butcher & Rohwer, 1989; Savalli, 1995; Endler, 1993,2000; Bennett & Owens, 2002; Owens, 2005), interspecific variation in carotenoid pigmentation is often associated with other ecological factors, but that the exact pattern of association is idiosyncratic to particular aspects of coloration and to particular phylogenetic hypotheses. Of most interest to the main topic of this particular study was our finding that, in all cases, dietary carotenoid availability was not the most important ecological correlate of interspecific variation in carotenoid pigmentation. Diet entered models consistently for plumage pigmentation, but not for bare part pigmentation.

Limitations of this study

We believe that the results of our study are noteworthy because it is the first large-scale, quantitative test for an interspecific association between carotenoid-based pigmentation and the carotenoid content of avian diets. But our results must also be treated cautiously because our study is limited in several ways, some of which are general to the comparative method and some of which are specific to this study. Of the limitations that are general to the comparative method the most important for this study are that comparative studies cannot show functional causality and therefore also cannot reveal in which direction such causality has occurred. So, while we can say that there are relationships between carotenoid pigmentation and various aspects of life history and ecology, we can only hypothesize about whether changes in life history and ecology have led to changes in pigmentation, or *vice versa*. Similarly, we cannot use comparative analyses to identify trade-offs among the various ecological and life history characteristics that are related to carotenoid pigmentation–we can only suggest where these might lie and test for them further through empirical studies. Finally, comparative studies are reliant on phylogenetic hypotheses, and are therefore only as good as the best available phylogenies for the taxa in question. Our study used the two of family level phylogenies currently available for birds and the major results remained consistent across these alternative hypotheses, but this is not to say that our results would not benefit from future refinements of our picture of avian inter-relationships.

The most important limitations that are specific to this study concern the indices of pigmentation and dietary carotenoids. We assumed that, within the limits we describe, a colour is indicative of the underlying pigment. This is not always the case, however (e.g. McGraw *et al.*, 2004), and while we have excluded families that we know do not use carotenoids for long-wavelength coloration, there may yet be other families for which biochemical analysis will prove that they too use pigments other than carotenoids to produce reds, oranges, and yellows. Having said that, given the number of families for which robust data is available, we feel the extra error variance contributed to our data will be relatively small. The disparity between actual and

perceived cause of a colour may have been particularly problematic for red bare parts where, while we endeavoured to distinguish between reds caused by pigments and reds resulting from the flushing of skin with blood, we may not always have been successful. We also assumed that the composition of a species' diet is indicative of carotenoid availability, however, researchers are only just beginning to understand the complexities of how birds absorb and utilize carotenoids. Our simple approach to availability does not take into account interspecific differences in these physiological characteristics. Finally, we did not include all possible ecological factors in our multiple regression models, and there may yet be others that will prove to be correlated with carotenoid pigmentation. This possibility remains to be tested in future studies.

General conclusions

In this study we discovered that, among avian families, dietary carotenoid intake is sometimes related to interspecific differences in carotenoid pigmentation, but that the relationship varies depending on the tissue deposition site and the hue of the pigments in question. In addition, other life history and ecological factors appear to be more important correlates of carotenoid pigmentation than diet. These findings do not undermine the conclusions of previous studies within species and genera that have supported a strong influence of diet on the production of carotenoid-based plumage colours. Our results do suggest, however, that the link between diet and carotenoid colour is highly context dependent.

The opportunities for future research on the origin and evolution of carotenoid pigmentation in birds are many, but two main observations strike us as the most immediate questions to address. First, while a fairly large proportion of all families appear to be able to use some form of carotenoid pigmentation, red or yellow alone were always less prevalent, regardless of the tissue in question (see Fig. 1). This observation suggests that while the expression of pure carotenoid-based coloration is costly for birds, blends of carotenoids with other elements (i.e. melanins or tissue structures) may be less costly to produce. It is possible that blended colours such as these require smaller quantities of carotenoids to produce, and therefore carry lower costs. This idea needs further examination through the use of biochemical and spectrophotometric techniques, as well as through controlled carotenoid supplementation experiments on birds producing both pure and blended carotenoid-based colours.

Finally, our results suggest that avian families not expressing carotenoid pigmentation may lack the genetic or physiological apparatus necessary for carotenoid expression, or that other aspects of their evolutionary history have made carotenoid pigmentation either less useful or even maladaptive. Our tests did not distinguish between families that have never evolved carotenoid pigmentation and those that have lost it, and it would be interesting to compare these groups with respect to their evolutionary relationships, life histories, and ecological characteristics.

Acknowledgments

We thank Peter Bennett, Rob Freckleton, Anne Goldizen, Geoff Hill, Hugh Possingham and Richard Zann for discussion and comments on earlier versions of this manuscript, Nick Isaac for statistical discussions, Keith Barker for giving us access to his phylogeny, and Albert Phillimore for formatting the amalgamated Cracraft-Barker phylogeny. We also thank the curators and librarians at the Smithsonian Institution (National Museum of Natural History, Division of Birds, Washington, DC, USA), Queensland Museum (Brisbane, Australia), University of Queensland (Brisbane, Australia), Alexander Koenig Museum of Zoology (Bonn, Germany), and Edward Grey Institute (Oxford, UK) for access to specimens and literature. This research was carried out with the aid of an Australian Postgraduate Award and a University of Queensland Graduate School Research Travel Award to V. Olson.

Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/jeb/jeb940/jeb940sm.htm

Appendix A1. Dietary carotenoid content and pigmentation indices for 141 families of birds.

Appendix A2. Diet categories used in the assessment of dietary carotenoid intake.

Appendix A3. Scores used to assess the openness of the ''typical'' habitats used by members of a given family.

Appendix A4. Scores assigned to nests based on both degree of accessibility and degree of concealment.

References

Andersson, S. 2000. Efficacy and content in colour signals. In: *Animal signals: signalling and signal design in animal communication* (Y. Espmark, T. Amundsen & G. Rosenqvist, eds), pp. 47–60. Tapir Academic Press, Trondheim, Norway.

Baker, R.E. & Parker, G.A. 1979. The evolution of bird coloration. *Phil. Trans. R. Soc. Lond. B* **287**: 63–130.

Barker, F.K., Cibois, A., Schikler, P., Feinstein, J. & Cracraft, J. 2004. Phylogeny and diversification of the largest avian radiation. *Proc. Nat. Acad. Sci. USA* **101**: 11040–11045.

Bendich, A. 1993. Biological functions of dietary carotenoids. *Ann. New York Acad. Sci.* **691**: 61–67.

Bennett, P.M. & Owens, I.P.F. 1997. Variation in extinction risk among birds: chance or evolutionary predisposition? *Proc. R. Soc. Lond. B* **264**: 401–408.

Bennett, P.M. & Owens, I.P.F. 2002. *Evolutionary ecology of birds: life histories, mating systems and extinction*. Oxford University Press, Oxford.

Blomberg, S. P., Garland, T. Jr. & Ives, A.R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Blount, J.D., Metcalfe, N.B., Birkhead, T.R. & Surai, P.F. 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300**: 125–127.

Brush, A.H. 1981. Carotenoids in wild and captive birds. In: *Carotenoids as colorants and vitamin A precursors* (J. C. Bauernfeind, ed.), pp. 539–562. Academic Press, New York.

Brush, A.H. 1990. Metabolism of carotenoid pigments in birds. *FASEB J.* **4**: 2969–2977.

Burley, N.T., Price, D.K. & Zann, R.A. 1992. Bill color, reproduction and condition effects in wild and domesticated zebra finches. *Auk* **109**: 13–23.

Butcher, G.S. & Rohwer, S. 1989. The evolution of conspicuous and distinctive coloration for communication in birds. *Curr. Ornithol.* **6**: 51–108.

Cracraft, J., Barker, F.K., Braun, M., Harshman, J., Dyke, G.J., Feinstein, J., Stanley, S., Cibois, A., Schikler, P., Beresford, P., Garcia-Moreno, J., Sorenson, M.D., Yuri, T. & Mindell, D.P. 2004. Phylogenetic relationships among modern birds (Neornithes): towards an avian tree of life. In: *Assembling the tree of life*, (J. Cracraft & M. J. Donoghue, eds), pp. 468–489. Oxford University Press, New York.

Colegrave, N. & Ruxton, G.D. 2003. Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. *Behav. Ecol.* **14**: 446–447.

Dyck, J. 1992. Reflectance spectra of plumage areas colored by green feather pigments. *Auk* **109**: 293–301.

Endler, J.A. 1993. The color of light in forests and its implications. *Ecol. Monogr.* **63**: 1–27.

Endler, J.A. 2000. Evolutionary implications of the interaction between animal signals and the environment. In: *Animal signals: signalling and signal design in animal communication*, (Y. Espmark, T. Amundsen & G. Rosenqvist, eds), pp. 11–46. Tapir Academic Press, Trondheim, Norway.

Faivre, B., Grégoire, A., Préault, M., Cézilly, F. & Sorci, G. 2003. Immune activation rapidly mirrored in a secondary sexual trait. *Science* **300**: 103.

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.

Folstad, I. & Karter, A. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**: 603–622.

Fox, D.L. 1979. *Biochromy: natural coloration of living things*. University of California Press, Berkeley.

Freckleton R.P., Harvey P.H. & Pagel M. 2002. Phylogenetic analysis and comparative data: A test and review of evidence. *Am. Nat.* **160**: 712–726.

Goodwin, T.W. 1984. *The biochemistry of the carotenoids. Vol. II. Animals*. Chapman & Hall, London.

Gray, D.A. 1996. Carotenoids and sexual dichromatism in North American passerine birds. *Am. Nat.* **148**: 453–480.

Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science* **218**: 384–387.

Hill, G.E. 1991. Plumage coloration is a sexually selected indicator of male quality. *Nature* **350**: 337–339.

Hill, G.E. 1992. Proximate basis of variation in carotenoid pigmentation in male house finches. *Auk* **109**: 1–12.

Hill, G.E. 1994a. House finches are what they eat: a reply to Hudon. *Auk* **111**: 221–225.

Hill, G.E. 1994b. Trait elaboration via adaptive mate choice: sexual conflict in the evolution of signals of male quality. *Ethol., Ecol. & Evol.* **6**: 351–370.

Hill, G.E. 1996. Redness as a measure of the production cost of ornamental coloration. *Am. Nat.* **154**: 589–595.

Hill, G.E. 2002. *A red bird in a brown bag: the function and evolution of colorful plumage in the house finch*. Oxford University Press, New York.

Hill, G.E., Montgomerie, R., Inouye, C.Y. & Dale, J. 1994. Influence of dietary carotenoids on plasma and plumage colour in the house finch: intra- and intersexual variation. *Func. Ecol.* **8**: 343–350.

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.

Hudon, J., Ouellet, H., Benito-Espinal, É., & Brush, A.H. 1996. Characterization of an orange variant of the bananaquit (*Coereba flaveola*) on La Désirade, Guadeloupe, French West Indies. *Auk* **113**: 715–718.

Johnson, K.P. & Lanyon, S.M. 2000. Evolutionary changes in color patches of blackbirds are associated with marsh nesting. *Behav. Ecol.* **11**: 515–519.

Latscha, T. 1990. *Carotenoids–their nature and significance in animal feeds*. F. Hoffmann-LaRoche Ltd., Basel, Switzerland.

Linville, S.U. & Breitwisch, R. 1997. Carotenoid availability and plumage coloration in a wild population of northern cardinals. *Auk* **114**: 796–800.

Lozano, G.A. 1994. Carotenoids, parasites, and sexual selection. *Oikos* **70**: 309–311.

Martin, T.E. & Badyaev, A.V. 1996. Sexual dichromatism in birds: importance of nest predation and nest location for females versus males. *Evolution* **50**: 2454–2460.

Mahler, B., Araujo, L.S. & Tubaro, P.L. 2003. Dietary and sexual correlates of carotenoid pigment expression in dove plumage. *Condor* **105**: 258–267.

Martins, E. P. & Housworth, E.A. 2002. Phylogeny shape and the phylogenetic comparative method. *Syst. Biol.* **51**: 873–880.

McGraw, K.J., Hill, G.E., Stradi, R. & Parker, R.S. 2001a. The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Physiol. Biochem. Zool.* **74**: 843–852.

McGraw, K.J., Stoehr, A.M., Nolan, P.M., & Hill, G.E. 2001b. Plumage redness predicts breeding onset and reproductive success in the house finch: a validation of Darwin's theory. *J. Avian Biol.* **32**: 90–94.

McGraw, K.J., Gregory, A.J., Parker, R.S. & Adkins-Regan, E. 2003. Diet, plasma carotenoids, and sexual coloration in the zebra finch (*Taeniopygia guttata*). *Auk* **120**: 400–410.

McGraw, K.J., Wakamatsu, K., Ito, S., Nolan, P.M., Jouventin, P., Dobson, F.S., Austic, R.E., Safran, R.J., Siefferman, L.M., Hill, G.E. & Parker, R.S. 2004. You can't judge a pigment by its color: carotenoid and melanin content of yellow and brown feathers in swallows, bluebirds, penguins and domestic chickens. *Condor* **106**: 390–395.

McNaught, M. & Owens, I.P.F. 2002. Interspecific plumage colour variation in birds: species recognition or light environment? *J. Evol. Biol.* **15**: 505–514.

Metz, K.J. & Weatherhead, P.J. 1992. Seeing red: uncovering coverable badges in red-winged blackbirds. *Anim. Behav.* **43**: 223–229.

Monroe, B.L. Jr. & Sibley, C.G. 1993. *A world checklist of birds*. Yale University Press, New Haven, CT.

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.

Owens, I. P. F. 2005. Ecological explanations for interspecific variation in plumage colour. In: *Avian Coloration* (G. Hill & K. McGraw, eds). Harvard University Press.

Owens, I.P.F. & Hartley, I.R. 1998. Sexual dimorphism in birds: why are there so many different forms of dimorphism? Proc. *R. Soc. Lond.* **265B**: 397–407.

Owens, I.P.F. & Short, R.V. 1995. Hormonal control of sexual dimorphism in birds: implications for new theories in sexual selection. *Trends Ecol. Evol.* **10**: 44–47.

Purvis, A. & Rambaut, A. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Computer Appl. Biosciences* **11**: 247–251.

Purvis, A., Gittleman, J.L., Cowlishaw, G. & Mace, G.M. 2000. Predicting extinction risk in declining species. *Proc. R. Soc. Lond.: Series* **267B**: 1947–1962.

Savalli. U. 1995. The evolution of bird coloration and plumage elaboration: a review of hypotheses. *Curr. Ornithol.* **12**: 141–190.

Scheidt, K. 1998. Absorption and metabolism of carotenoids in birds, fish and crustaceans. In: *Carotenoids Vol. 3: biosynthesis and metabolism*, (G. Britton, S. Liaanen-Jensen & H. Pfander, eds), pp. 285–358. Birkhäuser-Verlag, Basel, Switzerland.

Shykoff, J.A. & Widmer, A. 1996. Parasites and carotenoid-based signal intensity: how general should the relationship be? *Naturwissenschaften* **83**: 113–121.

Sibley, C.G. & Ahlquist, J.E. 1990. *Phylogeny and classification of birds*. Yale University Press, New Haven, CT, USA.

Slagsvold, T. & Lifjeld, J.T. 1985. Variation in plumage colour of the great tit *Parus major* in relation to habitat, season and food. *J. Zool. (Lond.)* **206**: 321–328.

Sokal, R.R. & Rohlf, F.J. 1981. *Biometry*, 2nd edn. W.H. Freeman & Co., New York.

Stradi, R., Pini, E. & Celentano, G. 2001. The chemical structure of the pigments in *Ara macao* plumage. *Comp. Biochem. Physiol.* **130B**: 57–63.

Tella, J.L., Figuerola, J., Negro, J.J., Blanco, G., Rodriguez-Estrella, A., Forero, M.G., Blazquez, M.C., Green, A.J. & Hiraldo, F. 2004. Ecological, morphological and phylogenetic constraints of interspecific variation in plasma carotenoid concentration in birds. *J. Evol. Biol.* **17**: 156–164.

Received 22 December 2004; revised 14 March 2005; accepted 18 March 2005