doi:10.1111/evo.13093

Evolutionary innovation and diversification of carotenoid-based pigmentation in finches

Russell A. Ligon,[1,2,3] Richard K. Simpson,[1] Nicholas A. Mason,[2,4] Geoffrey E. Hill,[5] and Kevin J. McGraw[1]

[1]*School of Life Sciences, Arizona State University, Tempe, Arizona 85287*

[2]*Laboratory of Ornithology, Cornell University, Ithaca, New York 14850*

[3]*E-mail: russell.ligon@gmail.com*

[4]*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853*

[5]*Department of Biological Sciences, Auburn University, Auburn, Alabama 36849*

Received June 24, 2015
Accepted October 8, 2016

The ornaments used by animals to mediate social interactions are diverse, and by reconstructing their evolutionary pathways we can gain new insights into the mechanisms underlying ornamental innovation and variability. Here, we examine variation in plumage carotenoids among the true finches (Aves: Fringillidae) using biochemical and comparative phylogenetic analyses to reconstruct the evolutionary history of carotenoid states and evaluate competing models of carotenoid evolution. Our comparative analyses reveal that the most likely ancestor of finches used dietary carotenoids as yellow plumage colorants, and that the ability to metabolically modify dietary carotenoids into more complex pigments arose secondarily once finches began to use modified carotenoids to create red plumage. Following the evolutionary "innovation" that enabled modified red carotenoid pigments to be deposited as plumage colorants, many finch species subsequently modified carotenoid biochemical pathways to create yellow plumage. However, no reversions to dietary carotenoids were observed. The finding that ornaments and their underlying mechanisms may be operating under different selection regimes—where ornamental trait colors undergo frequent reversions (e.g., between red and yellow plumage) while carotenoid metabolization mechanisms are more conserved—supports a growing empirical framework suggesting different evolutionary patterns for ornaments and the mechanistic innovations that facilitate their diversification.

KEY WORDS: **Ancestral state reconstruction, character evolution, phylogeny, pigments, plumage coloration.**

To understand the evolution of sexually selected colors, it is essential to determine the mechanisms of production and information content of different color expressions (McGraw et al. 2010; Hill and Johnson 2012). For various color ornaments, it has been proposed that trait elaboration signals information about foraging ability (Endler 1980), nutritional condition (Frischknecht 1993; Hill and Montgomerie 1994; Kemp 2008), social status (Rohwer 1975; Whiting et al. 2003; Dijkstra et al. 2007), immunocompetence (Folstad and Karter 1992), parasite resistance (Hamilton and Zuk 1982; Milinski and Bakker 1990), or sexual attractiveness (Weatherhead and Robertson 1979; Prum 1997; Smith et al. 2004). These hypotheses are not mutually exclusive, however, and for the best-studied species, such as Trinidad Guppies (*Poecilia reticulata*) and House Finches (*Haemorhous mexicanus*), it appears that colorful ornaments can signal multiple attributes (Houde 1997; Hill 2002).

Studies tracing the evolution of ornamental trait expression across multiple taxa hold the potential to help us understand basic principles of trait elaboration, particularly when traits are considered within a framework that provides predictions about ornament elaboration and complexity (Omland and Hofman 2006). For instance, some authors have speculated that indicator traits such as ornamental coloration are elaborated through an evolutionary arms race between males and females in which females demand the most costly and elaborate forms of traits while males evolve strategies to reduce the cost of trait production and hence undercut signal honesty (Williams 1966; Krebs and Dawkins 1984; Hill 1994; Balmford et al. 1994). The predicted outcome of this

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

hypothesized arms race is that, over evolutionary time, condition-dependent ornamental traits should become more elaborate and more costly. Reversals in ornament elaboration should occur only when the costs or benefits of producing the ornament change (Hill 1994; Wiens 2001; Badyaev 2004).

Carotenoid pigmentation of avian plumage is an ideal trait for studying the constraints and evolutionary pressures associated with ornament exaggeration in animals. Carotenoids are large, lipid-soluble molecules that are responsible for much of the yellow, orange, or red coloration in animals (Goodwin 1984), and these pigments have been studied extensively as colorants of bird feathers (McGraw 2006). Plumage carotenoids vary chemically among bird species in several important ways that may influence the costs and information content of ornate coloration. First, carotenoids vary in the hue they generate due to the length of the chromophore (i.e., the number of bonds in conjugation). For instance, lutein is a short-chromophore carotenoid that generates a yellow appearance, whereas astaxanthin is a long-chromophore carotenoid that confers a red hue. Variation in carotenoid concentration can also influence coloration and particularly chroma (Saks et al. 2003), but the concentration of carotenoids does not appear to exert strong influence on interspecific variation in color (Friedman et al. 2014a, b). Second, though all avian species acquire carotenoids exclusively from their diet, significant variation exists among species regarding the way they modify and incorporate these carotenoids into their feathers (Badyaev et al. 2015). Some species deposit the carotenoids that they ingest (e.g., lutein) unmodified into feathers, skin, and scales (Isaksson 2009), whereas others metabolize ingested carotenoids and incorporate these modified carotenoids (e.g., astaxanthin) as colorants into the integument (Brush 1990). Third, whereas dietary carotenoids used as colorants tend to be very similar in form (e.g., xanthophylls such as lutein and zeaxanthin), metabolically modified carotenoids come in two common forms: (1) canary xanthophylls, which generate yellow coloration, and (2) keto-carotenoids (including astaxanthin), which generate a range of red coloration.

It has been hypothesized that coloration generated by modifying carotenoids represents a more elaborate ornamental display than coloration derived from deposition of dietary pigments (Hill 2002). Furthermore, metabolically derived coloration has also been hypothesized to represent a fundamentally more costly trait than dietarily derived color (Hudon 1991; Hill 1994, 1996, 2002). The fact that some bird species replace simple dietary pigments in the integument with metabolically modified pigments suggests that there are significant benefits to balance the costs of such substitutions. When metabolic conversions of carotenoids change the hue of the pigment from yellow to red, the potential benefits of more stimulating visual signals are obvious (Hill 1996, 2000). Much more puzzling, however, is the modification of yellow dietary pigments into different yellow pigments that are deposited in feathers to create yellow plumage, as in American goldfinches *Spinus tristis* (McGraw and Gregory 2004). Equally confounding, some species modify yellow dietary carotenoids to create new yellow carotenoids that are deposited with, and masked by, red pigments (Hudon 1991; Andersson et al. 2007; LaFountain et al. 2013; Friedman et al. 2014b). Examining differential carotenoid use and modification among species within a comparative framework should enable the quantitative evolutionary analyses required to better understand the varied, changable pigmentary strategies that have evolved among birds (sensu Omland and Hofmann 2006).

To date, most comparative studies of the evolution of carotenoid-based signals have relied upon feather hue to characterize the carotenoid state of the plumage of avian species. Such studies have provided new insights into the environmental factors associated with differential expression of carotenoid plumage coloration (Gray 1996; Badyaev and Hill 2000; Olson and Owens 2005), the evolutionary lability of patterns and colors across taxa (Omland and Lanyon 2000; Cardoso and Mota 2008; Kiere et al. 2009; Prager and Andersson 2010; Friedman et al. 2011), and the evolutionary correspondence between signal design and receiver physiology (Endler et al. 2005; Bleiweiss 2014). Only recently, however, has the evolution of carotenoid plumage pigments been investigated from a biochemical perspective. Prum et al. (2012), for example, examined the biochemical basis for cotinga coloration (Cotingidae) and uncovered novel pigments and metabolic pathways used by cotingas to achieve orange, red, and purple plumage. Similarly, Friedman et al. (2014a,b) investigated carotenoid evolution in New World orioles (Friedman et al. 2014a) as well as caciques and meadowlarks (Friedman et al. 2014b). Among caciques and meadowlarks, Friedman et al. (2014b) found two independent origins of red carotenoid plumage arising via different pigmentary mechanisms. Additionally, among New World orioles Friedman et al. (2014a) found that all yellow orioles relied solely upon dietary lutein as a plumage colorant, whereas orange orioles, red caciques, and red meadowlarks incorporated metabolically modified red and metabolically modified yellow pigments into their plumage. More recently, Badyaev et al. (2015) conducted a comprehensive analysis of carotenoid networks and evolution in a wide diversity of birds, uncovering differential patterns of gains and losses depending on carotenoid type and revealing the importance of dietary input on subsequent carotenoid modifications. Collectively, these studies illustrate the ways that understanding the mechanistic underpinnings of ornamental trait evolution can provide novel insights into the selective forces shaping ornamental diversity.

To gain a better understanding of the evolution of carotenoid pigment ornamentation in birds, we used comparative analyses to address two hypotheses: (1) evolutionary innovations in

carotenoid ornamentation (e.g., the ability to incorporate metabolically modified pigments into plumage) should rarely be lost once gained, and (2) carotenoid plumage pigments should evolve in an ordered fashion. Our first hypothesis sets up two competing predictions that differ depending on which selective pressure acting on carotenoid pigmented plumage is strongest. If selection occurring at the level of plumage coloration has driven the evolution of carotenoid plumage ornaments, then we should see minimal losses of red coloration once it has evolved. Under this selection regime, red coloration represents an evolutionary "optimum" and serves as a more complex and visually stimulating display color. Alternatively, if selection at a level of the mechanisms responsible for creating different ornamental colors has driven the evolution of carotenoid plumage, then we should see that the ability to metabolically modify dietary pigments should not be lost after it has evolved. This second prediction holds only if metabolically modified yellows are indistinguishable from dietary yellows. Under our second hypothesis, we predict that the use of metabolically modified carotenoid pigments should evolve after the use of dietary carotenoid pigments, as evolutionary innovations (*sensu* Endler et al. 2005) create the opportunity to display new colors (e.g., red) and new color combinations (e.g., mixtures of red and yellow pigments to create oranges) previously unavailable but which potentially increase the visual stimulation of such ornaments.

To assess these two hypotheses, we used biochemical data on the carotenoid content of feathers—both published and newly obtained for this study—in conjunction with a recently published phylogeny (Zuccon et al. 2012) to reconstruct the evolution of carotenoid pigmentation in true finches (Fringillidae). Additionally, we investigated broad patterns of plumage color evolution using color plates. We explicitly limited our analysis to feathers because (1) the evolution of feather pigmentation represents an ornamentation pathway unique to birds, and (2) much less information is available regarding the mechanisms responsible for the coloration of eyes, bills, legs, and other "bare parts" of birds compared to feathers, due in part to analytical difficulties (McGraw et al. 2002) and poor pigment preservation in bare parts of museum skins (K. J. McGraw, pers. obs.). We conducted our analyses in fringillid finches because this group is the best-studied family of passerine birds in terms of data available regarding the carotenoid pigments used to color feathers (e.g., Stradi et al. 1995b, 1997; Stradi 1998) as well as for the number of species that have been studied with regard to the function of carotenoid-based coloration (e.g., Eley 1991; Hill 1991; Johnson et al. 1993; Senar 2006). Interestingly, this group also exhibits some of the highest evolutionary rates of carotenoid elaboration known in birds (Badyaev et al. 2015). We conducted ancestral state reconstructions of carotenoid plumage to assess various evolutionary models and generated stochastic character mapping simulations to estimate the number of transitions between each state (Revell 2012) for each model of carotenoid evolution.

Methods

CAROTENOID CLASSIFICATION

For our analysis of pigment evolution in the true finches (family: Fringillidae, order: Passeriformes), we assigned carotenoid pigment categories to species using (1) published descriptions of feather pigments and (2) high-performance liquid chromatography (HPLC) analyses (following procedures in McGraw et al. 2003a). For the HPLC analyses, we analyzed the feathers from 14 additional species, chosen to fill taxonomic gaps (Table 1). We used a three-step gradient solvent system to analyze xanthophylls and carotenes in a single run, at a constant flow rate of 1.2 mL min^{-1}: first, isocratic elution with 42:42:16 (v/v/v) methanol : acetonitrile : dichloromethane for 11 min, followed by a linear gradient up to 42:23:35 (v/v/v) methanol : acetonitrile : dichloromethane through 21 min, held isocratically at this condition until 30 min, and finishing with a return to the initial isocratic condition from 30 to 48 min. In total, we used biochemical data from 52 Fringillidae species, out of the 93 (56%) species with taxonomic relationships identified by Zuccon et al. (2012). We classified each species in the following categories: (1) no carotenoids in plumage ($n = 7$); (2) dietary carotenoids in plumage ($n = 9$); (3) metabolically modified red carotenoids in plumage ($n = 20$); and (4) endogenously modified yellow carotenoids in plumage ($n = 16$). In some species, males and females or members of different age-classes differ in the types of carotenoids used as feather colorants. Females of several species, for example, use yellow carotenoids as plumage colorants, while males of the same species use modified red carotenoids. When there was variation in pigment use within a species, we assigned the carotenoid category that occurred in the adult males. However, in the case of *Haemorhous mexicanus* and *Pyrrhula erythaca*, there is carotenoid variation within adult males, in that some males possess only modified yellow pigments while others possess modified red pigments. We assigned the character state for these species as modified red carotenoids; however, the results were qualitatively unchanged when these species were assigned a modified yellow state.

COLOR CLASSIFICATION

To analyze broad-scale patterns of plumage color evolution in Fringillid finches, we assigned plumage color categories to species using color plates in the Handbook of Birds of the World (del Hoyo et al. 2010). Because our focus was on putative carotenoid colors (i.e., yellows, oranges, reds), we scored species lacking any such colors as having "no carotenoid colors." For species that possessed putative carotenoid-derived plumage

Table 1. Fringillidae species used in our ancestral state reconstructions.

Species	Plumage pigments	Carotenoid class	Source
Fringilla montifringilla	Lutein	Dietary	7*
Fringilla coelebs	Lutein and zeaxanthin	Dietary	1, 2
Euphonia minuta	Lutein and zeaxanthin	Dietary	7**
Euphonia violacea	Lutein and zeaxanthin	Dietary	7**
Euphonia laniirostris	Lutein and zeaxanthin	Dietary	7**
Euphonia xanthogaster	Lutein and zeaxanthin	Dietary	7**
Euphonia rufiventris	Lutein and zeaxanthin	Dietary	7**
Euphonia musica	Lutein and zeaxanthin	Dietary	7**
Mycerobas carnipes	Lutein	Dietary	7
Eophona migratoria	None	None	7
Hesperiphona vespertina	Lutein	Dietary	12
Coccothraustes coccothraustes	None	None	7***
Carpodacus sibiricus	3-Hydroxy-echinenone, adonirubin, astaxanthin	Modified red	1, 2, 3, 4, 7*
Carpodacus thura	3-Hydroxy-echinenone, oxo-rubixanthin	Modified red	2
Carpodacus roseus	3-Hydroxy-echinenone, adonirubin, astaxanthin, papilioeritrinone, oxo-rubixanthin, alpha-doradexanthin	Modified red	1, 2, 3, 4, 5
Carpodacus rubicilloides	3-Hydroxy-echinenone, oxo-rubixanthin	Modified red	2, 3, 4
Carpodacus vinaceus	Astaxanthin, alpha-doradexanthin, 3'-dehydro-lutein, adonirubin, lutein, canthaxanthin, 3-hydroxy-echienone, echinenone	Modified red	3
Carpodacus pulcherrimus	Astaxanthin, alpha-doradexanthin, adonirubin, 3-hydroxy-echinenone, 4-oxo-rubixanthin, papilioerythrinone	Modified red	2
Haematospiza sipahi	3-Hydroxy-echinenone, oxo-rubixanthin, astaxanthin	Modified red	2
Haemorhous mexicanus	Astaxanthin, alpha-doradexanthin, adonirubin, canthaxanthin, 3-hydroxy-echinenone, echinenone, 4-oxo-rubixanthin, 4-oxo-gazaniaxanthin (*plus lutein, 3'-dehydrolutein, and canary xanthophylls A & B in yellow birds)*	Modified red	9
Crithagra rufobrunneus	None	None	11†
Crithagra mozambicus	Canary xanthophylls A and B	Modified yellow	1, 2
Crithagra leucopygius	None	None	11†
Crithagra mennelli	None	None	11†
Linurgus olivaceus	Canary xanthophylls A, B, C, and D	Modified yellow	7*
Loxia leucoptera	3-Hydroxy-echinenone, 4-oxo-rubixanthin, 4-oxo-gazaniaxanthin (*plus canary xanthophylls A & B in females*)	Modified red	1, 2, 10
Loxia curvirostra	3-Hydroxy-echinenone, 4-oxo-rubixanthin, 4-oxo-gazaniaxanthin (*plus canary xanthophylls A & B in females*)	Modified red	1, 2, 4, 5, 10
Acanthis hornemanni	3-Hydroxy-echinenone, canthaxanthin, adonirubin, astaxanthin, rubixanthin	Modified red	3
Acanthis flammea	3-Hydroxy-echinenone, adonirubin, astaxanthin	Modified red	1, 2, 3, 4, 5
Linaria cannabina	3-Hydroxy-echinenone, canthaxanthin, adonirubin, astaxanthin	Modified red	1, 2, 3, 4
Carduelis citrinella	Canary xanthophylls A and B	Modified yellow	1, 2, 6

(Continued)

Table 1. Continued.

Species	Plumage pigments	Carotenoid class	Source
Carduelis carduelis	Canary xanthophylls A and B (*canary xanthophylls C and D in red head feathers only, red plumage comes via specialized carotenoid binding to feather keratin*)	Modified yellow	1, 2, 6
Serinus pusillus	Canary xanthophylls A and B (*red plumage comes via specialized carotenoid binding to feather keratin*)	Modified yellow	1, 2, 6
Serinus serinus	Canary xanthophylls A and B	Modified yellow	1, 2, 5, 6
Serinus canaria	Canary xanthophylls A and B	Modified yellow	2
Spinus tristis	Canary xanthophylls A and B	Modified yellow	8
Spinus spinus	Canary xanthophylls A and B	Modified yellow	1, 2, 5, 6
Spinus pinus	Canary xanthophylls A, B, C, and D	Modified yellow	7***
Spinus cucullata	Alpha-doradexanthin and canthaxanthin	Modified red	1, 2
Spinus atrata	Canary xanthophylls A and B	Modified yellow	1, 2
Rhynchostruthus socotranus	Canary xanthophylls A and B	Modified yellow	7*
Rhodospiza obsoleta	Alpha-doradexanthin and canthaxanthin	Modified red	1, 2
Chloris chloris	Canary xanthophylls A and B (*some lutein*)	Modified yellow	1, 2, 5, 6
Chloris sinica	Canary xanthophylls A and B (*some lutein*)	Modified yellow	6
Chloris spinoides	Canary xanthophylls A and B (*some lutein*)	Modified yellow	6
Pyrrhula pyrrhula	Astaxanthin, alpha-doradexanthin, adonirubin, canthaxanthin, papilioeritrinone	Modified red	1, 2, 4, 5
Pyrrhula erythaca	Canthaxanthin, 3-hydroxy-echinenone (*plus canary xanthophylls A & B in orange birds*)	Modified red	2
Pinicola enucleator	Alpha-doradexanthin, adonirubin, canthaxanthin, 3-hydroxy-echinenone, 4-oxo-rubixanthin (*plus lutein and 3'-dehydrolutein in females*)	Modified red	1, 2, 4, 5, 10
Bucanetes githaginea	Astaxanthin, alpha-doradexanthin, adonirubin, canthaxanthin	Modified red	2
Leucosticte nemoricola	None	None	11†
Procarduelis nipalensis	Astaxanthin, 3-hydroxy-echinenone (*plus lutein and canary xanthophylls A & B in females*)	Modified red	2
Pyrrhoplectes epauletta	Lutein, 3'-dehydrolutein	Modified yellow	7*

Each species in this table either possesses red, orange, or yellow plumage that has been analyzed biochemically *or* lacks red, orange, or yellow plumage completely.

***Plumage samples obtained from the American Museum of Natural History.**

****Plumagesamples obtained from the Cornell University Museum of Vertebrates (Table S3).**

*****Plumagesamples obtained from the Auburn University Museum of Natural History.**

†**Carotenoid status of "none" assigned to these taxa based on lack of any apparent carotenoid-pigmented plumage (red, orange, yellow).**

1, Stradi (1998); 2, Stradi (1999); 3, Stradi et al. (1997); 4, Stradi et al. (2001); 5, Stradi et al. (1995a); 6, Stradi et al. (1995b); 7, present study; 8, McGraw et al. (2001); 9, Inouye et al. (2001); 10, Stradi et al. (1996); 11, del Hoyo et al. (2010); 12, McGraw et al. (2003b).

colors, we scored plumage color as either "yellow" or "red" (which included any color with longer wavelength hues, e.g., oranges, pinks, and reds; Table S4).

PHYLOGENETIC ANALYSES

We performed all phylogenetic analyses of carotenoid plumage pigments in fringillid finches using R (R Core Development Team 2012) and the R packages *ape* (Paradis et al. 2004), and *phytools* (Revell 2012). We obtained the gene alignment matrix from the recently published phylogeny on Fringillidae, which used two mitochondrial DNA regions (*ND2* and *ND3*) and three nuclear DNA loci (intron 2 of the myoglobin gene: *myo*, intron 6 and 7 of ornithine decarboxylase gene: *ODC*, and intron 11 of the glyceraldehyde-3-phosphodehydrogenase gene: *GAPDH*; Zuccon et al. 2012). We identified the optimal partitioning strategy for introns and codon positions of the two mtDNA gene regions with PartitionFinder version 1.1.0 (Lanfear et al. 2012). For the nuclear loci, the favored partitioning scheme separated *GAPDH* in its own partition (K80 + G nucleotide substitution model) and joined *myo* and *ODC* in a combined partition (HKY

+ G). The favored partitioning scheme combined mtDNA gene regions with separate partitions for each codon position (*ND2* and *ND3* codon 1: GTR + I + G; *ND2* and *ND3* codon 2: HKY + I + G; *ND2* and *ND3* codon 3 GTR + G). Using this partitioning scheme, we generated an ultrametric, time-calibrated phylogeny using BEAST version 2.3.0 (Bouckaert et al. 2014) with a relaxed clock and mutation rates derived from (Lerner et al. 2011). We ran three separate Metropolis-coupled MCMC chains for 30×10^6 generations and discarded the first 10% as burn-in. We assessed the convergence of each run by examining stationarity among the model parameters and ensuring that the estimated sample sizes (ESS) for all parameters exceeded 200. We combined post-burn-in phylogenies to generate a maximum clade credibility (MCC) tree and a posterior distribution of phylogenies. We pruned the trees to create two sets of phylogenies for use with carotenoid and color classifications that had different numbers of missing taxa.

ANCESTRAL STATE RECONSTRUCTIONS

To investigate potential differences in evolutionary patterns between carotenoid pigment types and plumage color, we performed stochastic character mapping (Bollback 2006; Revell 2012) using two different datasets: (1) carotenoid classifications and (2) color classifications. Such comparison is conceptually valid only if there is not a one-to-one correspondence between carotenoid pigment type and plumage color. Analysis of a small dataset of passerine birds that use carotenoids to create yellow plumage (several species that rely on dietary pigments, several species that rely on metabolically modified pigments) provides evidence for substantial spectral and perceptual overlap in the yellow coloration produced by these different means (Table S1, Fig. S1). This overlap suggests that it is not possible to accurately categorize plumage mechanisms based on color alone, and validates the utility of the evolutionary comparisons of signal class (i.e., plumage color) and underlying mechanisms (i.e., carotenoid types).

For the carotenoid classification dataset, we performed stochastic character mapping with 12 different evolutionary models (Fig. 1) and compared these models using Akaike Information Criterion (AIC) scores. For the color classification set, we compared the performance of six different models (Fig. S2) using AIC scores. For both datasets, we first assessed model performance by conducting 50 ancestral state reconstructions for each model in which we sampled a random phylogeny from the post-burn-in posterior distribution of trees. We used a continuous-time reversible Markov model fitted to our Q matrix (i.e., Q = "empirical") and estimated the prior distribution on the root node of the tree based on tip character states (i.e., pi = "estimated"). We then averaged the log likelihood of each set of reconstructions for all models and calculated the corresponding AIC scores and AIC weights (AIC_w). After determining the AICw of each model, we ran 1000 simulations of stochastic character mapping in which the number of simulations for each model was weighted proportionally to their AICw. We sampled a random phylogeny from the post-burn-in distribution for each simulation to account for uncertainty in topology and branch lengths. We then averaged the number of character state transitions across the weighted distribution of simulations and visualized the output on the MCC tree. Finally, we combined the estimated number of transitions for each character state changes with inferred ancestral state reconstructions mapped onto the MCC tree to propose a pathway for the evolution of plumage carotenoids in finches.

Results

We found varying levels of support for the different models of carotenoid evolution considered in this study, with no single model being a clear "best" model (Table 2). The model with the strongest support explaining carotenoid evolution was the symmetrical model ($AIC_w = 0.38$) followed by the equal rates model ($AIC_w = 0.23$). The remaining models each had an AIC_w less than 0.20. Similarly, the models examining plumage color evolution were roughly equivocal, though the equal rates model had the highest support ($AIC_w = 0.39$; Table S2). For both datasets (carotenoid type and color), we incorporated models into our sets of 1000 stochastic character mapping simulations proportionally to their AIC_w scores.

The most likely ancestral carotenoid state for fringillids was "dietary yellow" (posterior probability = 0.83; Fig. 2A). Across the 1000 stochastic character mapping simulations of carotenoid types in fringillids, the median number of carotenoid state changes was 16 or an average of 23.11 ± 0.85 (standard error). Specifically, the median number of transitions from "no carotenoids" to dietary yellow was 1 (mean = 2.43 ± 0.16; Fig. 2B), while the median number of transitions from "dietary carotenoids" to no carotenoids was 3 (mean = 3.43 ± 0.14; Fig. 2B). Most SIMMAP simulations generated a single transition from dietary yellow to "modified red" (median = 1, mean = 0.74 ± 0.03; Fig. 2B) and no transitions from modified red to dietary yellow (median = 0, mean = 0.66 ± 0.05; Fig. 2B). Likewise, most simulations revealed no transitions from dietary yellow to modified yellow (median = 0, mean = 0.47 ± 0.04; Fig. 2B) and transitions from modified yellow to dietary yellow were similarly rare (median = 0, mean = 0.47 ± 0.04; Fig. 2B). In contrast, transitions between modified red and modified yellow were frequent (median = 3, mean = 3.42 ± 0.05; Fig. 2B), as were transitions between modified yellow and modified red (median = 4, mean = 4.73 ± 0.08; Fig. 2B). Changes from modified red carotenoids to no carotenoids were rare (median = 0, mean = 0.9 ± 0.04; Fig. 2B), and gains of modified red

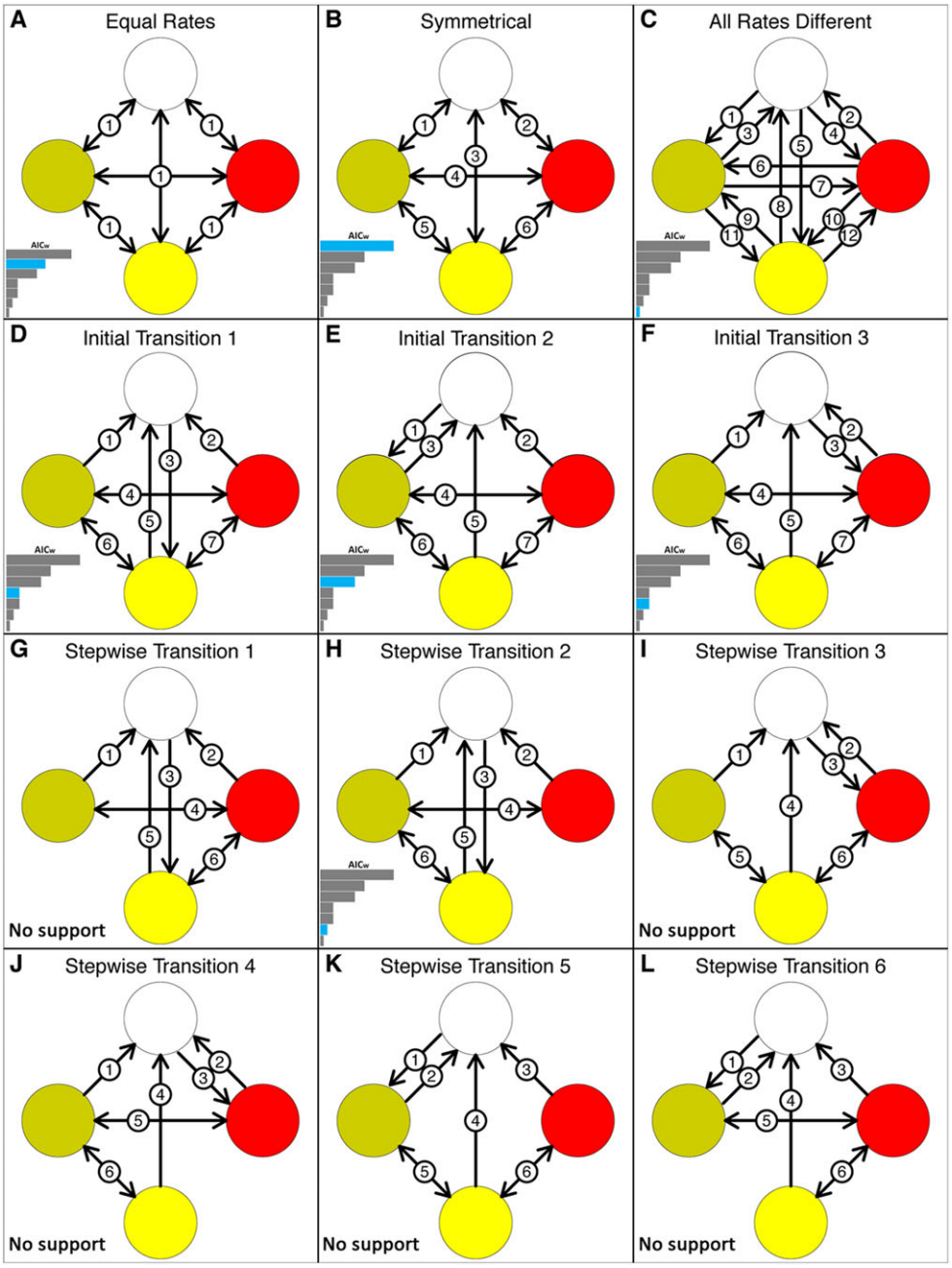

Figure 1. **Twelve different carotenoid evolution models tested via stochastic character mapping. Plumage lacking any carotenoids is indicated by the white circle, dietary carotenoids indicated by the bright yellow circle, modified red pigments by the red circle, and modified yellow pigments by the off-yellow circle. Each number within figure panels corresponds to a different rate shift parameter. Arrows with heads on both ends indicate symmetrical transition rates. Model support (AIC_w weight) values are indicated in the bottom left of each panel for all models with AIC_w values greater than zero.**

carotenoids from no carotenoids were also rare (median = 0, mean = 0.84 ± 0.09; Fig. 2B). Finally, losses of modified yellow carotenoids to no carotenoids were common (median = 4, mean = 3.95 ± 0.08; Fig. 2B), but gains of modified yellow carotenoids from no carotenoids were rare (median = 0, mean = 1.07 ± 0.05; Fig. 2B).

Congruent with our findings regarding the evolutionary history of carotenoid plumage types, the most likely ancestral plumage color for fringillids was yellow (posterior probability = 0.73; Fig. S2A). Across the 1000 stochastic character mapping simulations of plumage coloration in fringillids, the median number of plumage color state changes was 21, or an average of 23.37

Table 2. Performance metrics of 12 different carotenoid evolution models.

Model name	Log likelihood	Parameters	AIC	ΔAIC	AIC_w
Equal rates	–50.56	1	103.13	1.03	0.23
Symmetrical	–45.05	6	102.10	0.00	0.38
All rates different	–41.83	12	107.67	5.57	0.02
Initial transition 1	–45.69	7	105.39	3.29	0.07
Initial transition 2	–44.83	7	103.66	1.56	0.18
Initial transition 3	–45.68	7	105.36	3.26	0.07
Stepwise transition 1	–49.84	6	111.68	9.58	0
Stepwise transition 2	–47.31	6	106.62	4.52	0.04
Stepwise transition 3	–62.22	6	136.43	34.33	0
Stepwise transition 4	–59.83	6	131.67	29.57	0
Stepwise transition 5	–70.70	6	153.40	51.30	0
Stepwise transition 6	–57.32	6	126.65	24.55	0

The model names correspond to those in Figure 1.

$\pm$ 0.20. Within the finches, transitions from yellow to red were quite common (median = 7, mean = 6.92 $\pm$ 0.05; Fig. S2B), and transitions from red to yellow were not infrequent (median = 2, mean = 2.36 $\pm$ 0.06; Fig. S2B). Additionally, gains of yellow (from plumage lacking red or yellow) were relatively common (median = 4, mean = 5.46 $\pm$ 0.17; Fig. S2B), while novel gains of red almost never occurred (median = 0, mean = 0.22 $\pm$ 0.02; Fig. S2B). Lastly, loss of yellow/red plumage color from a yellow state were relatively common (median = 7, mean = 7.1 $\pm$ 0.07; Fig. S2B), while losses from red were rare (median = 1, mean = 1.69 $\pm$ 0.04; Fig. S2B).

Our ancestral state reconstructions (Fig. 2A) combined with estimates of the number of evolutionary transitions between carotenoid types (Fig. 2B) suggest an evolutionary history of carotenoid innovation and diversification in fringillids (Fig. 3). We suggest that the evolutionary progression of plumage carotenoids in fringillid finches began with dietary yellow followed by a single transition to modified red carotenoids. This character state change enabled frequent transitions between modified red and modified yellow carotenoids. Additionally, there were several losses of carotenoid pigmented plumage from the dietary yellow carotenoid state, several losses from the modified yellow state, and only a single likely loss of modified red carotenoid coloration. Most models suggested there were no reversions from either modified carotenoid pigment state to dietary yellow (Fig. 3).

Discussion

The simultaneous comparison of ornament evolution and the evolution of those mechanisms underpinning such ornaments can provide important insight into the varied and diverse factors that promote and maintain signal diversity in nature. Using this approach, we found that the evolution of colorful carotenoid plumage in fringillid finches is a labile evolutionary process, with numerous transitions between red and yellow feathers as well as several complete losses of carotenoid pigmentation. Interestingly, the general mechanisms responsible for the different classes of color appear to be less labile than the colors themselves: following the evolutionary innovation of carotenoid modification, no finch species exhibited a reversion (sensu Cronk 2009) to dietary carotenoids as primary plumage colorants.

In contrast to an evolutionary reversion to the plesiomorphic character state of dietary carotenoid incorporation, which was never observed following the evolution of carotenoid pigment modification, the complete loss of carotenoid pigmentation occurred frequently (median = 7; Fig. 2) among frigillid finches. Reversals and losses are typically distinct processes of unequal likelihood (Cronk 2009) arising from the enormous diversity of mutations that can lead to a complete breakdown in pigmentary processes (i.e., loss-of-function mutations) relative to those required for a specific recreation of ancestral genotypes and phenotypes (Dollo's Law; Cronk 2009). For example, loss-of-function mutations can impede the pathways responsible for melanin pigmentation of mammals and fish (Rees 2000; Gross and Wilkens 2013) or those responsible for anthocyanin production/deposition in flowers (Rausher 2008). Just as loss-of-function mutations can facilitate rapid adaptation to dark environments (e.g., in cave fish; Gross and Wilkens 2013) or changes in pollinators (Rausher 2008), similar mutations may enable some finches to conserve carotenoids for vital physiological processes when social or environmental conditions no longer favor the expression of carotenoid plumage ornaments. Additionally, the observed differences between carotenoid plumage loss and reversion further illustrate the variable selective pressures acting on plumage coloration in finches and the relative inertia of carotenoid modification mechanisms within those lineages that retain carotenoid-pigmented

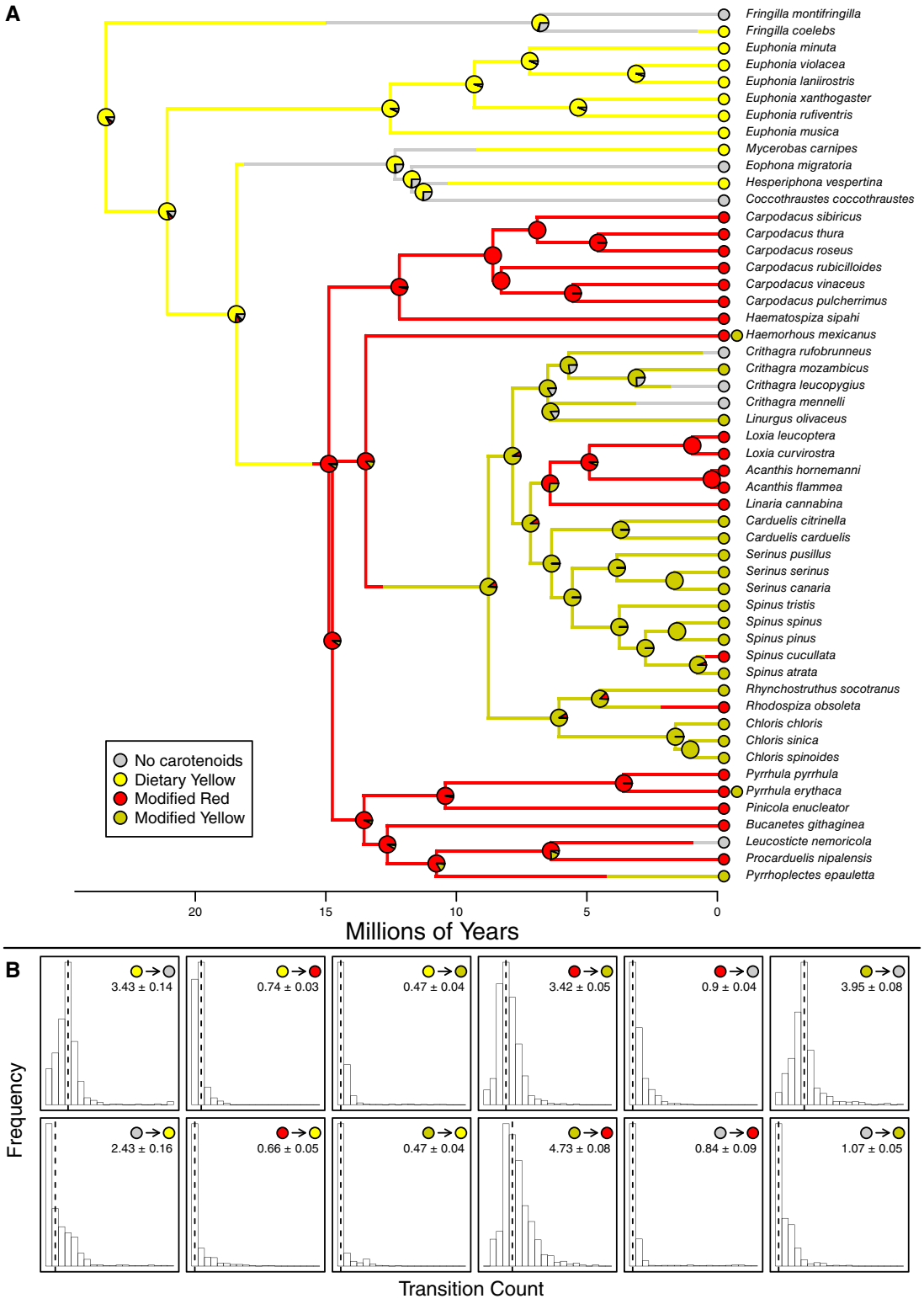

Figure 2. (A) Ancestral state reconstruction of carotenoid plumage states in Fringillidae. The number of simulations included for each model is proportional to their AICw. Colored circles at the tips of each branch represent the carotenoid plumage state of each extant species. Pie charts on each node indicate the proportion of each character state summed across the posterior distribution of simulations. A time scale, estimated with a relaxed molecular clock, is shown below the phylogeny. (B) Histograms of carotenoid character state transitions across stochastic character mapping simulations included in the posterior distribution. Median values are indicated by dashed vertical lines. The circular symbols used in (B) correspond to the carotenoid pigment character states identified in (A).

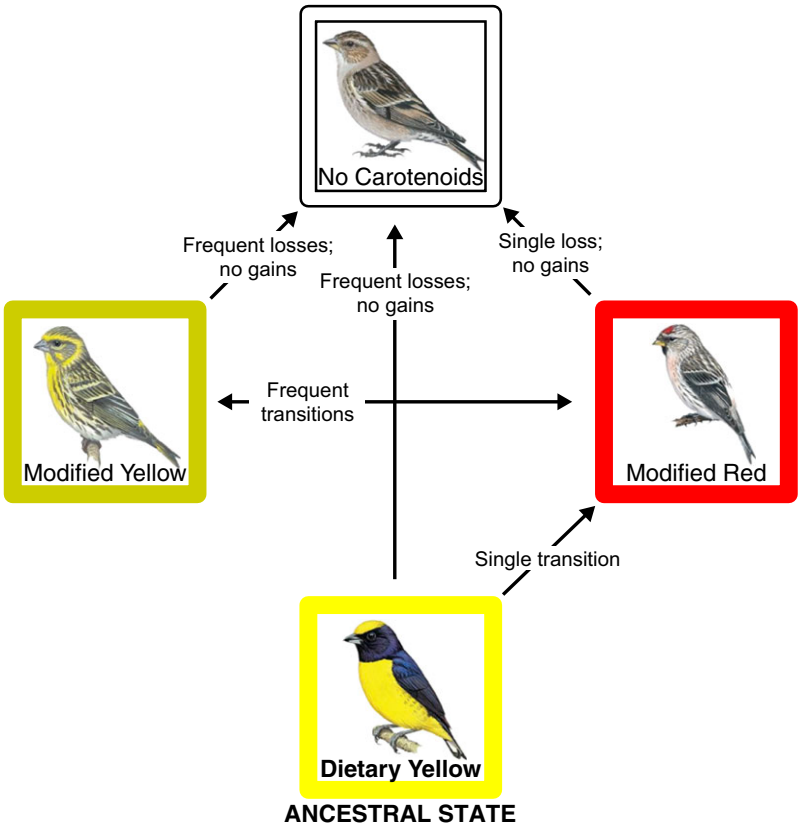

Figure 3. Summary of carotenoid character state changes throughout the evolutionary history of Fringillidae.

plumage. The lack of observed reversals in carotenoid metabolization may reflect net benefits (e.g., physiological or signaling) associated with this strategy, or may simply be a nonadaptive consequence of phylogenetic inertia.

As a consequence of inertia or adaptive benefits associated with new opportunities in signaling space, mechanistic innovations that increase ornament elaboration frequently persist once they are introduced into lineages (Prager and Andersson 2009; Robillard and Desutter-Grandcolas 2011; Maia et al. 2013; though patterns can vary by sex, Maia et al. 2016). The elaboration and diversification of signals enabled by novel production mechanisms can facilitate evolutionary flexibility in response to changing selection pressures, whether these pressures are ecological (e.g., Endler et al. 2005), social (e.g., Hill and McGraw 2004), or the result of random variation in receiver preference (e.g., Prum 2010). Our current investigation of plumage pigment evolution suggests that, in the case of fringillid finches, an important innovation in ornamental feather coloration was the evolution of the mechanisms required to metabolically modify dietary carotenoids into either red or yellow pigments. This finding supports the prediction that broad-scale selection has favored the evolution of pigmentary modification mechanisms that allow a diverse array of plumage colorants to be synthesized from a relatively restricted subset of dietary carotenoids (Lopes et al. 2016). However, this finding fails to support the prediction of directional selection on redder plumage.

Based on the presumed costs of carotenoid metabolism and the increasing complexity of mechanisms involved in pigment utilization (Hill 1996, 2000; Badyaev et al. 2015), we originally predicted an ordered evolutionary pathway of plumage carotenoid pigments, progressing from complete lack of carotenoid pigments, to use of unaltered dietary carotenoids, to the incorporation of metabolically modified carotenoids into feathers. Our results only partially support this idea because the most likely ancestral state within Fringillidae appears to be dietary yellow carotenoids (Figs. 2, 3). However, our multimodel informed ancestral state reconstruction suggests that modified plumage carotenoids evolved following dietary carotenoids, with modified yellow carotenoids appearing as plumage colorants only after the innovation of modified red pigments. This pattern sets up an interesting comparison with meadowlarks, where modified yellow carotenoids (canary xanthophylls) only occur in species that *also* possess the ability to modify dietary carotenoids into red ketocarotenoids—though not all red meadowlarks or caciques deposit modified yellow pigments into their plumage (Friedman et al. 2014b). Similarly, modified yellow carotenoids co-occur with modified red carotenoids in a number of other avian species, some with red plumage (Emberizidae and Thraupidae, Hudon 1991; Passeridae, Andersson

et al. 2007; Cotingidae, Prum et al. 2012; Oriolidae, LaFountain et al. 2013) and some with orange (Icteridae, Hudon 1991; Parulidae, McGraw 2006; Cotingidae, Prum et al. 2012). In cases where combinations of red ketocarotenoids and yellow canary xanthophylls produce orange plumage colors (e.g., as in orioles, Friedman et al. 2014a), the co-occurrence of pigment types makes sense from a signaling perspective. However, why some species produce metabolically modified yellow carotenoids when these pigments are masked by red carotenoids remains an outstanding question.

If modified yellow pigments exert subtle, as yet undetected, influences on ornamental coloration, then perhaps chromatic advantages can explain the use of modified yellow carotenoids (instead of dietary pigments) as colorants in the yellow plumage of so many fringillid finches (Fig. 2). Even among carotenoid-pigmented birds, however, the identity and metabolic modification of particular plumage colorants only tell part of the story. For example, the same pigment (canthaxanthin) can produce red, red-orange, and even purple plumage in different species, depending on molecular alignment and interaction with proteins (Mendes-Pinto et al. 2012). Similarly, two species in the present study (*Serinus pusillus* and *Carduelis carduelis*) rely exclusively on modified yellow pigments to produce red plumage, demonstrating a rare evolutionary pathway to achieve red feathers within this taxon (though this phenomenon has previously been described for red-shouldered widowbirds *Euplectes axillaris*; Andersson et al. 2007). If the mechanisms that enable modified yellow carotenoids to imbue red coloration, perhaps specialized carotenoid-keratin bonds (Stradi et al. 1995b), also alter the visual characteristics of yellow feathers pigmented with canary xanthophylls, then chromatic or signaling benefits associated with this strategy may explain the prevalence of yellow finches that use modified yellow carotenoids as plumage colorants. Canary xanthophylls also provide yellow coloration for a number of nonfinch species (reviewed in McGraw 2006), and investigating the chromatic properties of dietary and modified yellow carotenoids in a rigorous, phylogenetically controlled framework could provide new insights into the underlying factors favoring the use of modified yellow carotenoids in place of dietary yellow carotenoids.

Our study focused specifically on feather pigmentation, but carotenoids are also important colorants of eyes, bills, legs, and mouth linings. Carotenoid pigmentation of such nonfeathered parts is a primitive trait in birds (Hill 2010). Pigmentation of feathers with carotenoids, on the other hand, is a derived avian character (Hill 2010), apparently requiring special adaptations found in only a subset of birds. Before birds evolved feather pigmentation, however, they might have evolved sophisticated mechanisms for carotenoid modification related to use of such pigments as colorants in skin, eyes, legs, or bills. Alternatively, the benefits associated with modifying dietary carotenoid pigments may have been unrelated to ornamentation of any kind. In fact, carotenoids are known to serve a physiological function as antioxidants (McGraw and Ardia 2003), immunomodulators (Chew 1993), and photoprotectants (Thomson et al. 2002). If metabolically modified forms of carotenoids conferred fitness benefits unrelated to external coloration, the ability to create them might has arisen well before the ability to incorporate them into growing feathers. Indeed, the ketocarotenoid astaxanthin is thought to be created in the eyes of all diurnal birds (Goldsmith et al. 1984; Hart 2001a,2001b), so the enzymatic mechanisms required for converting dietary carotenoids are likely ancestral among birds (Lopes et al. 2016). Thus, the apparent gain of a new character such as incorporation of metabolized carotenoids into feathers may not represent a novel enzymatic gain per se, but rather a cis regulatory change for an existing system pigment metabolism pathway (True and Carroll 2002; Lopes et al. 2016).

In this study, we evaluated the evolution of carotenoid plumage pigmentation within the fringillid finches. We tested two hypotheses: (1) when evolutionary innovations that increase ornament elaboration are introduced, they should persist; and (2) carotenoid plumage pigments should evolve in an ordered pathway. Through ancestral state reconstructions and estimated trait transition frequencies, we found some level of support for both hypotheses. Though transitions between red and yellow pigments were relatively common, carotenoid modification never reverted to dietary pigment use once it evolved within the finches. In contrast, the complete loss of carotenoid ornamentation occurred multiple times. Carotenoid modification arose after the incorporation of dietary carotenoids as plumage colorants, and the ancestral finch likely was able to deposit unmodified dietary carotenoids directly into its plumage. Given that carotenoid metabolism varies in other taxa (discussed above), the universality of our carotenoid-specific implications are difficult to predict. However, the broader patterns of ornamental plasticity and mechanistic inertia are potentially applicable to, and testable in, a wide array of signaling systems. Understanding the adaptive benefits of the mechanistic processes involved in ornamental expression within and outside of signaling contexts will therefore be a valuable contribution to our understanding of the selection pressures the visual appearance of animals.

ACKNOWLEDGMENTS

We thank J. D. Ligon, M. G. Meadows, M. W. Butler, and three anonymous reviewers for helpful comments on earlier versions of this article and J. Johnson for thought-provoking discussions on the topic of carotenoid metabolization. We thank L. Revell for assistance with comparative methods. The analysis of the carotenoid content from museum specimens was made possible by feather donations from the Smithsonian Institution (Division of Birds, National Museum of Natural History), American Museum of Natural History, and Cornell Museum of Vertebrates (Table S3). Additionally, we thank D. Zuccon for providing the Fringillidae phylogeny

and C. Dardia at the Cornell University Museum of Vertebrates for help in providing additional specimens for us to sample. RAL was supported by the ASU Graduate College Completion Fellowship and NSF grants 1401236 and 1523895 during the creation of this article. NAM was supported by an EPA STAR Fellowship (F13F21201). We have no conflicts of interest to report.

LITERATURE CITED

Andersson, S., M. Prager, and E. I. A. Johansson. 2007. Carotenoid content and reflectance of yellow and red nuptial plumages in widowbirds (*Euplectes* spp.). Funct. Ecol. 21:272–281.

Badyaev, A. V. 2004. Paradox of an ideal sexual trait: exaggerated, yet honest. Developmental perspective on the evolution of sexual ornaments. Evol. Ecol. Res. 6:975–991.

Badyaev, A. V., and G. E. Hill. 2000. Evolution of sexual dichromatism: contribution of carotenoid versus melanin-based coloration. Biol. J. Linn. Soc. 69:153–172.

Badyaev, A. V., E. S. Morrison, V. Belloni, and M. J. Sanderson. 2015. Tradeoff between robustness and elaboration in carotenoid networks produces cycles of avian color diversification. Biol. Direct 10: 1–22.

Balmford, A., I. L. Jones, and A. L. R. Thomas. 1994. How to compensate for costly sexually selected tails: the origin of sexually dimorphic wings in long-tailed birds. Evolution 48:1062–1070.

Bleiweiss, R. 2014. Physical alignments between plumage carotenoid spectra and cone sensitivities in ultraviolet-sensitive (UVS) birds (Passerida: Passeriformes). Evol. Biol. 41:404–424.

Bollback, J. P. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinform. 7:88.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10:e1003537.

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

Cardoso, G. C., and P. G. Mota. 2008. Speciational evolution of coloration in the genus *Carduelis*. Evolution 62:753–762.

Chew, B. P. 1993. Role of carotenoids in the immune-response. J. Dairy Sci. 76:2804–2811.

Cronk, Q. C. B. 2009. Evolution in reverse gear: the molecular basis of loss and reversal. Cold Spring Harb. Symp. Quant. Biol. 74:259–266.

del Hoyo, J., A. Elliot, and D. A. Christie, eds. 2010. Handbook of birds of the world. Vol. 15. Weavers to New World warblers. Lynx Edicions, Barcelona.

Dijkstra, P. D., R. Hekman, R. W. Schulz, and T. G. G. Groothuis. 2007. Social stimulation, nuptial colouration, androgens and immunocompetence in a sexual dimorphic cichlid fish. Behav. Ecol. Sociobiol. 61:599–609.

Eley, C. C. 1991. Status signalling in the western greenfinch (*Carduelis chloris*). Ph.D. diss., University of Sussex, Brighton, U.K.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76–91.

Endler, J. A., D. A. Westcott, J. R. Madden, and T. Robson. 2005. Animal visual systems and the evolution of color patterns: sensory processing illuminates signal evolution. Evolution 59:1795–1818.

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

Friedman, N. R., L. M. Kiere, and K. E. Omland. 2011. Convergent gains of red carotenoid-based coloration in the New World blackbirds. Auk 128:1–10.

Friedman, N. R., K. J. McGraw, and K. E. Omland. 2014a. History and mechanisms of carotenoid plumage evolution in the New World orioles (Icterus). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 172–173:1–8.

———. 2014b. Convergence and parallelism in the evolution of carotenoid pigmentation in caciques and meadowlarks. Evolution 68:791–801.

Frischknecht, M. 1993. The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. Evol. Ecol. 7: 439–450.

Goldsmith, T. H., J. S. Collins, and S. Licht. 1984. The cone oil droplets of avian retinas. Vision Res. 24:1661–1671.

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

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

Gross, J. B., and H. Wilkens. 2013. Albinism in phylogenetically and geographically distinct populations of Astyanax cavefish arises through the same loss-of-function Oca2 allele. Heredity 111:122–130.

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

Hart, N. S. 2001a. Variations in cone photoreceptor abundance and the visual ecology of birds. J. Comp. Physiol. A 187:685–598.

———. 2001b. The visual ecology of avian photoreceptors. Prog. Retin. Eye Res. 20:675–703.

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

———. 1994. Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. Behav. Ecol. 5:64–73.

———. 1996. Redness as a measure of the production cost of ornamental coloration. Ethol. Ecol. Evol. 8:157–175.

———. 2000. Energetic constraints on expression of carotenoid-based plumage coloration. J. Avian Biol. 31:559–566.

———. 2002. A red bird in a brown bag: the function and evolution of ornamental plumage coloration in the house finch. Oxford Univ. Press, New York.

———. 2010. National geographic bird coloration. National Geographic, Washington, DC.

Hill, G. E., and J. D. Johnson. 2012. The vitamin a-redox hypothesis: a biochemical basis for honest signaling via carotenoid pigmentation. Am. Nat. 180:E127-E150.

Hill, G. E., and K. J. McGraw. 2004. Correlated changes in male plumage coloration and female mate choice in cardueline finches. Anim. Behav. 67:27–35.

Hill, G. E., and K. J. McGraw, eds. 2006. Bird coloration. Vol. II. Function and evolution. Harvard Univ. Press, Cambridge, MA.

Hill, G. E., and R. Montgomerie. 1994. Plumage colour signals nutritional condition in the house finch. Proc. R. Soc. Lond. B 258:47–52.

Houde, A. E. 1997. Sex, color, and mate choice in guppies. Princeton Univ. Press, Princeton, NJ.

Houde, A., and J. A. Endler.1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. Science 248:1405–1408.

Hudon, J. 1991. Unusual carotenoid use by Western Tanager (*Piranga ludoviciana*) and its evolutionary implications. Can. J. Zool. 69:2311–2320.

Inouye, C. Y., G. E. Hill, R. Stradi, and R. Montgomerie. 2001. Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. Auk 118:900–915.

Isaksson, C. 2009. The chemical pathway of carotenoids: from plants to birds. Ardea 97:125–128.

Johnson, K., R. Dalton, and N. Burley. 1993. Preferences of female American goldfinches (*Carduelis tristis*) for natural and artificial male traits. Behav. Ecol. 4:138–143.

Kemp, D. J. 2008. Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. Evolution 62:2346–2358.

Kiere, L. M., C. M. Hofmann, J. J. Price, T. W. Cronin, and K. E. Omland. 2009. Discrete evolutionary color changes in caciques suggest different modes of carotenoid evolution between closely related taxa. J. Avian Biol. 40: 605–613.

Krebs, J., and R. Dawkins. 1984. Animal signals: mind-reading and manipulation. Pp. 380–402 *in* Behavioural ecology: an evolutionary approach. 2nd ed. Blackwell Scientific Publications, Oxford, UK.

LaFountain, A. M., H. A. Frank, and R. O. Prum. 2013. Carotenoids from the crimson and maroon plumages of Old World orioles (Oriolidae). Arch. Biochem. Biophys. 539:126–132.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29:1695–1701.

Lerner, H. R. L., M. Meyer, H. F. James, M. Hofreiter, and R. C. Fleischer. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of hawaiian honeycreepers. Curr. Biol. 21:1838–1844.

Lopes, R. L., J. D. Johnson, M. B. Toomey, M. S. Ferreira, P. M. Araujo, J. Melo-Ferreira, L. Andersson, G. E. Hill, J. C. Corbo, and M. C. Carneiro. 2016. Genetic basis for red coloration in birds. Curr. Biol. 26:1–8.

Maia, R., D. R. Rubenstein, and M. D. Shawkey. 2013. Key ornamental innovations facilitate diversification in an avian radiation. Proc. Natl. Acad. Sci. USA 110:10687–10692.

———. 2016. Selection, constraint, and the evolution of coloration in African starlings. Evolution 70:1064–1079.

McGraw, K. J. 2006. Mechanics of carotenoid-based coloration. Pp. 177–242 *In* G. E. Hill and K. J. McGraw, eds. Bird coloration. Harvard Univ. Press, Cambridge, MA.

McGraw, K. J., and D. R. Ardia. 2003. Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. Am. Nat. 162:704–712.

McGraw, K. J., and A. J. Gregory. 2004. Carotenoid pigments in male American Goldfinches: what is the optimal biochemical strategy for becoming colorful? Biol. J. Linn. Soc. 83:273–280.

McGraw, K. J., G. E. Hill, R. Stradi, and R. S. Parker. 2001. 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., E. Adkins-Regan, and R. S. Parker. 2002. Anhydrolutein in the zebra finch: a new, metabolically derived carotenoid in birds. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 132:811–818.

McGraw, K. J., G. E. Hill, and R. S. Parker. 2003a. Carotenoid pigments in a mutant cardinal: implications for the genetic and enzymatic control mechanisms of carotenoid metabolism in birds. Condor 105:587–592.

McGraw, K. J., M. D. Beebee, G. E. Hill, and R. S. Parker. 2003b. Lutein-based plumage coloration in songbirds is a consequence of selective pigment incorporation into feathers. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 135:689–696.

McGraw, K. J., A. A. Cohen, D. Costantini, and P. Horak. 2010. The ecological significance of antioxidants and oxidative stress: a marriage between mechanistic and functional perspectives. Funct. Ecol. 24:947–949.

Mendes-Pinto, M. M., A. M. LaFountain, M. C. Stoddard, R. O. Prum, H. A. Frank, and B. Robert. 2012. Variation in carotenoid-protein interaction in bird feathers produces novel plumage coloration. J. R. Soc. Interface 9:3338–3350.

Milinski, M., and T. C. M. Bakker. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. Nature 344:330–333.

Olson, V. A., and I. P. F. Owens. 2005. Interspecific variation in the use of carotenoid-based coloration in birds: diet, life history and phylogeny. J. Evol. Biol. 18:1534–1546.

Omland, K. E. and C. M. Hofmann. 2006. Adding color to the past: ancestral state reconstruction of bird coloration. *In* G. E. Hill and K. J. McGraw, eds. Bird coloration. Vol. II. Function and evolution. Harvard Univ. Press, Cambridge, MA. pp. 417–454.

Omland, K. E. and S. M. Lanyon. 2000. Reconstructing plumage evolution in orioles (Icterus): repeated convergence and reversal in patterns. Evolution 54:2119–2133.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Prager, M., and S. Andersson. 2009. Phylogeny and evolution of sexually selected tail ornamentation in widowbirds and bishops (*Euplectes* spp). J. Evol. Biol. 22:2068–2076.

———. 2010. Convergent evolution of red carotenoid coloration in widowbirds and bishops (*Euplectes* spp.). Evolution 64:3609–3619.

Prum, R. O. 1997. Phylogenetic tests of alternative intersexual selection mechanisms: trait macroevolution in a polygynous clade (Aves: Pipridae). Am. Nat. 149:668–692.

Prum, R. O. 2010. The Lande-Kirkpatrick mechanism is the null model of evolution by intersexual selection: implications for meaning, honesty, and design in intersexual signals. Evol. 64:3085–3100.

Prum, R. O., A. M. LaFountain, J. Berro, M. C. Stoddard, and H. A. Frank. 2012. Molecular diversity, metabolic transformation, and evolution of carotenoid feather pigments in cotingas (Aves: Cotingidae). J. Comp. Physiol. B 182:1095–1116.

Rausher, M. D. 2008. Evolutionary transitions in floral color. Int. J. Plant Sci. 169:7–21.

Rees, J. L. 2000. The melanocortin 1 receptor (MC1R): more than just red hair. Pigment Cell Res. 13:135–140.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217–223.

Robillard, T., and L. Desutter-Grandcolas. 2011. Evolution of calling songs as multicomponent signals in crickets (Orthoptera: Grylloidea: Eneopterinae). Behaviour 148:627–672.

Rohwer, S. 1975. The social significance of avian winter plumage variability. Evolution 29:593–610.

Saks, L., K. McGraw, and P. Horak. 2003. How feather colour reflects its carotenoid content. Funct. Ecol. 17:555–561.

Senar, J. C. 2006. Color displays as intrasexual signals of aggression and dominance. pp. 87–136 *In* G. E. Hill and K. J. McGraw, eds. Bird coloration. Vol. II. Function and evolution. Harvard Univ. Press, Cambridge, MA. pp. 87–136.

Smith, C., I. Barber, R. J. Wooton, and L. Chittka. 2004. A receiver bias in the origin of three-spined stickleback mate choice. Proc. R. Soc. Lond. B 271:949–955.

Stradi, R. 1998. The colour of flight: carotenoids in bird plumage. Solei Gruppo Editorale Informatico, Milan.

———. 1999. Pigmenti e sistematica degli uccelli. Pp. 117–146 *in* L. Brambilla, G. Canali, E. Mannucci, R. Massa, N. Saino, R. Stradi, and G. Zerbi, eds. Colorio in volo: il piumaggio degli uccelli. Universita degli Studi di Milano, Milan.

Stradi, R., G. Celentano, and D. Nava. 1995a. Separation and identification of carotenoids in bird's plumage by high-performance liquid

chromatography—diodearray detection. J. Chromatogr. B Biomed. Appl. 670:337–348.

Stradi, R., G. Celentano, E. Rossi, G. Rovati, and M. Pastore. 1995b. Carotenoids in bird plumage—I. The carotenoid pattern in a series of Palearctic Carduelinae. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 110:131–143.

Stradi, R., E. Rossi, G. Celentano, and B. Bellardi. 1996. Carotenoids in bird plumage: the pattern in three Loxia species and *Pinicola enucleator*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 113:427–432.

Stradi, R., G. Celentano, M. Boles, and F. Mercato. 1997. Carotenoids and bird plumage: the pattern in a series of red-pigmented Carduelinae. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 117:85–91.

Stradi, R., E. Pini, and G. Celentano. 2001. Carotenoids in bird plumage: the complement of red pigments in the plumage of wild and captive Bullfinch (*Pyrrhula pyrrhula*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 128:529–535.

Thomson, L. R., Y. Toyoda, A. Langner, F. C. Delori, K. M. Garnett, N. Craft, C. R. Nichols, K. M. Cheng, and C. K. Dorey. 2002. Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. Invest. Opthamol. Visual Sci. 43:3538–3549.

True, J. R., and S. B. Carroll. 2002. Gene co-option in physiological and morphological evolution. Annu. Rev. Cell Dev. Biol. 18:53–80.

Weatherhead, P. J., and R. J. Robertson. 1979. Offspring quality and the polygyny threshold: "the sexy son hypothesis." Am. Nat. 113:201–208.

Whiting, M. J., K. A. Nagy, and P. W. Bateman. 2003. Evolution and maintenance of social status-signalling badges: experimental manipulation in lizards. Pp. 47–82 *in* S. F. Fox, J. K. McCoy, and T. A. Baird, eds. Lizard social behavior. John Hopkins Univ. Press, Baltimore, MD.

Wiens, J. J. 2001. Widespread loss of sexually selected traits: how the peacock lost its spots. Trends Ecol. Evol. 16:517–523.

Williams, G. C. 1966. Adaptation and natural selection. Princeton Univ. Press, Princeton, NJ. p.184.

Zuccon, D., R. Prŷs-Jones, P. C. Rasmussen, and P. G. P. Ericson. 2012. The phylogenetic relationships and generic limits of finches (Fringillidae). Mol. Phylogenet. Evol. 62:581–596.

Associate Editor: J. Storz
Handling Editor: M. Servedio

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Yellow colors created with metabolically modified carotenoids (red) overlap substantially with yellow colors created with unmodified, dietary carotenoids (blue).

Figure S2. Five different plumage color evolution models tested via stochastic character mapping.

Figure S3. (A) Ancestral state reconstruction of plumage colors in Fringillidae.

Table S1. Bird species with yellow plumage for which the biochemical nature of the carotenoids used to create yellow plumage is known or inferred from closely related species.

Table S2. Performance metrics of twelve different carotenoid evolution models.

Table S3. Specimen IDs obtained from the Cornell University Museum of Vertebrates.

Table S4. Subjective, human-based description of putative carotenoid-based colors in finches.