Title: Carotenoid metabolism strengthens the link between feather coloration and individual quality

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Abstract

Thirty years of research has made carotenoid coloration a textbook example of an honest signal of individual quality, but tests of this idea are surprisingly inconsistent. Here, to investigate sources of this heterogeneity, we perform meta-analyses of published studies on the relationship between carotenoid-based feather coloration and measures of individual quality. To create color displays, animals use either carotenoids unchanged from dietary components or carotenoids that they biochemically convert before deposition. We hypothesize that converted carotenoids better reflect individual quality because of the physiological links between cellular function and carotenoid metabolism. We show that feather coloration is an honest signal of some, but not all, measures of quality. Where these relationships exist, we show that converted, but not dietary, carotenoid coloration drives the relationship. Our results have broad implications for understanding the evolutionary role of carotenoid coloration and the physiological mechanisms that maintain signal honesty of animal ornamental traits.

Introduction

Red, yellow, and orange carotenoid-based color displays are among the most widespread and conspicuous ornamental traits in animals. Carotenoid coloration is frequently an important criterion in mate choice [1,2], and researchers have found associations between carotenoid coloration and various measures of individual quality in studies of fish, reptiles, and birds [3–6]. Based on these observations, researchers hypothesized that assessment of the carotenoid coloration of prospective mates provides key information about individual quality and enables choices that increase fitness [7,8]. However, the mechanism that links carotenoid coloration to individual quality, and hence the specific information content of carotenoid displays, remains unresolved and contentious [5,9].

In addition to being colorful, carotenoids are thought to be relevant and essential molecules used in cellular processes such as immunocompetence and vitamin A synthesis, as well as antioxidants [10,11]. Understanding the connections between individual quality and carotenoid ornamentation demands an understanding of the biochemistry of the pigments involved (Box 1, [12,13]). Carotenoids cannot be synthesized by animals *de novo;* whether they are used as external colorants or serve functions in cellular pathways, all carotenoids must ultimately be obtained from the diet [14]. Moreover, animals typically ingest only the yellow carotenoid pigments (*e.g.,* lutein and zeaxanthin; Fig. 1). To display carotenoid-based red coloration, most animals have to bio-convert yellow pigments to red [15,16]. The quality of an individual color display is a product of the biochemical pathways by which carotenoids are absorbed, transported, metabolized, and deposited [17] , so it follows that these biochemical pathways are central in creating the connections between ornamental coloration and individual condition [7].

The dual role of carotenoids, both as colorants and potentially as important components of core cellular processes, is the foundation of the resource allocation trade-off hypothesis [9], which proposes that carotenoid coloration links to individual quality through tradeoffs in use of carotenoids for body

maintenance versus ornamentation [18–20]. Under this hypothesis, carotenoids are needed both for body maintenance and for ornamentation and thus only individuals with large stores of carotenoids or with low demands for body maintenance due to superior health can afford to allocate sufficient carotenoids for production of full ornamentation. The resource allocation tradeoff hypothesis does not predict a difference in the signal content of coloration derived from metabolically modified versus dietary pigments.

Alternatively, carotenoid coloration may serve as a reliable signal of individual quality because the mechanisms involved in the metabolic conversion of carotenoid pigments (Fig. 1; Box 1) are intimately linked to vital cellular pathways [9,12,21]. This shared pathway hypothesis predicts that regardless of the carotenoid resources that are available, disruption of core cellular processes, and particularly cellular respiration, will reduce production of carotenoid ornamentation [7,22]. Moreover, under the shared pathway hypothesis, metabolism of dietary carotenoids for ornamentation should create stronger connections between cellular processes that give rise to quality and carotenoid-based signals because the pathways required for carotenoid metabolism are sensitive to the cellular environment [12,23]. The process of transforming dietary carotenoids to ketocarotenoids (Box 1) could be a key mechanism responsible for maintaining honesty from converted yellow and red carotenoid-based feather coloration, but this idea has not been rigorously tested.

Studies of the function and evolution of carotenoid coloration have frequently focused on birds and particularly on plumage. Sufficient studies have now been published to evaluate patterns among studies for associations between individual quality and feather coloration resulting from carotenoid pigments that emerge from fundamentally different biochemical processing. In this quantitative synthesis, we categorize carotenoids used for feather coloration as ‘dietary’ if the pigments are present in food and deposited in feathers without further modifications (Fig. 1). Pigments in this category are absorbed,

89 transported, and deposited but they undergo no metabolic conversion. The two most common dietary
90 pigments used as colorants in birds are lutein and zeaxanthin. Alternatively, we categorize carotenoid as
91 'converted' if they are derived from dietary carotenoids that are metabolically oxidized internally by the
92 bird to form ketocarotenoids before deposition to feathers (Fig. 1; Box 1). Common examples of
93 converted carotenoids include echinenone, canthaxanthin, and canary xanthophylls [16].

94 Previous reviews of condition-dependence of carotenoid pigmentation in birds have not considered
95 whether the biochemical processes involved in carotenoid pigmentation—and specifically whether or
96 not dietary carotenoids are metabolically converted— affects the relationship between coloration and
97 condition [6,24,25]. We hypothesized that the strength of the relationship between coloration signal and
98 individual quality is dependent on whether the color display involved metabolic conversion of dietary
99 carotenoids. We predicted that if the mechanisms of carotenoid metabolism are linked to basic cellular
100 function that comprise individual quality [9,26], feather coloration requiring carotenoid metabolism would
101 have a stronger positive relationship with measures of individual quality than feather coloration derived
102 from deposition of unaltered dietary carotenoids [12,23]. Additionally, metabolism of carotenoids requires
103 the maintenance of enzyme systems (Box 1; [27]) and perhaps additional transport of carotenoids [13] that
104 may create stronger links between coloration and system performance for ornaments produced from
105 converted pigments versus unaltered dietary pigments.

106 We tested our hypothesis that carotenoid metabolism strengthens the link between feather coloration
107 and individual quality using meta-analysis. We quantitatively synthesized published results on the
108 relationships between individual quality and plumage coloration of passerines produced via dietary
109 versus converted carotenoids. Overall, we find that, without partitioning between carotenoid types,
110 carotenoid-based feather coloration is positively associated with individual quality. However, including
111 carotenoid type as a predictor reveals that converted, and not dietary, carotenoids are driving the

relationship between coloration and quality. Thus, the physiological processes involved in carotenoid metabolism may be an important mediator in maintaining honesty from carotenoid-based coloration.

Results

The final dataset included 191 effect size estimates from 50 published studies of 19 passerine bird species (Supplementary Data 1). Detailed results from each model are listed in Table 2. Mean effect size estimates and 95% credible intervals (CI) are reported unless otherwise noted. Mean estimates with credible intervals that do not include zero are a statistically significant effect at $\alpha = 0.05$. Using sampling variance as a predictor, results from the Egger's regression did not imply funnel plot asymmetry from the residuals of the overall meta-analytic model (Supplementary Fig. 1; $t_{189} = 1.98$, $p = 0.05$).

Carotenoid coloration as an honest signal of quality

Consistent with previous quantitative and qualitative reviews that did not account for carotenoid type, we found a small positive correlation between richness of coloration and individual quality overall, but note that the 95% CI slightly overlaps zero ($Zr = 0.161$, 95% CI: -0.079 to 0.368; $I^2 = 98.10\%$). However, when we parsed the data between species with plumage coloration resulting from converted versus dietary carotenoids, we found that the relationship between richness of coloration and individual quality held only for species that used converted pigments (Converted: $Zr = 0.263$, 95% CI: 0.062 to 0.491; Dietary: $Zr = 0.089$, 95 % CI: -0.123 to 0.292; Fig. 2a). On average, we found that dietary carotenoid coloration is a weaker predictor of overall quality than converted carotenoid coloration ($\beta_{(Dietary)} = -0.158$, 95% CI: - 0.388 to 0.097), though the credible intervals from this comparison slightly overlap zero.

Body condition

Measures of body condition were not reliably positively correlated with carotenoid-based feather coloration ($Zr = 0.065$, 95% CI: -0.218 to 0.352, Fig. 2b), and we found no clear difference in the relationship between carotenoid types (Converted: $Zr = 0.084$, 95% CI: -0.158 to 0.358; Dietary: $Zr = 0.107$, 95% CI: -0.186 to 0.388).

Immune function and oxidative physiology

On average across all measures of immune function and oxidative physiology, we found that feather coloration was not a reliable predictor (Zr = 0.102, 95% CI: -0.210 to 0.362; Fig. 2c) with no clear difference in the relationship between carotenoid types (Converted: Zr = 0.098, 95% CI: -0.186 to 0.367; Dietary: Zr = 0.113, 95% CI: -0.135 to 0.371). When we treated these two categories separately, the results were qualitatively similar (Supplementary Table 2); neither dietary nor converted carotenoids were robust signals of immune function or measures of oxidative physiology.

Parasite resistance

When no distinction was made between carotenoid types, richness of coloration was positively correlated with parasite resistance (Zr = 0.243, 95% CI: -0.074 to 0.510). When we added carotenoid type as a moderator to our model we found that coloration from dietary carotenoids was weakly correlated with parasite resistance (Zr = 0.009, 95% CI: -0.242 to 0.371; Fig. 2d). In contrast, coloration from converted carotenoid-based plumage was strongly positively associated with parasite resistance (Zr = 0.435, 95% CI: 0.175 to 0.689; Fig. 2d) and was a more reliable signal than coloration from dietary carotenoid-pigmented feathers ($\beta_{(Dietary)}$ = -0.420, 95% CI: -0.692 to -0.162). When measures of parasite resistance were pooled with the immune category, our model return qualitatively similar results (Supplementary Table 3); effect sizes from dietary coloration were not robustly associated with measures of immune function, but effect sizes from converted coloration strongly correlated with measures of immune function.

Reproductive and parental quality

We found that overall, carotenoid coloration positively correlated with aspects of reproductive and parental quality (Zr = 0.223, 95% CI: -0.099 to 0.462; Fig. 2e), but that the pattern was driven by

converted carotenoid feather coloration (Converted: *Zr* = 0.337, 95% CI: 0.021 to 0.618; Dietary: *Zr* = 0.0.09, 95% CI: -0.183 to 0.312). However, converted feather color was only a marginally better predictor of quality than dietary feather color ($\beta_{(Dietary)}$ = -0.227, 95% CI: -0.536 to 0.077).

Species-level analysis

The strength of the relationship between feather coloration and combined measures of individual quality was dependent on the individual species (Fig. 3). However, we found only a weak phylogenetic signal among carotenoid types (I^2= 2.24%*)* in the overall analysis (Table 2).

The role of sex on effect size estimates

We found that the sex from which effect sizes were calculated did not influence the magnitude of the meta-analytic mean (Dietary female vs male: β= 0.008, 95% CI: -0.140 to 0.170; Converted female vs male: β= 0.101, 95% CI: -0.07 to 0.29).

Residual heterogeneity

Heterogeneity in our overall meta-analytic model was high (I^2 = 98.12%; see ref. [28] for benchmarks). Including carotenoid type as a moderator in our overall model reduced heterogeneity (I^2 = 72.43; Table 2). More variance was partitioned from the model by the addition of quality category and carotenoid type as model moderators (I^2 = 72.40; Table 2). A high degree of heterogeneity remained after we included carotenoid type and quality category as moderators in the model indicating that other variables not included in this analysis may be influencing the relationship between feather coloration and aspects of individual quality[29].

Discussion

Carotenoid coloration is among the most frequently cited examples of a condition-dependent signal of quality assessed during mate choice [1,30], but the relationship between various measures of condition and different forms of carotenoid coloration is not consistent across studies [31–33]. We hypothesized that some of the noise in the comparative data might be due to a failure to consider whether the color display under study was derived from unmodified dietary pigments or pigments that had been metabolically converted from dietary pigments. We predicted that feather displays derived from converted carotenoids would be better signals of quality if carotenoid metabolism creates stronger connections between color displays and the cellular processes that underlie the state of condition in the animal. [12,21] The patterns we observed in our meta-analyses indicate that converted carotenoid feather coloration indeed had stronger relationships with measures of individual quality than dietary carotenoid feather coloration.

The results of our meta-analyses show that whether the carotenoid coloration is dietary or converted is an important predictor of the likelihood that a plumage color display will be correlated to a measure of individual quality. When we assessed all studies of carotenoid feather coloration, without regard for whether the pigments were dietary or converted, there was a non-significant trend across studies for a positive association between carotenoid coloration and individual quality (Fig. 2a). When we examined whether the type of carotenoid (converted or dietary) used for plumage coloration had an effect on the strength of the relationship between carotenoid coloration and condition, we found that there was a strong and statistically significant correlation between measures of quality and coloration for birds that deposit converted carotenoids to feathers. In contrast, there was much weaker condition dependency for birds that deposited only dietary carotenoids to feathers, and the relationship was not significantly different from zero. We did not observe a clear phylogenetic pattern in the strength of the relationship

between coloration and quality. These results support our hypothesis that carotenoid metabolism is a meaningful factor that predicts the strength of correlation between coloration and measures of individual quality (Fig 3).

These results have important implications for understanding the mechanisms that link carotenoid coloration and individual condition. Endler [34] first proposed that carotenoid coloration in animals could serve as an honest signal of quality because carotenoids are scarce resources that are challenging to accrue. Experiments with guppies (*Poecilia reticulata*) [35] and house finches (*Haemorhous mexicanus*) [36] showed that depriving individuals of carotenoids during ornament production caused loss of color, but whether resource limitation was the basis for signal honesty in natural environments remained contentious [37–39]. The patterns revealed in our meta-analysis suggest that honest signaling arises from more than simply access to carotenoid resources, whereby all forms of carotenoid pigmentation should serve as signals of quality. Rather, the strong association between display of converted carotenoid pigments and individual quality suggest that something about the process of carotenoid modification creates a link between pigmentation and individual condition [7,9,21,22].

Perhaps one of the most ubiquitous statements made of carotenoids is their capacity to serve as powerful antioxidants and immune boosters [10,40,41]. However, the specific roles that carotenoids might play *in vivo* in free radical scavenging or immune system function are uncertain and contentious [42–45]. A previous meta-analysis by Simons et al. [6] examined the relationship between carotenoid content and multiple measures of immunocompetence and oxidative stress in birds to test the idea that carotenoid content or coloration is an honest signal of these qualities. They found that circulating carotenoid levels in the plasma related to only two out of seven measures: PHA response — a commonly used skin swelling assay of immunocompetence — and antioxidant capacity. Additionally, carotenoid coloration of

228 feathers or skin was not a reliable signal for six out of seven proxies of immune function or oxidative
229 stress [6]. Our results are generally consistent with those of Simons et al. [6] in that feather coloration was
230 weakly correlated with measures of immune function and oxidative physiology, and was not a robust
231 signal on average (Fig. 2c). Furthermore, whether coloration was derived from dietary or converted
232 carotenoids had no effect on the honesty of coloration as a signal of immune function. The complex
233 pathways involved in innate and acquired immunity may obscure meaningful interpretation of
234 immunocompetence when it is measured only as the relative abundances of different types of white
235 blood cells in circulation.

236 Unlike measures of immune function, studies on internal or surface parasites in birds benefit from a
237 simple methodology that is straightforward to interpret. One of the earliest and widely cited examples
238 of honest signaling of parasite resistance from feather coloration was Hamilton and Zuk's [46] study that
239 proposed that 'bright' feather coloration evolved to signal heritable parasite resistance. This seminal
240 study served as the cornerstone for new ideas about why ornaments are assessed during mate choice.
241 We found that birds with converted carotenoid feather coloration had a strong relationship with
242 parasite resistance. In contrast, birds that directly deposited carotenoids from their diet showed a weak
243 and unreliable correlation with parasite resistance (Fig. 2d). The mechanisms involved in the
244 transformation of dietary carotenoids might act as a signal of the underlying genetic or physiological
245 mechanisms that provide parasite resistance [47].

246 The stronger associations between reproduction and plumage color derived from converted versus
247 dietary pigments could be a consequence of stronger associations between individual condition and
248 ornamentation produced by converted versus dietary carotenoids. The proxies for reproductive and
249 parental quality that were used in this study are a composite of the strategies and performances of both
250 the ornamented male bird and its mate [48–50]. Mate choice for feather coloration should be stronger

when the signal of quality is stronger, so the stronger association between reproduction and converted carotenoids could arise through stronger choice for such signals. Moreover, if the stronger preference for coloration resulting from converted carotenoids means that such birds attract higher quality mates then color based on converted carotenoids would be more strongly linked to reproductive success. For example, in many species of perching birds, males directly participate in nesting so there should also be direct effects of male condition on reproductive success. Any of these factors could have contributed to the stronger associations of color produced by converted carotenoids and reproduction.

The proxies for quality used in nearly every study of carotenoid coloration are rooted in the ability to efficiently produce ATP via oxidative phosphorylation at a level necessary to meet physiological demand. Thus far, the mechanism by which carotenoid coloration links to quality has remained speculative. Direct measures of both the allocation of specific pools of carotenoids to physiological function versus ornament production and the effects of direct manipulation of mitochondrial performance on carotenoid production are needed to definitively test the resource allocation and shared pathway hypotheses.

The patterns that we revealed though our meta-analysis indicate that feather coloration from converted carotenoids are better indicators of individual condition than dietary carotenoids. One could reasonably extrapolate from this observation to propose that metabolism of feather carotenoids evolved in response to female mate preferences for the best indicators of condition. But the conversion of yellow dietary to yellow converted pigments, which present the same visual display, presents an enigma. Why don't males that display with converted yellow pigments just skip the conversion and load up on dietary pigments? Badyaev et al. [51] examined patterns of feather growth and coloration and hypothesized that metabolized carotenoids caused less stochastic variation in feather structure than dietary carotenoids. In other words, dietary pigments can affect the integrity of feathers and perhaps the stochastic variation

they engender can undermine the quality of the sexual signal. In light of our finding that converted pigments provide a better indicator of condition, it will be interesting to further test such hypotheses for how developmental pathways might favor converted over dietary carotenoids.

Using a meta-analysis and meta-regressions of bird studies that investigated the association between carotenoid-based feather coloration and individual condition, we found support for the hypothesis that this type of plumage coloration serves as an honest indicator of individual quality [52]. The strength of this signal, however, is greater for species that convert dietary carotenoids to ketolated carotenoids before utilizing them for feather coloration.

Our results are not consistent with the resource allocation hypothesis because dietary carotenoids are the pigments primarily implicated in immune enhancement and free radical scavenging, and yet they were not consistently associated with proxies for individual quality. Our results are consistent, however, with the hypothesis that conversion of dietary carotenoids acts to strengthen the relationship between feather coloration and measures of quality and may be an important mechanism in maintaining honesty from carotenoid-based ornaments.

Feathers are not the only structures that are colored by carotenoids. Many bird, reptile, and fish taxa exhibit bare-part carotenoid color signals [53–56]. Future work could focus on understanding the differences in signal honesty from bare-part carotenoid coloration among different taxa to test alternative predictions of our hypothesis that carotenoid metabolism strengthens the link between coloration and individual quality.

Methods

Literature search

A literature search of the *Web of Science* and *Google Scholar* databases using alternative spellings and combinations of the keywords including “carotenoid”, “color”, “condition”, “signal”, “feather” and “quality”, returned over 1,600 potentially eligible published articles. This search was based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses statement (PRISMA[57]; Supplementary Fig. 2). We also considered as potentially eligible articles, data from studies included in published meta-analyses of bird coloration [6,24] and references therein. The online database search was last performed on 11 April 2017 on titles, abstracts and keywords in both databases. We did not request unpublished data sets from colleagues, because of the risk of biasing the estimates of effect size (see ref. [58]).

It is important to note that carotenoid coloration is not limited to feathers; indeed, carotenoid coloration from bare-parts including beaks, legs, and combs of birds has been the focus of excellent research on the evolution of honest signaling [53,59–61]. However, to best address how carotenoid metabolism affects honest signaling, we focused our study on a single trait, feather coloration, to avoid confounding biological factors such as blood flow [62], carotenoid esterification [61], and differences in requisite enzymes [27,63] that are relevant to bare-part coloration, but not feather coloration. These factors could obscure meaningful interpretation of the relationship among coloration measures, carotenoid metabolism, and individual quality from bare-part coloration.

Because we were interested in the signal content of carotenoid-based plumage, we focused on studies that quantified feather color using standardized color metrics (see ‘Color metrics’ below) of natural (i.e., un-supplemented) adult bird color levels. Therefore, we excluded studies from our meta-analysis for any of the following reasons: only coloration of non-feathered structures was measured (e.g. wattles, legs,

beaks); a non-passerine species was studied; only plasma concentrations of carotenoids were measured; or only nestling or juvenile coloration was studied. We did not include measures of feather brightness as it is sensitive to factors unrelated to pigmentation (see below). Additionally, to be included in our meta-analysis, studies must have investigated at least one of the following proxies of individual quality: (1) nutritional condition, (2) immune function or oxidative capacity, (3) parasite resistance, or (4) reproductive or parental quality (Table 1).

Data extraction and coding

We used the correlation coefficient, Pearson's *r*, as the effect size metric to describe the association between measurements of feather coloration and aspects of individual quality. The effect size metric was extracted directly from each study when available (*n*=9). For cases in which studies did not provide Pearson's *r* (*n*=43), the reported test statistics (*F, ρ, χ²*, τ, t, and means and standard deviations or standard errors) were used to estimate *r* [64]. Pearson's *r* was transformed to Fisher's *Z* for statistical analyses to meet normality assumptions of linear models. Researchers commonly refer to *r* = 0.1, 0.3 and 0.5 as small, medium, and large effect sizes ([65]; these benchmarks are equivalent for *Zr* values), respectively. The sign of the correlation coefficient was changed in some cases to facilitate comparisons across metrics of individual quality; for example, some measures —such as parasite load— were often negatively correlated with richness of coloration but indicated a positive relationship with quality [66].

Original studies often include multiple effect size estimates because of the measurement of several life history traits associated with individual condition or performance (*e.g.*, immune function or fledging success) and/or from different color metrics (see below). When more than one effect size is reported these data points cannot be assumed to be independent. To deal with this issue, we ran multi-level, random-effects meta-analytic models that allowed us to include multiple, non-independent effect sizes

per study. This was accomplished by including the study identity and species identity as random effects [67].

Carotenoid type

Coloration of feathers was categorized as dietary if feather carotenoids identified for that species were only those that are typically found in passerine diets (e.g., lutein or zeaxanthin) [16,68]. Carotenoids were categorized as converted if feather carotenoids identified for that species were those that are oxidative products of dietary carotenoids, typically 4-keto-carotenoids and canary xanthophylls (Box1 [16,68]). We referenced published studies that characterized the carotenoids present in feathers of each species included in our meta-analysis (Supplementary Table 4). For instances in which we could not identify the feather carotenoids of a particular species (n =3) we used published data from a sister-species of the same genus that displayed the same feather color.

Color metrics

Standing variation in richness of color between individuals is requisite for assessing quality from carotenoid-based ornaments. Common metrics used to quantify this variation in feather reflectance include comparisons to standard color charts (e.g., Munsel), calculations of hue, chroma and brightness or composite metrics such as principal components (PCA) from spectrophotometer data or digital photographs. Hue describes the unique spectral color (e.g., “red”, “orange”, “yellow”) and chroma describes the saturation or spectral purity of the color display relative to total reflectance across the visible range of the electromagnetic spectrum [69]. We extracted the response of all color variables used to assess the relationship between color and a measure of quality (e.g., condition, immune function) from each study. We did not include measures of brightness in our analyses because it is strongly influenced by the physical structure of the feather which may be altered by abrasion and wear and is difficult to interpret for carotenoid content [70].

363

364 **Meta-analysis technique**

365 To evaluate the strength of correlation between all measures of individual quality and overall feather
366 color richness, we first used a Bayesian mixed-effects meta-analytic model without distinguishing
367 between carotenoid types (i.e., "Combined"; intercept only model). To further partition heterogeneity,
368 we conducted a Bayesian meta-regression analysis with carotenoid type included as a categorical
369 moderator to test the effect of the source of ornamental coloration (either dietary or converted
370 carotenoids) on the overall relationship between color and quality. We then performed a second meta-
371 regression analysis to test the strength of association of each quality category with both dietary and
372 converted carotenoid-based plumage. We used a third meta-regression model to quantify the
373 relationship between coloration and quality for each bird species examined in the data set. Lastly, we
374 used a fourth meta-regression model that included an interaction between the carotenoid-type and sex
375 to investigate the role of sex in the relationship between color measures and quality. We used the
376 MCMCglmm package [71,72] in program R version 3.3.0 [73] to conduct the Bayesian mixed-effects meta-
377 analyses.

378 To adequately correct for non-independence, it is necessary to model the correlations among effect
379 sizes. For instance, effect sizes for immune response and fledging success are likely to be correlated
380 when the traits were measured from the same group of individuals. Unfortunately, such correlations are
381 almost never reported in the original studies, and are seldom accounted for in meta-analytic models
382 (but see ref. [74]), potentially biasing general findings. Thus, to conservatively account for such
383 correlations, we report multi-level models that assume all correlations to be 0.5. Additionally, in
384 Supplementary Table 1, we report the results of the same models assuming correlations of zero. We
385 note that qualitatively the results are very similar among these meta-analytic models.

386 We also accounted for the evolutionary history of the bird species used in the study by including a
387 phylogenetic random effect to the models. Our meta-analytic models used avian phylogenetic trees with
388 the Ericson backbone from ref. [75]. To account for uncertainty in the phylogenetic reconstruction, we
389 used a sample of 1,000 trees, so that each tree was sampled at iteration, *t*. We calculated phylogenetic
390 heritability, H^2, as an index of phylogenetic signal. H^2 can be defined as the proportion of phylogenetic
391 variance in relation to the sum of all other variance components, with the exception of sample error
392 variance. When the unit of analysis is at the species level, H^2 is equivalent to Pagel's λ [76].

393 For all multi-level models, we used parameter expanded priors (*V*=1, nu=1, alpha.mu = 0, alpha.V =
394 1000) for all random effects. We used a combination of total iterations and burn-in period so that the
395 posterior distributions consisted of 1,000 samples for the model parameters (see Supplementary Data 2
396 for full code and model details). We report point estimates from the models based on the posterior
397 means, and considered moderator estimates to be statistically significant if the 95% credible interval
398 (95% CI) did not overlap zero. We calculated an modified version of the I^2 statistics (following ref. [67]) to
399 estimate heterogeneity in multi-level meta-analytic models. This procedure allocates the proportion of
400 variance not attributable to sampling variance to the random factors of the model. In our models, these
401 are the variance in effect sizes due to the four following components: phylogenetic relatedness,
402 differences among species, differences among studies, and differences residual variation (within-study
403 variation). Adding the total variation (percentages) from each of these components yields the original I^2
404 proposed by ref. [28].

405 To test for potential publication bias, we visually evaluated funnel plot asymmetry of effect size as a
406 function of sample size. Then, we conducted an Egger's regression [77] to test statistically for publication
407 bias. The analyses of bias for the multi-level models was conducted on the meta-analytic residuals (see
408 ref. [67]), as this safeguards that we meet the assumption of independence.

Code availability

Data availability

All data and R code relevant to this study are available as supplementary information.

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582

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589 **Statement of authorship**

590 RW, GH, and AW designed the study. RW collected data. RW, AMT, and ES performed the meta-
591 analyses. RW and GH wrote the first draft of the manuscript, and all authors contributed substantially to
592 revisions.

593 **Competing interests**

594 The authors declare no competing financial interests.

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Figure legends

Figure 1. To produce feather coloration, passerine birds either use dietary carotenoids unaltered or use carotenoid pigments that are metabolically derived from dietary pigments. Depicted are the proposed metabolic pathways by which the common dietary carotenoids in the diets of passerine birds can be converted into the red and yellow ketolated carotenoids found in feathers [16,78,79] . Birds shown are representative of coloration from converted and dietary carotenoids. House finch (lower) use converted red carotenoids, American goldfinch (upper) display converted yellow carotenoids, and wood warblers (middle) display dietary yellow carotenoids. A few bird species directly ingest red carotenoids, such as astaxanthin, from their diet (not shown).

Figure 2. The weighted mean correlation (*Zr*) betwen feather color richness and measures of individual quality. The strength of the association was calculated for all published studies without consideration of the carotenoid type in the feathers of the study bird (Combined, gray circles), for only studies of bird species with plumage coloration derived from converted carotenoids (red circles), and for only studies of bird species with plumage coloration derived from dietary carotenoids (yellow circles). Proxies of quality were divided into (b) body condition, (c) immune and oxidative physiology, (d) parasite resistance, and (e) aspects of parental and reproductive quality. Circle size is inversely proportional to the variance of the mean effect size. Horizontal lines represent 95% credible intervals. Effect size estimates with credible intervals that do not include zero are statistically significant effects (α =0.05).

Figure 3. The contribution of each species to the overall relationship between feather color richness and measures of individual quality. Number of effect sizes per species are in parentheses. Circles represent weighted mean correlation (*Zr*) effect sizes for species with plumage coloration from converted (red)

624 and dietary carotenoids (yellow). Circle size is inversely proportional to the variance of the mean effect
625 size. Horizontal lines represent 95% credible intervals. Effect size estimates with credible intervals that
626 do not include zero are statistically significant effects ($\alpha = 0.05$).

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Table 1. Commonly used proxies for quality to evaluate the relationship between carotenoid-based feather coloration and individual quality.

Category	Metric	Relationship with Quality	Example Reference
Condition	Body Mass	+	Dunn et al 2010
	Size Adjusted Body Mass	+	Chui et al 2011
	Ptilochronology	+	Senar et al 2003
Immune and Oxidative Physiology	Anti-Oxidant Capacity	+	Losdat et al 2011
	Antibody	+	Aguilera et al 2007
	Disease Survival	+	Van Oort and Dawson 2005
	Environmental Pollutant	-	Dauwe and Eens 2008
	HL Ratio	-	Maney et al 2008
	Pathogen Challenge	-	Brawner et al 2000
	Oxidative Damage	-	Freeman-Gallant et al 2011
	PHA response	+	Vinkler et al 2011
	WBC count	+	Horak et al 2004
Parasite	Load	-	Chui et al 2011
	Resistance	+	Hill and Brawner 1998
Reproduction	Clutch Size	+	Estep et al 2006
	Feeding Rate	+	Sundberg and Larsson 1994
	Fledgling number	+	Crary and Rodewald 2012
	Fledgling Success	+	Wolfenbarger 1999
	Lay Date	-	Sundberg 1995
	Nest Attentiveness	+	Matysiokova and Remes 2010

630

631 **Table 2.** Results from mixed-effects multilevel phylogenetic meta-analyses that assume effect sizes

632 within studies are correlated (*r=0.5)*

Analysis	*k*	*m*	Mean (*Zr)*	Lower CI (2.5%)	Upper CI (97.5%)	I^2(%)	Heterogeneity (Var_{study}) %	Heterogeneity ($Var_{species}$) %	Heterogeneity (phylogeny) %
Overall	191	19	0.1609	-0.0797	0.368	98.10	0.68	1.17	2.24
Carotenoid Type:						72.43			
Converted	92	12	**0.263**	**0.0622**	**0.492**				
Dietary	99	7	0.089	-0.1235	0.292				
Category (combined)						94.39			
Condition	35	12	0.0645	-0.2184	0.352				
Immune and Oxidative	42	10	0.1015	-0.2107	0.362				
Parasite Resistance	49	10	0.2433	-0.074	0.510				
Reproductive and Parental Quality	65	9	0.223	-0.099	0.462				
Category (Converted)						72.4			
Condition	18	8	0.084	-0.158	0.358				
Immune and Oxidative	21	6	0.097	-0.185	0.367				
Parasite Resistance	30	8	**0.435**	**0.174**	**0.688**				
Reproductive and Parental Quality	23	3	**0.337**	**0.021**	**0.618**				
Category (Dietary)									
Condition	17	4	0.107	-0.186	0.387				
Immune Function	21	4	0.113	-0.135	0.371				
Parasite Resistance	19	3	0.009	-0.241	0.283				
Reproductive and Parental Quality	42	6	0.095	-0.183	0.311				

633 *k = number of effect sizes, m = number of species.* Category (combined) represents estimates from a meta-
634 analytic model with life history trait category as a predictor, while Category (Converted) and Category
635 (Dietary) represent estimates from a meta-analytic model with an interaction between life history trait
636 category and type of carotenoid as predictor variables. Effect sizes in bold are considered to be
637 statistically significantly different from 0, as the 95% credible interval did not overlap 0.

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Box 1: Carotenoid structure, nomenclature, and metabolic transformations.

Carotenoids used for coloration in most animals are C_{40} tetraterpenoids. They consist of a central polyene chain — a system of conjugated carbon bonds that comprises most of the ***chromophore*** (*i.e.* part of the molecule that reflects light) — with ionone rings at either end (Fig 1). These hydrocarbon carotenoids are called ***carotenes*** whose specific names are derived from the types of ionone end rings present. β-carotene (β, β-carotene) contains two β-ionone rings, while α-carotene (β, ε -carotene) contains one β-ionone and one ε-ionone ring. Carotenoids containing at least one unmodified β-ionone ring can be cleaved by most animals to yield retinal and thus have pro-vitamin A potential. Modifications to the end rings though oxidation reactions determine the function and color of the carotenoid by increasing its polarity and/or chromophore length. The addition of conjugated double bonds lengthens the chromophore and increases peak light absorption from shorter to longer wavelengths, causing a shift from yellow towards red color (***a bathochromic shift***).

Carotenes can be modified by the addition of oxygen (as hydroxyl or ketone functional groups) to carbons 3 or 4 of the ionone end rings through oxygenation or dehydrogenation reactions. These oxygenated carotenoids are broadly known as ***xanthophylls.*** Specific xanthophyll names are determined by the presence of either one or more hydroxyl groups (***hydroxy-carotenoids***) and/or ketone groups (***keto-carotenoids***) to the ionone rings. Zeaxanthin (3,3'-dihydroxy-β-carotene) and lutein (3,3'-dihydroxy- α -carotene) are common hydroxy-carotenoids that are abundant in the diet of many herbivorous and insectivorous animals. In contrast, keto-carotenoids such as echinenone (4-keto-β-carotene), canthaxanthin (4,4'-diketo-β-carotene), and astaxanthin (3,3'-dihydroxy-4,4'diketo -β-carotene) are mostly absent from animal diets. Instead, keto-carotenoids are produced either through ketolation of hydroxy-carotenoids or through hydroxylation and ketolation of carotenes and are responsible for most of the vibrant red hues of animal integuments. However, not all keto-carotenoids yield red coloration; ***canary xanthophylls***—ketolated products of lutein and zeaxanthin that are derived

664 from dehydrogenation of the existing hydroxyl groups —produce a rich yellow color used by some
665 songbirds as feather pigments. The mechanism by which the ketone is formed includes a change from β-
666 ionone rings to ε-ionone rings which shortens the conjugated system (shortens the chromophore)
667 causing canary xanthophylls appear yellow and not red.

668 Despite the prevalence and importance of carotenoids in animals, the genetic architecture and
669 physiological mechanisms involved in carotenoid metabolism have only recently been identified. In
670 2016, two independent lab groups characterized the genetic basis for red bill and red feather coloration,
671 dubbed the *redness* gene [27,63]. This gene encodes a cytochrome P450 oxidoreductase *CYP2J19* that
672 catalyzes the oxidative transformation of dietary carotenoids to hydroxy- or keto-carotenoids
673 Identification of the particular mechanisms and cellular locations involved in hydroxylation and
674 ketolation of carotenoids in animals is currently underway.

675

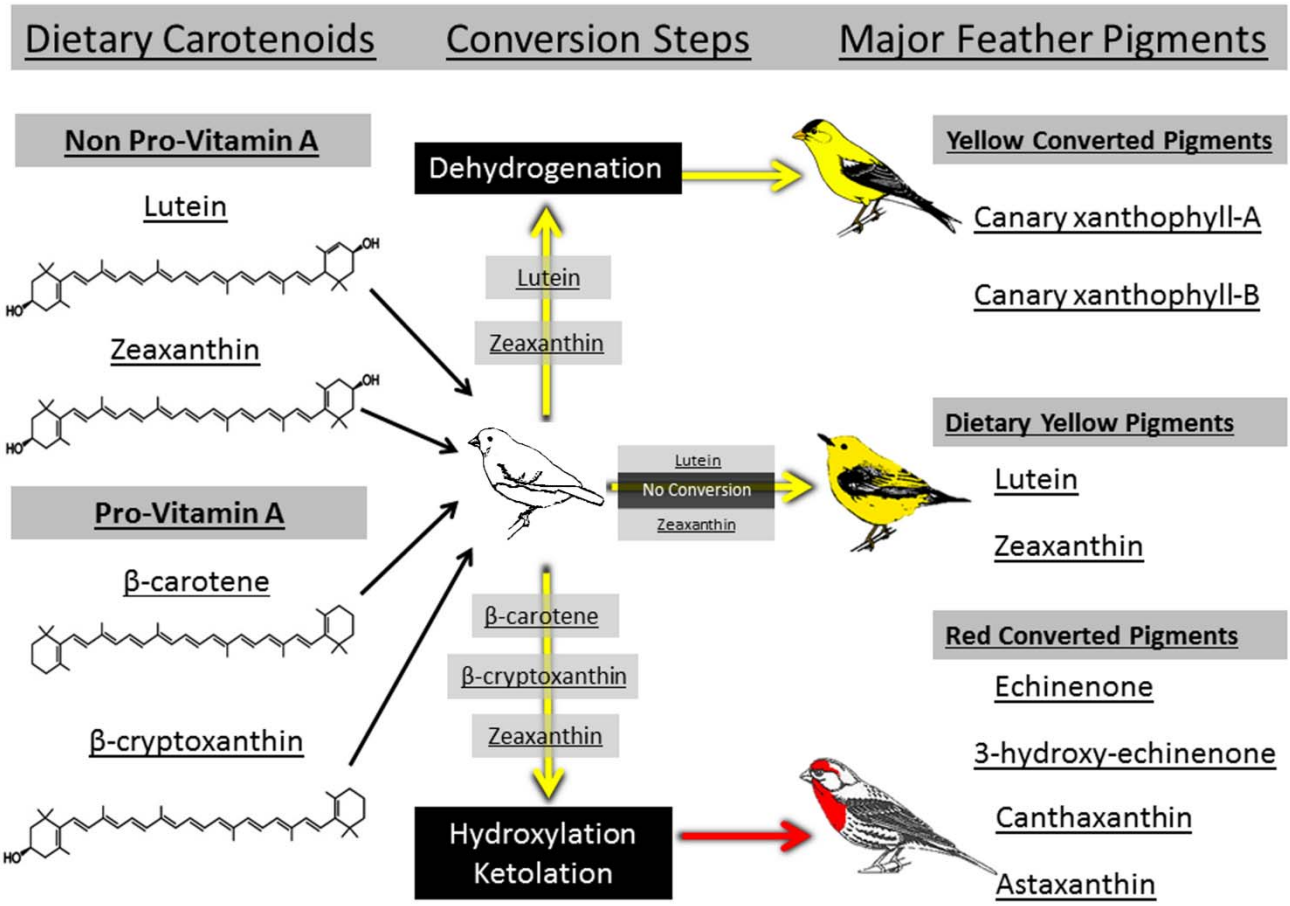

Dietary Carotenoids
Conversion Steps
Major Feather Pigments
Non Pro-Vitamin A
Lutein
Zeaxanthin
Pro-Vitamin A
β-carotene
β-cryptoxanthin
Dehydrogenation
Lutein
Zeaxanthin
Lutein
No Conversion
Zeaxanthin
β-carotene
β-cryptoxanthin
Zeaxanthin
Hydroxylation
Ketolation
Yellow Converted Pigments
Canary xanthophyll-A
Canary xanthophyll-B
Dietary Yellow Pigments
Lutein
Zeaxanthin
Red Converted Pigments
Echinenone
3-hydroxy-echinenone
Canthaxanthin
Astaxanthin

Figure 1

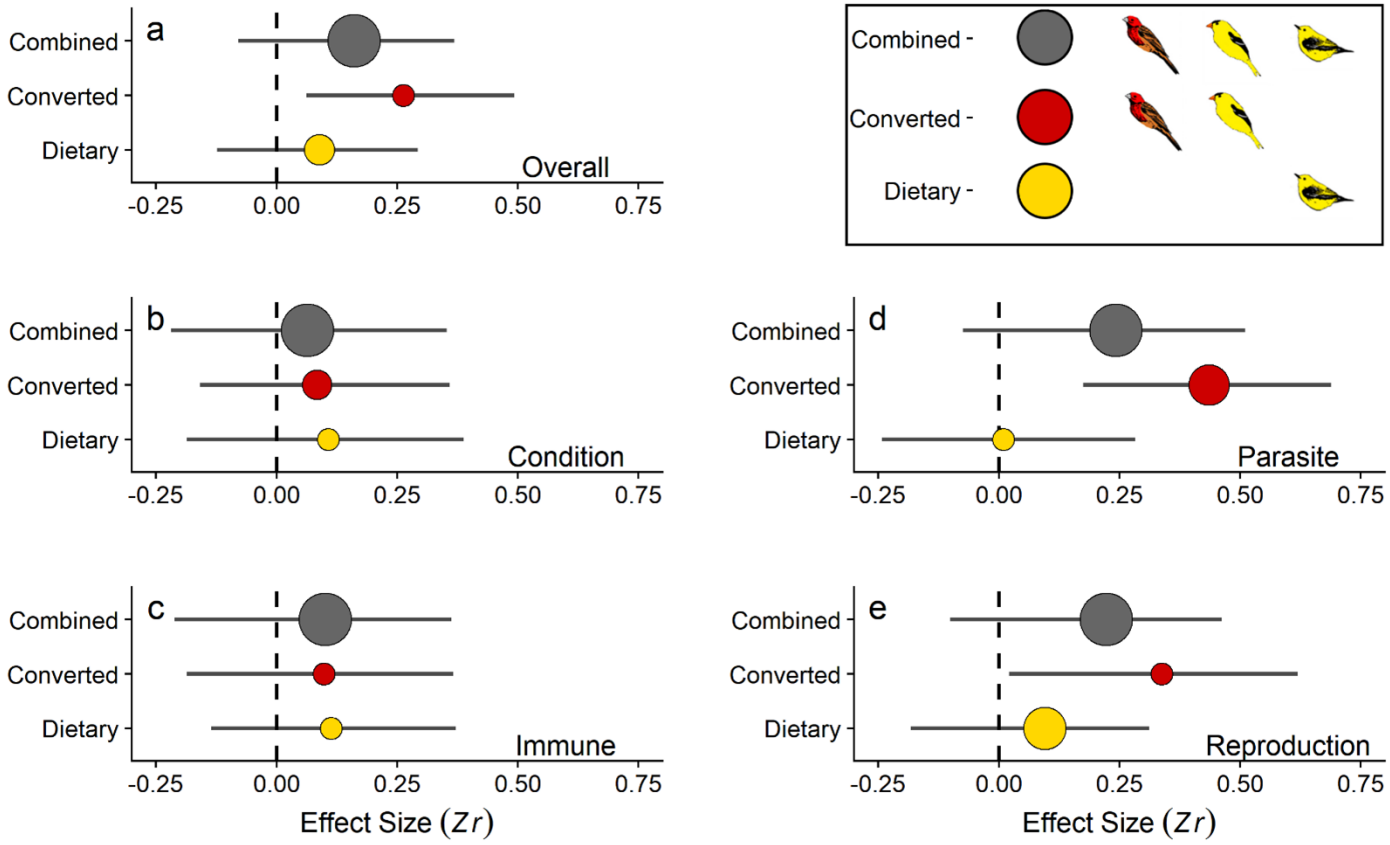

a
Combined
Converted
Dietary
Overall
-0.25
0.00
0.25
0.50
0.75
b
Condition
c
Immune
d
Parasite
e
Reproduction
Effect Size (Zr)

Figure 2

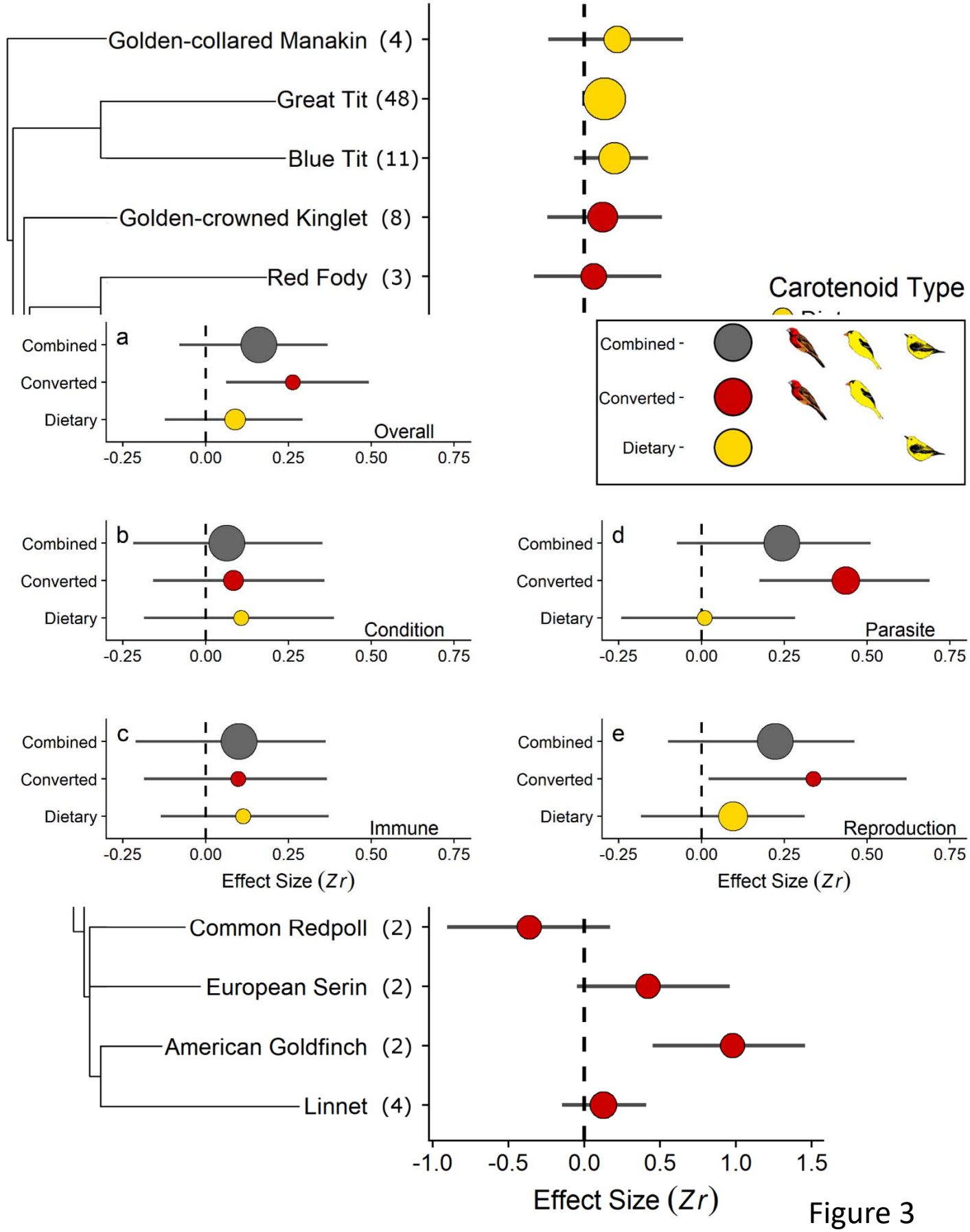
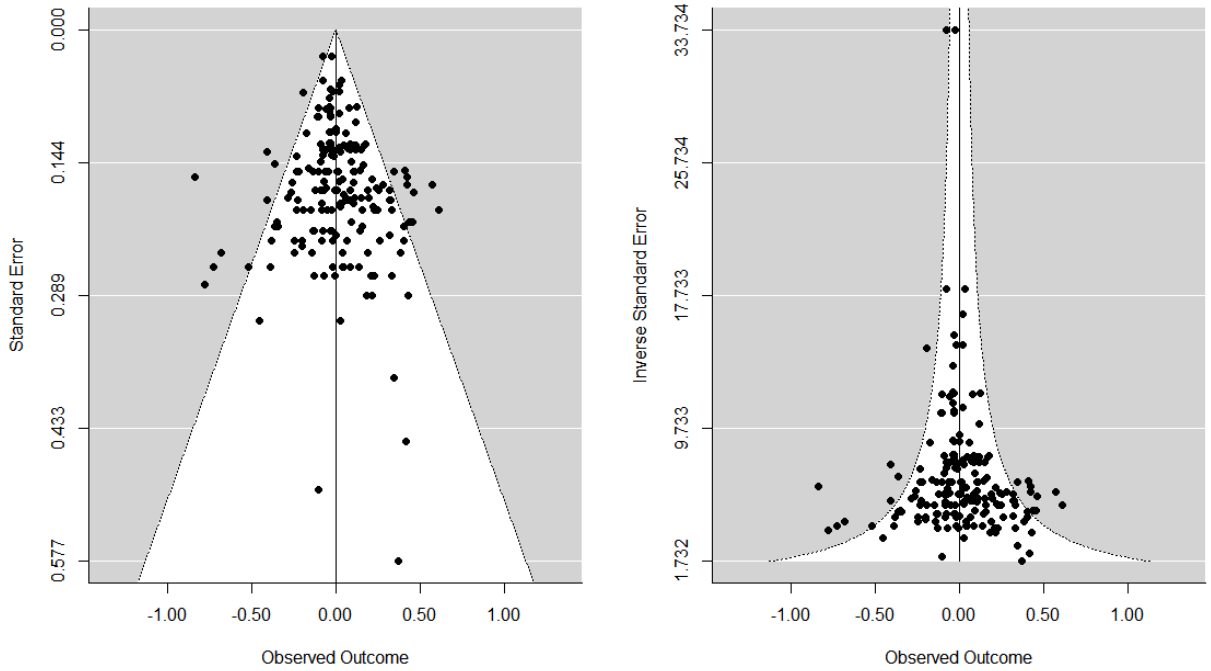

Golden-collared Manakin (4)
Great Tit (48)
Blue Tit (11)
Golden-crowned Kinglet (8)
Red Fody (3)
Carotenoid Type
Combined
Converted
Dietary
a
Overall
b
Condition
c
Immune
d
Parasite
e
Reproduction
-0.25
0.00
0.25
0.50
0.75
Effect Size (Zr)
Common Redpoll (2)
European Serin (2)
American Goldfinch (2)
Linnet (4)
-1.0
-0.5
0.0
0.5
1.0
1.5
Effect Size (Zr)

Figure 3



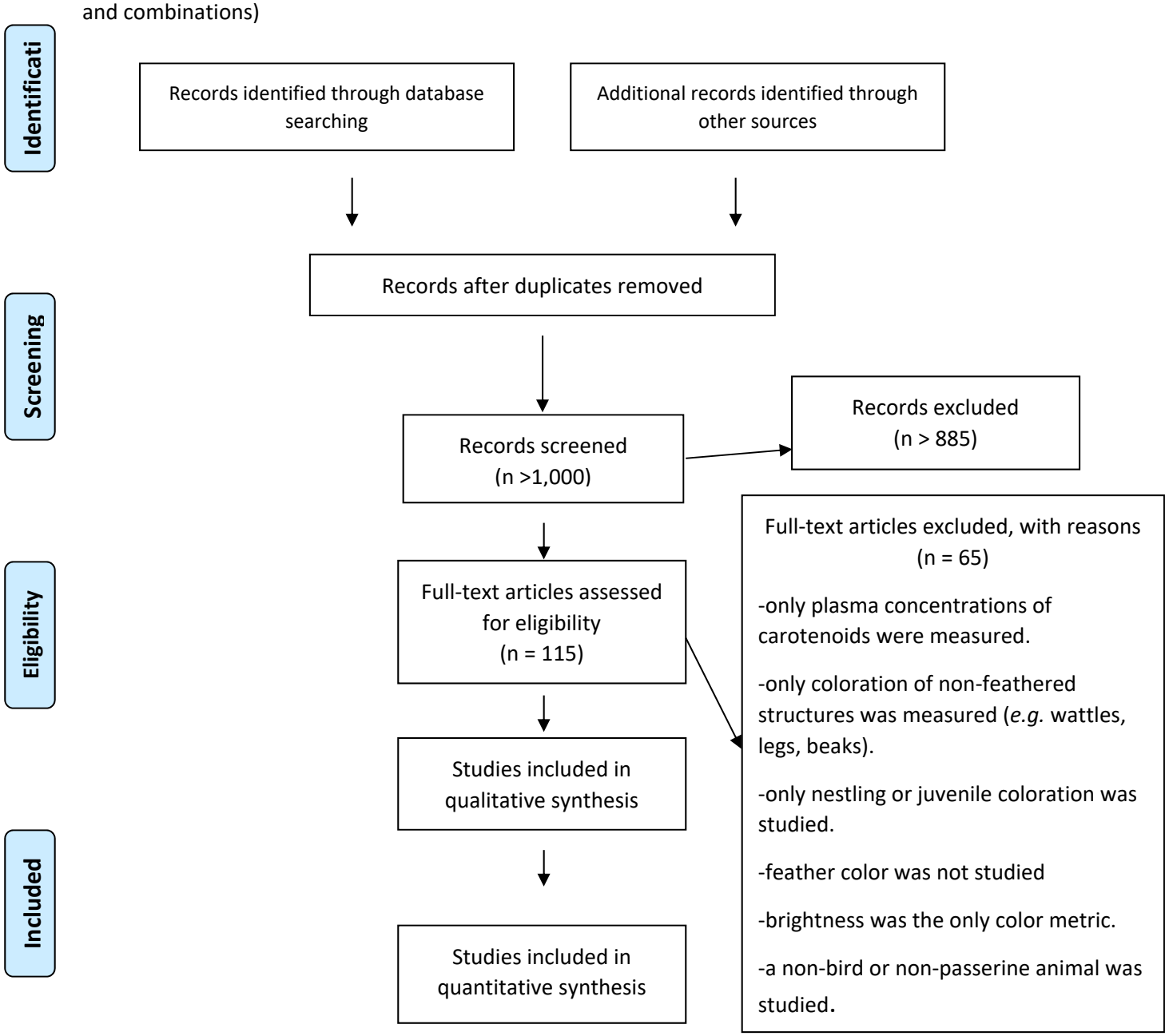
Supplementary Figure 1. Funnel plots of meta-analytic residuals. Effect size (Zr) plotted against standard error (left) and precision (right; the inverse of the standard error).

PRISMA 2009 Flow Diagram

Corresponding Article Title: Carotenoid metabolism strengthens the link between feather coloration and individual quality

Search terms:

("carotenoid", "color", "condition", "signal", "feather", "quality" and all possible alternative spellings and combinations)



Supplementary Figure 2. PRISMA flow diagram.

Studies included in the meta-analysis

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Supplementary Table 1. Model assuming effect sizes are NOT correlated

Analysis	*k*	*m*	Mean (*Zr*)	Lower CI (2.5%)	Upper CI (97.5%)	I^2(%)	Heterogeneity (Var_{study}) %	Heterogeneity ($Var_{species}$) %	Heterogeneity (phylogeny) %

Overall	191	19	0.178	-0.038	0.414	64.67	36.26	10.48	17.91
Carotenoid Type:						61.26			
Converted	92	12	**0.255**	**0.03**	**0.506**				
Dietary	99	7	0.077	-0.134	0.318				
Category (combined)						66.27			
Condition	35	12	0.069	-0.148	0.328				
Immune Function	42	10	0.142	-0.077	0.406				
Parasite Resistance	49	10	0.237	-0.02	0.467				
Reproductive and Parental Quality	65	9	0.225	-0.02	0.451				
Category (Converted)						57.91			
Condition	18	8	0.104	-0.161	0.39				
Immune Function	21	6	0.133	-0.162	0.414				
Parasite Resistance	30	8	**0.423**	**0.135**	**0.688**				
Reproductive and Parental Quality	23	3	**0.368**	**0.067**	**0.677**				
Category (Dietary)									
Condition	17	4	0.106	-0.163	0.385				
Immune Function	21	4	0.129	-0.154	0.384				
Parasite Resistance	19	3	0.029	-0.256	0.276				
Reproductive and Parental Quality	42	6	0.08	-0.147	0.365				

k = number of effect sizes, m = number of species. Category (combined) represents estimates from a meta-analytic model with life history trait category as a predictor, while Category (Converted) and Category (Dietary) represent estimates from a meta-analytic model with an interaction between life history trait category and type of carotenoid as predictor variables. Effect sizes in bold are considered to be statistically significantly different from 0, as the 95% credible interval did not overlap 0.

Supplementary Table 2. Model with measures of oxidative physiology split from immune category

Analysis	*k*	*m*	Mean (*Zr*)	Lower CI (2.5%)	Upper CI (97.5%)
Category (Converted)					
Condition	18	8	0.077	-0.238	0.347
Immune	20	6	0.095	-0.205	0.42
Oxidative	1	1	0.107	-0.515	0.724
Parasite	30	8	**0.438**	**0.173**	**0.751**
Reproductive and Parental Quality	23	3	**0.333**	**0.011**	**0.647**
Category (Dietary)					
Condition	17	4	0.101	-0.196	0.397
Immune	12	4	0.045	-0.286	0.359
Oxidative	9	2	0.222	-0.116	0.592
Parasite	19	3	0.0026	-0.256	0.298
Reproductive and Parental Quality	42	6	0.089	-0.211	0.358

k = number of effect sizes, m = number of species. Category (Converted) and Category (Dietary) represent estimates from a meta-analytic model with an interaction between life history trait category and type of carotenoid as predictor variables. Effect sizes in bold are considered to be statistically significantly different from 0, as the 95% credible interval did not overlap 0.

Supplementary Table 3. Model with parasite measures lumped with immune category, but oxidative measures in a separate category

	k	*m*	Mean (*Zr*)	Lower CI (2.5%)	Upper CI (97.5%)
Category (Converted)					
Condition	18	8	0.077	-0.138	0.341
Immune	50	11	**0.318**	**0.094**	**0.56**
Oxidative	1	1	0.082	-0.534	0.752
Reproductive and Parental Quality	23	3	**0.354**	**0.092**	**0.661**
Category (Dietary)					
Condition	17	4	0.097	-0.195	0.339
Immune	31	5	0.02	-0.196	0.268
Oxidative	9	2	0.236	-0.107	0.526
Reproductive and Parental Quality	42	6	0.103	-0.115	0.36

k = number of effect sizes, m = number of species. Category (Converted) and Category (Dietary) represent estimates from a meta-analytic model with an interaction between life history trait category and type of carotenoid as predictor variables. Effect sizes in bold are considered to be statistically significantly different from 0, as the 95% credible interval did not overlap 0.

Supplementary Table 4. Types of carotenoids in feathers of species included in the meta-analyses.

Species	Color	Carotenoid Type	Reference
American Goldfinch	Yellow	Converted	[51]
Blue Tit	Yellow	Dietary	[52]
Cirl Bunting	Yellow	Dietary *	[52]
Common Redpoll	Red	Converted	[52,53]
Common Rosefinch	Red	Converted	[54]
Common Yellowthroat	Yellow	Dietary	[55]
European Greenfinch	Yellow	Converted	[52]
European Serin	Yellow	Converted	[56]
Golden-Collared Manakin	Yellow	Dietary *	[57]
Golden-crowned Kinglet	Yellow	Converted	[4]
Great Tit	Yellow	Dietary	[58]
House Finch	Red	Converted	[58]
Kentucky Warbler	Yellow	Dietary *	[55]
Linnet	Red	Converted	[54]
Northern Cardinal	Red	Converted	[53]
Red Fody	Red	Converted	[58]
Red-winged Blackbird	Red	Converted	[59]
Southern Red Bishop	Red	Converted	[58]
Yellowhammer	Yellow	Dietary	[58]

* No published reports for this species, carotenoid type estimated by comparison to sister-species of the same genus that displays the same feather color.

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