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Carotenoid-Dependent Signals and the Evolution of Plasma Carotenoid Levels in Birds

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ABSTRACT: Sexual selection has resulted in a wide array of ornaments used in mate choice, and such indicator traits signal quality honestly when they bear costs, precluding cheating. Carotenoid-dependent coloration has attracted considerable attention in this context, because investing carotenoids in coloration has to be traded off against its physiological functions; carotenoids are antioxidants and increase immunocompetence. This trade-off is hypothesized to underlie the honesty of carotenoid-dependent coloration, signaling the “handicap” of allocating carotenoids away from somatic maintenance toward sexual display. Utilizing recent advances in modeling adaptive evolution, we used a comparative approach to investigate the evolution of plasma carotenoid levels using a species-level phylogeny of 178 bird species. We find that the evolutionary optimum for carotenoid levels is higher in lineages that evolved carotenoid-dependent coloration, with strong attraction toward this optimum. Hence, carotenoids do not appear to be limiting, given that higher carotenoid levels readily evolve in response to the evolution of carotenoid-dependent coloration. These findings challenge the assumption that carotenoids are a scarce resource and thus also challenge the hypothesis that physiological resource value of carotenoids underlies honesty of carotenoid-dependent traits. Therefore, the comparative evidence suggests that other factors, such as the acquisition and incorporation of carotenoids, are involved in maintaining signal honesty.

Keywords: signaling, ornaments, sexual selection, pigments, honesty.

Introduction

Elaborate sexual ornaments are generally considered to have evolved through sexual selection, either to attract the opposite sex or for use in intrasexual competition (Andersson and Iwasa 1996). Mate choice for elaborate sexual ornamentation can provide direct and indirect fitness benefits (Kokko et al. 2006), with the choice of an attractive

mate resulting in offspring that are attractive and/or otherwise of high quality. Indirect benefits (the gain of attractive offspring via choice for an average preferred phenotype; Maan and Seehausen 2011) can cause ornaments to evolve via Fisherian runaway selection (Fisher 1930), resulting in trait exaggeration, until either a trait is fixed in the population or costs underlying further exaggeration allow the trait to evolve into an indicator trait. Mate choice for indicator traits is expected to evolve only if cheating is precluded, which can be achieved by the fitness costs of developing or maintaining such traits. These costs can arise from energetic or resource investment in the ornament (Zahavi 1975; Grafen 1990) or a suite of other mechanisms (e.g., social punishment; reviewed in Számadó 2011). The nature of the costs that maintain signal honesty has attracted considerable attention (Schantz et al. 1999; Verhulst et al. 1999; Kotiaho 2001; Roberts et al. 2004; Emlen et al. 2012).

Carotenoid-dependent coloration—predominantly yellow and orange to red colors present in reptiles, fish, and mammals and especially common in birds (McGraw 2006)—represents a widespread class of sexual signals. Carotenoid-dependent coloration features in mate choice (Sundberg 1995; Künzler and Bakker 2001; Pike et al. 2007; Simons and Verhulst 2011; Toomey and McGraw 2012) and signals phenotypic quality; more intense coloration has been linked to reproduction and survival (Hill 1991; Nolan et al. 1998; Hōrak et al. 2001; Prévault et al. 2005; Pike et al. 2007; Simons et al. 2012a), immune function, and oxidative stress state (meta-analysis in Simons et al. 2012b). Carotenoid molecules are responsible for pigmenting these traits, and consequently plasma carotenoid levels are often positively correlated to the intensity of sexual coloration within species (Simons et al. 2012b); supplementation has also been shown to increase coloration (Blount et al. 2003; Simons et al. 2012b).

Carotenoids are exclusively derived from the diet (Olson and Owens 1998) and are generally considered to be a

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limiting physiological resource given their potential to act as antioxidants and/or immune-supporting agents (Lozano 1994; Olson and Owens 1998; Schantz et al. 1999; Vinkler and Albrecht 2010; Svensson and Wong 2011). Building on this assumption, it is thought that allocation of carotenoids away from maintenance toward coloration underlies honesty of carotenoid-dependent signals. This hypothesis posits that individuals advertise their ability to allocate carotenoids away from the physiological processes that carotenoids would influence positively (i.e., the carotenoids' resource value) toward signal pigmentation ("handicap signaling") and thereby advertise their genetic and/or phenotypic quality (Kokko et al. 2006). However, costly acquisition of carotenoids to pigment traits could also maintain honesty (Olson and Owens 1998; Hill and Johnson 2012). Behaviorally, individuals may show plasticity in foraging and favor carotenoid-enriched diets, as demonstrated experimentally in great tits (*Parus major*; Senar et al. 2010). Additionally, differences in levels of food intake and/or metabolic uptake and processing of ingested carotenoids can influence their availability for both ornamentation and physiological functions. Hence, fitness costs of carotenoid-dependent ornaments may arise from the costs of carotenoid uptake, due to time and/or energy invested in carotenoid acquisition, which would also maintain signal honesty. In addition, carotenoids may also prove toxic at high levels or in certain contexts, such as under oxidative stress (Hartley and Kennedy 2004; Simons et al. 2014a), which conflicts with the notion that allocating carotenoids to integumentary structures necessarily trades off with physiological functioning. Finally, signal honesty can also arise from localized metabolic costs for incorporation into colorful integumentary structures (McGraw 2004). Thus, although the physiological resource value hypothesis for the honesty of carotenoid visual signals has garnered much attention, alternative hypotheses are also likely.

The causal relationship between carotenoids, oxidative stress state, and immunocompetence (as shown in a recent meta-analysis of carotenoid supplementation studies; Simons et al. 2012b) may also arise via costly acquisition. When carotenoid availability is increased experimentally, costly acquisition may be down-regulated, allowing the energetic or resource investment into carotenoid acquisition to be allocated toward immunocompetence and battling oxidative stress instead. Thus, costs associated with honest signaling of carotenoid-dependent traits may be based either on the value of carotenoids as a physiological resource or on costs related to their acquisition. This distinction has important consequences for our understanding of the evolution of carotenoid ornaments, because the mechanisms underlying honesty in both scenarios are different. When carotenoids are used as a physiological re-

source, allocation of carotenoids away from specific physiological needs and toward coloration will be responsible for maintaining honesty. In contrast, if the acquisition of carotenoids itself is either energetically limiting, behaviorally limiting, or subject to context-dependent detrimental effects, constraints on the investment in the acquisition of carotenoids itself will underlie honesty. In addition, carotenoid acquisition might not have reached the evolutionary optimum where physiological and ornamental investments of this resource are at an equilibrium, in which case allocation to ornaments is not costly and trade-offs are not responsible for the maintenance of honesty, and choice for carotenoid-dependent coloration would likely be driven by Fisherian ("sexy son") mechanisms (Kokko et al. 2006).

Recent advances in phylogenetic comparative methods make it possible to test such hypotheses in a macroevolutionary framework. Trait evolution across evolutionary time can be modeled as Brownian motion (BM) and Ornstein-Uhlenbeck (OU) processes (Butler and King 2004). BM is a random walk model with each time-step determined by a sample from a normal distribution with a standard deviation (σ^2) representing the rate of "random drift" (O'Meara et al. 2006). OU extends BM models by introducing an optimum (θ) with an attraction parameter (α), representing the strength of attraction toward the optimum and usually interpreted in terms of selection toward an optimal value or constraints limiting evolution away from it (Hansen 1997). Recently, these models have been incorporated into a general framework in which these parameters can be allowed to vary across a phylogeny, such as under evolutionary regimes determined by a coevolving phenotypic or ecological trait (Beaulieu et al. 2012). This framework provides the exciting possibility to test specific adaptive hypotheses in comparative data. Here we examine the evolution of plasma carotenoid levels in relation to carotenoid-dependent trait evolution in birds. Resource- or pigment-based honesty mechanisms of carotenoid-dependent coloration provide distinctly different predictions for the evolutionary model to be favored.

Hypothesis 1

When carotenoids are simply strictly limiting color signal elaboration, we expect a BM model with similar rates of change (σ^2) between lineages with and without carotenoid-dependent traits to be favored. Although commonly interpreted as a "pure drift" model, a BM model may also reflect the pattern of lineages evolving toward phylogenetically structured, lineage-specific adaptive optima, which are randomly structured in relation to the considered factors (Revell et al. 2008). In this scenario, the level of carotenoids does not limit the evolution of carotenoid-

dependent coloration, and within- and among-species carotenoid levels are limited by unknown constraints, which are likely to vary at a fine scale because of ecological factors (such as dietary carotenoid availability).

Hypothesis 2

When carotenoids are a limiting resource, we expect a single optimum OU model to be favored. Selection to maintain or increase plasma carotenoid level is independent of the presence of carotenoid-dependent ornaments, because carotenoids provide physiological resource benefits irrespective of the presence of sexual signaling. Thus we expect strong attraction toward this optimum, with possibly higher attraction (or lower evolutionary rates) in lineages that exhibit carotenoid-dependent traits, because sexual selection within these lineages is predicted to enhance selective pressures, thereby maintaining carotenoid levels closer to the shared optimal value.

Hypothesis 3

When carotenoids are not a limiting resource and are mostly used as a pigment, we expect an OU model with two optima—one lower optimum with low attraction (weak stabilizing selection) in lineages lacking carotenoid-dependent traits and a higher optimum with stronger attraction in lineages that do exhibit carotenoid-dependent traits—to be preferred. If carotenoids are not a physiological resource, natural selection should not drive the evolution of plasma carotenoid levels to be high in the absence of carotenoid-dependent sexual signals. In contrast, in lineages with carotenoid-dependent traits, sexual selection is expected to select for higher carotenoid levels (higher optima), which will mostly be used for signal pigmentation.

Methods

Plasma Carotenoid Levels

We searched the literature using Google Scholar for articles reporting plasma carotenoid levels in birds, with the latest search dating to December 2011. Plasma carotenoid levels in birds are positively related to carotenoid levels in the liver (Figueroa et al. 2005; Møller et al. 2005; McGraw et al. 2006; Butler and McGraw 2010; McGraw and Toomey 2010; Galvan et al. 2012), although less consistently so to carotenoids in fat (Figueroa et al. 2005; McGraw and Toomey 2010). The liver and fat are the main storage organs for carotenoids (Negro et al. 2001; Hill and Johnson 2012), and plasma carotenoid levels are therefore a reliable proxy of total carotenoid stores.

Our search returned 53 articles containing information on 251 carotenoid plasma level means for 178 different species of birds (deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.kg007>; Simons et al. 2014b). When we obtained multiple estimates of plasma carotenoid level per species, we took a weighted average (for the square root of the sample size). Carotenoid levels followed a log-normal distribution and were transformed accordingly ($\log_{10}(x + 1)$) before the analyses.

Carotenoid-Dependent Coloration

We scored whether the species for which we collected carotenoid plasma level estimates exhibited carotenoid-dependent traits based on previous studies and reports and on photographs when such information was not available (Simons et al. 2012b). We judged the presence of carotenoid-dependent traits by its characteristic yellow, orange, or red color (Gray 1996; Tella et al. 2004; Olson and Owens 2005; Galvan et al. 2012), excluding those derived from other described pigments (e.g., psittacofulvins; McGraw and Nogare 2004). The scoring was done by two experimenters blind to the collected plasma carotenoid levels. Furthermore, we validated our scoring using two published sets of actual carotenoid content of ornaments (Fox 1976; McGraw 2006). The pictures were obtained using Google image search using the scientific name of the species as the search query (Simons et al. 2012b). In addition, we scored whether the coloration was present in plumage or in the integument.

Phylogeny

We used the complete avian species-level time-calibrated phylogeny by Jetz et al. (2012) for our comparative analyses. This tree was constructed under a Bayesian framework using a supermatrix approach based on a previously established higher-order relationship as a “backbone” (Hackett et al. 2008), considering both phylogenetic and taxonomic information, with the species lacking genetic sequences placed in the tree according to their taxonomic classification and a birth-death model (Jetz et al. 2012). This approach, while representing our best knowledge of the avian tree of life and being mostly congruent with other independent phylogenies constructed using different approaches, can lead to biases resulting from taxonomic imprecision or incorrect model choice when placing them in the phylogeny. However, our data set included only three of 178 species (*Circus pygargus*, *Chiroxiphia lanceolata*, and *Myiarchus panamensis*) lacking genetic data and thus grafted taxonomically to the tree. Therefore, and given that these species are relatively well resolved taxo-

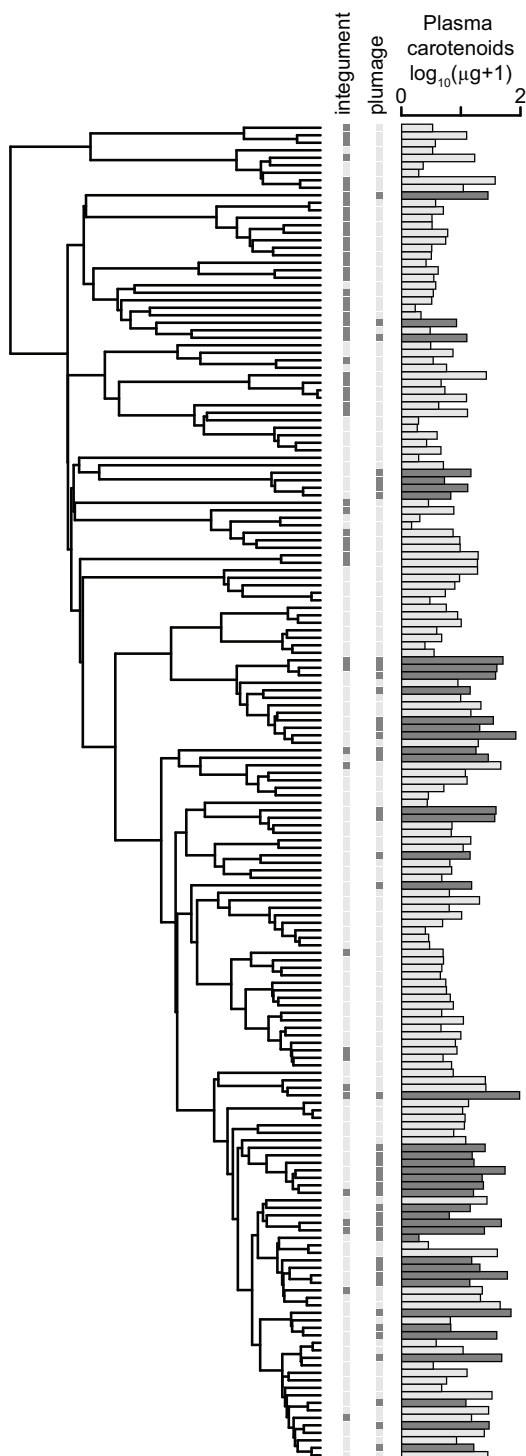


Figure 1: Maximum clade credibility tree from Jetz et al. (2012) pruned to the species included in this study, indicating the presence of integumentary and plumage carotenoid-dependent traits (squares) and circulating carotenoid levels (bars) for those species. A randomly drawn sample of 500 phylogenetic trees from the posterior distribution transformed to a total root-to-tip distance of 1 was used in all comparative analyses.

nominally within their groups, we kept all species in our analyses.

Nonetheless, to integrate across competing and uncertain scenarios deriving from the phylogenetic reconstruction, we sampled 500 trees from the posterior distribution and obtained a representation of topologies and relative branch lengths proportional to their posterior probabilities, which were transformed to a total root-to-tip distance of 1 and used in the comparative analyses described below.

Evolutionary Models

To test for the effects of the presence or absence of carotenoid-dependent traits (present in plumage, integument, or the combined measure), we used phylogenetically controlled generalized least squares (PGLS) models (Grafen 1989). Plasma carotenoid level was included as the response variable, and the presence or absence of integument and plumage carotenoid traits, as well as their interaction, were included as predictor variables. We conducted PGLS analyses considering an OU model of trait evolution (Martins and Hansen 1997), with parameter estimates for the predictor variables and the OU α estimated under maximum likelihood using the R packages APE (Paradis et al. 2004) and nlme (Pinheiro et al. 2013). Maximum likelihood estimates of the parameters were obtained across all trees, and the sum of the among-tree and mean within-tree variance was used to statistically test for the effects of the response variables while accounting for phylogenetic and estimate uncertainties.

To estimate the evolutionary regimes determined by the presence or absence of carotenoid-dependent traits, we used stochastic character mapping (Huelsenbeck et al. 2003) to reconstruct the evolution of carotenoid signals. This method produces stochastic regime mappings of transitions between states (carotenoid ornaments present or absent) following a continuous-time Markov chain Monte Carlo (MCMC) model. We applied a hierarchical Bayesian approach to incorporate uncertainty from both phylogenetic and regime reconstruction. To choose adequate priors for the regime reconstruction, we conducted an MCMC analysis to sample overall rate values, using the posterior distribution of these rates to obtain the α and β parameters for the best-fitting gamma distribution. The estimated parameters were then used to determine the gamma distribution used as the prior for the stochastic character mapping of the presence of plumage traits ($\alpha = 10$, $\beta = 7$), integument traits ($\alpha = 8.3$, $\beta = 11$), or any carotenoid trait ($\alpha = 11.5$, $\beta = 22.5$), and a uniform prior for both states at the root. For each of the 500 sampled trees from the posterior distribution, we ran the stochastic character mapping reconstruction for 1,000 generations after a burn-in of equal length, sampling every

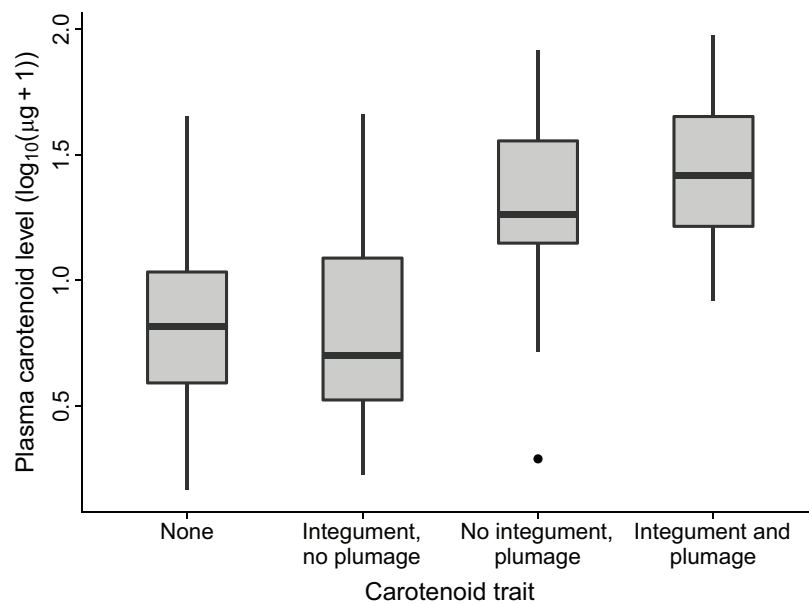


Figure 2: Distribution of circulating carotenoid levels in relation to the presence of carotenoid-dependent integumentary and plumage traits. Phylogenetically controlled analysis revealed a significant influence of plumage but not integumentary, carotenoid traits in plasma carotenoid levels (see text for details).

100 generations for a total of 10 regime reconstructions for each tree, using SIMMAP 1.5 (Bollback 2006) and the `make.simmap` function from the R package `phytools` (Revell 2012).

Therefore, a total of 5,000 trees representing combinations of phylogenetic and ancestral state reconstructions were used to fit the evolutionary models. We used this approach to reconstruct evolutionary regimes determined by the presence of any carotenoid trait (integument and/or plumage) and by the presence of plumage traits and integument traits separately. For each regime set of reconstructions, we fitted seven different evolutionary models under a generalized Hansen model framework, encompassing derivations from BM and OU (Butler and King 2004; Beaulieu et al. 2012; Lapiedra et al. 2013), using the package `OUwie` (Beaulieu et al. 2012) in R (R Development Core Team 2008). BM models considered included a model with a single (carotenoid trait-independent) evolutionary rate (σ^2) across the tree (BM1) and a model with separate rates of plasma-level carotenoid evolution for each regime (lineages with and without carotenoid-dependent traits; BMS). We also considered five different OU models. The first included a single, trait-independent optimum (θ) for plasma carotenoid levels (OU1), whereas a second considered carotenoid trait-dependent optima, with two optima for the different regimes (OUM). Both of these models still estimated the same attraction parameter (α) and rate of random drift (σ^2) across lineages with and without

carotenoid traits. The third model allowed both optima and attraction parameter α to vary according to the evolutionary regime while fitting the same rate of evolution σ^2 (OUMA), whereas the fourth allowed the rate of evolution to vary between the two optima with a shared attraction parameter across regimes (OUMV). Finally, a model in which all parameters—optima, rates, and attraction—were allowed to vary across the regimes (OUMVA) was considered. Models were estimated considering the estimated intraspecific means. Model choice was conducted using relative likelihoods based on the second-order corrected Akaike Information Criterion (AICc), which allows for the comparison of nested and nonnested models (Butler and King 2004), and model-averaged parameters weighted for their relative support across the different models were calculated (Lapiedra et al. 2013).

Results

Levels of circulating plasma carotenoids were associated with the presence of plumage carotenoid traits (PGLS estimate \pm SE: 0.39 ± 0.08 , $t_{176} = 5.12$, $P < .001$) but not with the presence of bare skin carotenoid traits (-0.003 ± 0.07 , $t_{176} = -0.05$, $P = .96$; figs. 1, 2). There was no interaction between the presence of both plumage and bare skin carotenoid traits on plasma carotenoids levels (PGLS interaction \pm SE: 0.16 ± 0.14 , $t_{176} = 1.12$, $P = .27$; fig. 2). PGLS analyses revealed a strong attraction

Table 1: Summary of model selection results across the sample of 5,000 trees

| Model | Preferred, % | AICc | Δ AICc | wAICc |
|--|--------------|------------------------|----------------------|---------------|
| Plumage carotenoid regime models: | | | | |
| BMS | 0 | 211.89 (191.43–235.73) | 84.7 (61.74–110.25) | 0 |
| OUM | 73.20 | 127.58 (112.15–141.82) | .39 (0–3.60) | .38 (.09–.54) |
| OUMA | 15.08 | 128.40 (113.18–142.32) | 1.20 (0–2.52) | .25 (.12–.44) |
| OUMV | 5.64 | 128.61 (113.23–142.35) | 1.42 (0–4.01) | .22 (.08–.37) |
| OUMVA | 5.64 | 129.72 (114.27–143.51) | 2.52 (0–4.10) | .15 (.07–.52) |
| Any carotenoid trait regime models: | | | | |
| BMS | 0 | 212.60 (191.66–236.71) | 85.40 (61.14–111.78) | 0 |
| OUM | 0 | 157.08 (147.03–168.25) | 29.88 (13.68–47.48) | 0 |
| OUMA | .34 | 154.40 (141.27–166.7) | 27.21 (8.37–45.88) | 0 (0–.01) |
| OUMV | .04 | 155.67 (144.33–166.8) | 28.48 (11.22–46.29) | 0 |
| OUMVA | .06 | 155.73 (142.14–168.12) | 28.53 (9.47–47.25) | 0 |
| Integument carotenoid regime models: | | | | |
| BMS | 0 | 214.13 (196.15–236.29) | 86.93 (64.51–111.08) | 0 |
| OUM | 0 | 159.10 (149.98–169.79) | 31.91 (16.01–48.85) | 0 |
| OUMA | 0 | 160.58 (151.39–171.7) | 33.39 (17.33–50.45) | 0 |
| OUMV | 0 | 161.07 (151.99–171.77) | 33.88 (18.01–50.79) | 0 |
| OUMVA | 0 | 161.80 (152.37–172.69) | 34.60 (18.44–51.93) | 0 |
| Regimes not determined by carotenoid traits: | | | | |
| Brownian motion | 0 | 213.57 (194.58–236) | 86.37 (64.43–110.9) | 0 |
| Ornstein-Uhlenbeck | 0 | 157.47 (148.18–168.24) | 30.28 (14.68–46.64) | 0 |

Note: The proportion of trees in which each model was preferred is presented alongside the mean and 95% quantiles for the second-order corrected Akaike Information Criterion (AICc). The difference between the model and the lowest AICc values (Δ AICc) and the Akaike weights (wAICc) calculated for each tree are presented. See text for definition of models.

parameter (α estimate \pm SE: 9.67 ± 2.50), resulting in low phylogenetic signal. Thus, circulating carotenoid levels are only affected by the presence of carotenoid-colored plumage traits, independent of the presence of other integumentary carotenoid-colored traits, and can vary considerably between closely related species (figs. 1, 2).

Overall, OU models with differential optima of plasma carotenoid levels for lineages lacking and exhibiting carotenoid-dependent traits were preferred over models that did not consider carotenoid-dependent trait regimes and over models that considered only rate variation between lineages with and without carotenoid traits (table 1). However, plumage-only regimes were strongly supported over “any carotenoid trait” models, which in turn outperformed “integument only” models (table 1). Together, these results suggest that the adaptive evolution of circulating carotenoid levels is influenced by evolutionary regimes determined solely by either the presence or absence of plumage carotenoid ornaments.

Given the strong preference for OU over BM models and for plumage over other trait regimes, we based our inferences on model-averaged parameters across OU models in the plumage-determined regimes (Lapiedra et al. 2013). The four OUM models, characterized by different optima and attraction and rate parameters across the re-

gimes determined by the presence of carotenoid-based plumage traits, together received the strongest support, being the preferred set of models in more than 99% of cases (table 1). However, there was some uncertainty between the four models that incorporated different optima, with the simplest model of different optima with the same rates and attraction parameters (OUM) receiving most of the support (table 1). The model-averaged parameter estimates strongly supported a higher optimum plasma carotenoid level for lineages exhibiting carotenoid-dependent plumage relative to those lacking such traits (figs. 3, 4). Lineages without carotenoid plumage tended to have lower evolutionary rates (σ^2) and attraction parameters (α) than lineages with carotenoid traits, but these differences were very small and not statistically significant (figs. 3, 4).

Discussion

Our results support the hypothesis that circulating carotenoid levels show adaptive evolution toward higher values associated with the presence of carotenoid-dependent plumage coloration. They are therefore in line with hypothesis 3, that carotenoid plumage reflects the ability to acquire these pigments rather than the allocation of a limited resource into signaling. The optimum of plasma ca-

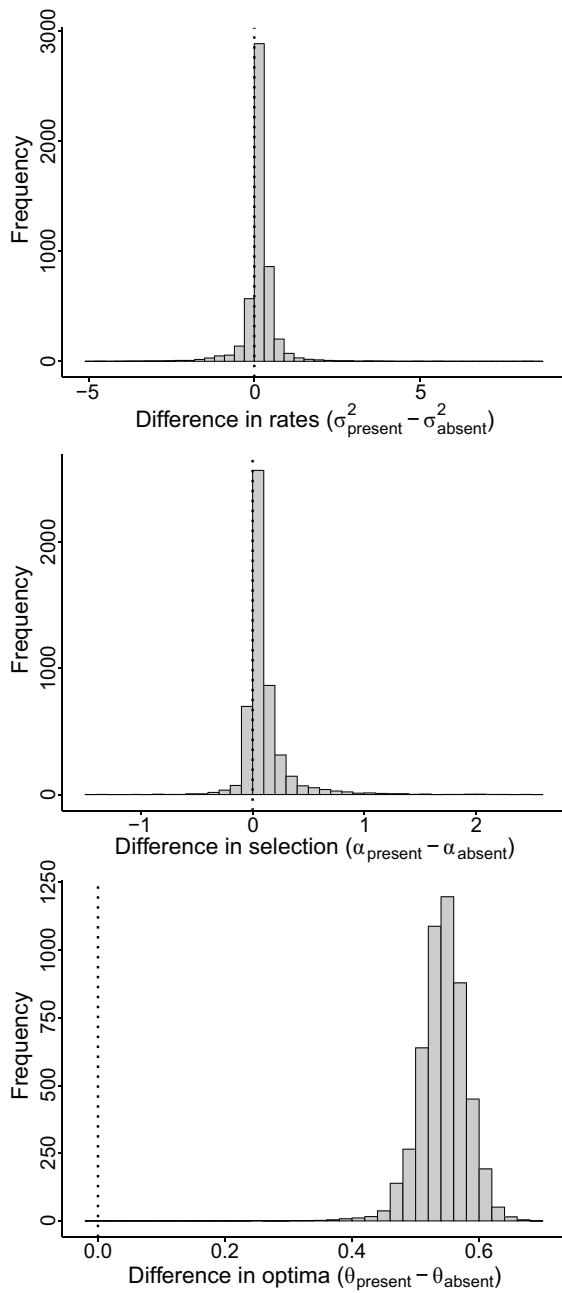


Figure 3: Histogram of the difference in the model-averaged parameters (σ^2 , α , and θ) for the evolution of circulating carotenoid levels in relation to selective regimes determined by the presence or absence of carotenoid-dependent plumage coloration across the posterior sample of the 5,000 trees used. The vertical dotted line indicates no difference in parameters (at zero). Optima of carotenoid levels are higher for lineages that exhibit carotenoid-dependent plumage (the tail-area probability of $\theta_{\text{present}} - \theta_{\text{absent}}$ relative to zero $<.001$), but no differences were observed between the attractions toward the optima (α) and rates (σ^2).

rotenoid levels is higher in lineages exhibiting carotenoid-dependent plumage traits, yet the attraction toward both optima is similar and high. The selection to maintain carotenoid levels in lineages that do not exhibit carotenoid-dependent plumage may be interpreted as reflecting the physiological resource value of carotenoids (hypothesis 2). However, increased acquisition of carotenoids is rapidly selected in the presence of carotenoid-dependent plumage, most likely via sexual selection. This suggests that trade-offs maintaining honesty of carotenoid-dependent signals must also consider the physiology associated with carotenoid acquisition (hypothesis 3).

It is surprising that higher plasma carotenoid levels are selected for only in species that exhibit carotenoid-dependent plumage but not integumentary coloration. Plumage pigmentation is mostly static, and carotenoids are only incorporated into feathers at set times, which are determined by molting patterns (Blount and McGraw 2007). Thus, reduced circulating carotenoid levels and/or selection on maintaining them might be expected, especially because most blood samples were taken outside the period of new feather formation. Elaborate plumage coloration may require larger carotenoid levels to assure carotenoid availability during a relatively short period (molt), when large amounts of carotenoids are incorporated. Furthermore, there is no evidence that the associations between signal expression and immunocompetence and oxidative stress state are weaker when carotenoid-dependent traits are expressed in plumage compared with integument (Simons et al. 2012b), which would be expected under hypothesis 2, given the differences in plasma carotenoid levels observed. This further supports the hypothesis that carotenoid signals, particularly plumage signals, can also reflect the ability to acquire carotenoids rather than the handicap of allocating scarce carotenoids toward pigmentation. In the case of integumentary coloration, another possible explanation for its weak relationship with plasma carotenoid levels could be that the continuous turnover and investment of carotenoids in bare skin traits reduces carotenoid levels in circulation. As a consequence, total carotenoids acquired could be higher for species with bare skin carotenoid traits, but the dynamic nature of allocation of pigments to such traits decouples ornamentation from circulating levels. Measuring actual carotenoid acquisition, turnover, and incorporation in sexual signals (e.g., using carotenoids labeled with stable isotopes; Canene-Adams and Erdman 2009) in species that express integument versus plumage coloration as well as investigating circulating levels continuously (before, during, and after molt) is thus critical to understand this pattern.

In the preferred evolutionary models, the attraction rate (α) for plasma carotenoid levels was considerably high, indicating that levels rapidly evolve toward their respective

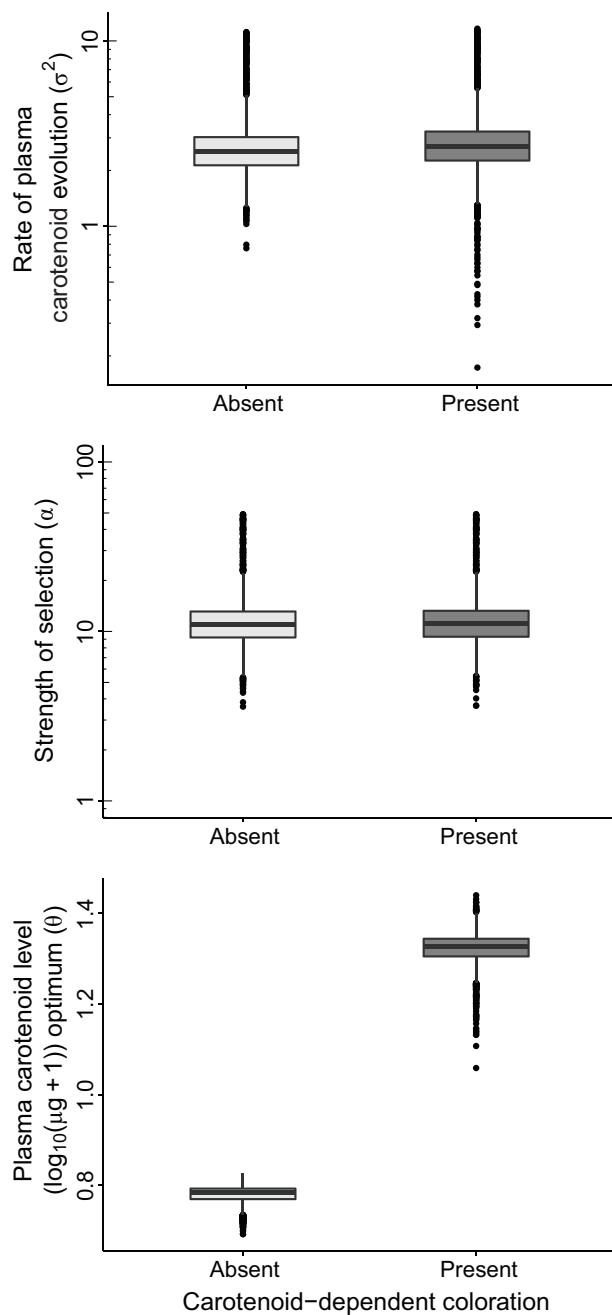


Figure 4: Boxplots of the model-averaged parameters (σ^2 , α , and θ) for the evolution of circulating carotenoid levels in relation to selective regimes determined by the presence (dark gray) or absence (light gray) of carotenoid-dependent plumage coloration across the posterior sample of the 5,000 phylogenetic trees used.

optima regardless of past evolutionary history and phylogenetic effects. Such fast evolution of plasma carotenoid levels has been demonstrated earlier in two sympatric morphs of sockeye salmon (*Oncorhynchus nerka*). Anad-

romous morphs experience relatively high carotenoid availability at sea and have genetically determined lower rates of carotenoid acquisition, whereas nonanadromous morphs experience lower carotenoid availability in freshwater and have genetically determined higher rates of acquisition (Craig and Foote 2001; Craig et al. 2005). Indeed, carotenoid uptake is an active process that may not always be maximized (Hill and Johnson 2012), contrary to the expectation if carotenoids are scarce and have high physiological value. Rapid evolution of carotenoid acquisition may also explain why diet has marginal effects on plasma carotenoid levels and the evolution of carotenoid-dependent traits, as demonstrated by earlier comparative work (Tella et al. 2004; Olson and Owens 2005; Galvan et al. 2012). These studies demonstrated that carotenoid-dependent coloration is associated with higher circulating carotenoid levels (Tella et al. 2004) but not liver levels (Galvan et al. 2012). Our results expand on these findings by showing that optima of plasma carotenoids are higher in species with carotenoid-dependent plumage but that attraction toward this optimum is similar in lineages lacking and exhibiting carotenoid-dependent traits.

At a microevolutionary level, there are four possible mechanistic scenarios underlying variation in carotenoid-dependent coloration: (1) differences in carotenoid acquisition, which is still evolving toward the optimum, may underlie variation in color intensity; or if carotenoid acquisition is near or at the optimum, the carotenoid-dependent trait may honestly signal quality based on either (2) the costs of acquisition or (3) the resource value of carotenoids. Finally, (4) the carotenoid-dependent trait may not be used in mate choice anymore, with its current presence reflecting historical (phylogenetic) patterns of sexual selection, maintained for reasons such as species or sex recognition (Holland and Rice 1998).

Mating preference for carotenoid-dependent signal intensity would be a reason to reject scenario 4, but mate choice studies for carotenoid-dependent traits are relatively rare (Simons and Verhulst 2011). Scenarios 1 and 2 can be distinguished from 3 by studying whether variation in carotenoid acquisition underlies color intensity variation. If there is little variation in carotenoid acquisition, then by default scenario 3 becomes more likely (i.e., that the resource value of carotenoids governs intraspecific variation in ornamental color, given that carotenoids are only predicted to be allocated away from crucial physiological needs when there is little scope for increasing carotenoid acquisition). Our results challenge scenario 3 at the macroevolutionary level, showing an association between plasma carotenoid levels and the presence of carotenoid deposition. The rapid response of carotenoid levels to sexual selection supports the hypothesis that pure acquisition and assimilation of carotenoids (scenarios 1 and 2) may

be key in carotenoid signaling. This hypothesis can also explain interspecific differences in the relationship between carotenoid levels and carotenoid-dependent signals with quality indices, as reported elsewhere for oxidative stress state and immunocompetence (Simons et al. 2012b). Moreover, carotenoid-dependent signals are predicted to be correlated with reproductive success under scenarios 1, 2, and 3 but not with indices of quality under scenario 1, which only bears indirect (“sexy son”) benefits.

Our macroevolutionary results posit exciting perspectives as to whether acquisition-based or resource-based honesty is the main driver of the honesty and evolution of carotenoid-based ornaments. For example, what are the ecological and physiological characters favoring evolution of either mechanism? The generality of both mechanisms and whether pure acquisition-based or resource-based honesty can be shown to operate will be crucial to understand the evolution of carotenoid ornaments. The comparative evidence presented here questions the dominant hypothesis that honest signaling of carotenoid-dependent traits is exclusively based on the resource value of carotenoids. Our results suggest instead that, at least in the interspecific level, there is a role for carotenoid acquisition and, more importantly, for the constraints that operate in shaping carotenoid acquisition.

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