

Evolutionary innovation and diversification of carotenoid-based pigmentation in finches

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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 metabolism 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 (*Haemorrhous 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



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, changeable 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 esti-

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

(Continued)

Table 1. Continued.

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

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

***Plumage samples 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 AIC_w 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 AIC_w. 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

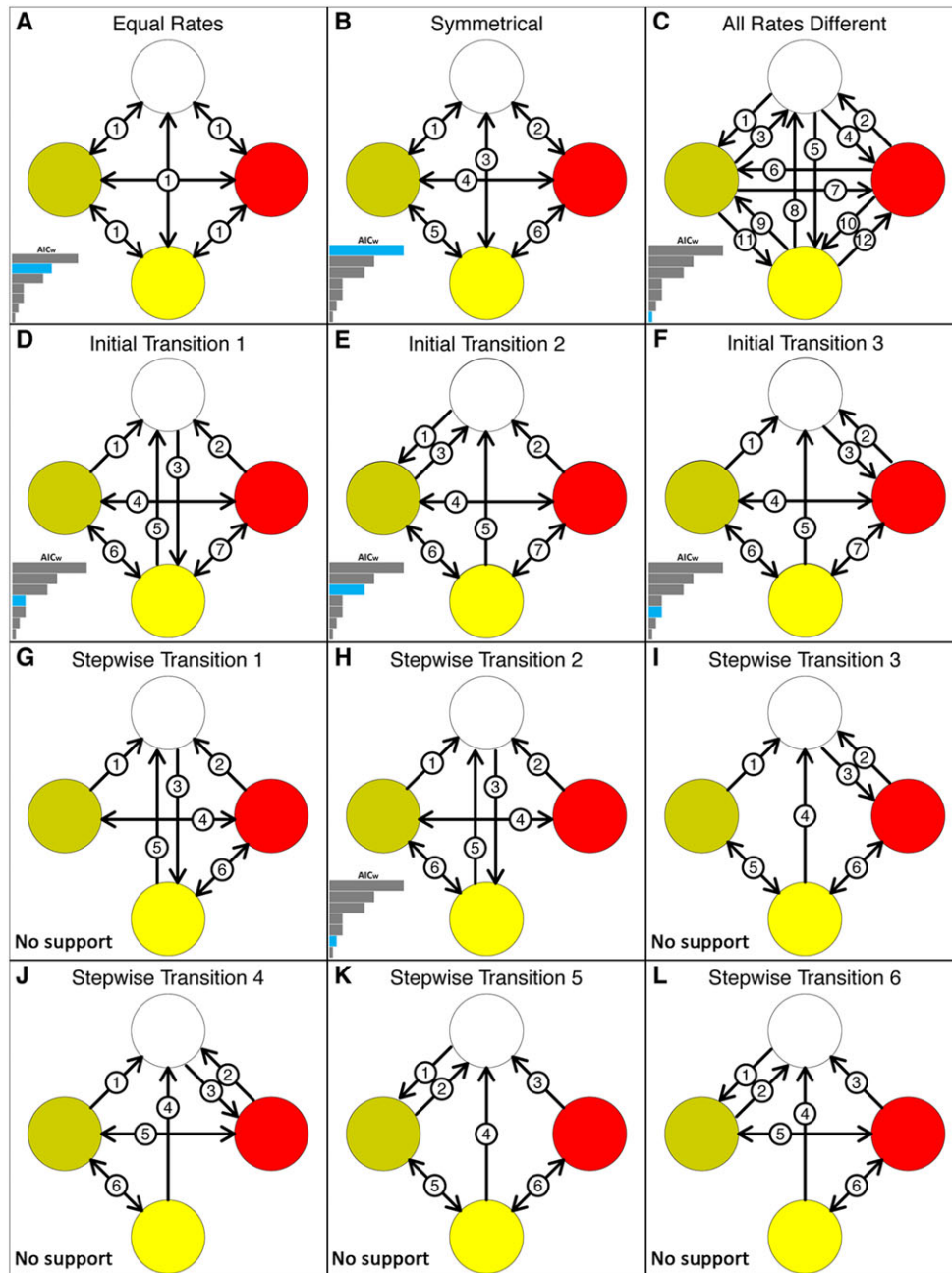


Figure 1. Twelve different carotenoid evolution models tested via stochastic character mapping. Plumage lacking any carotenoids is indicated by the white circle, dietary carotenoids indicated by the bright yellow circle, modified red pigments by the red circle, and modified yellow pigments by the off-yellow 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	Δ AIC	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.

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

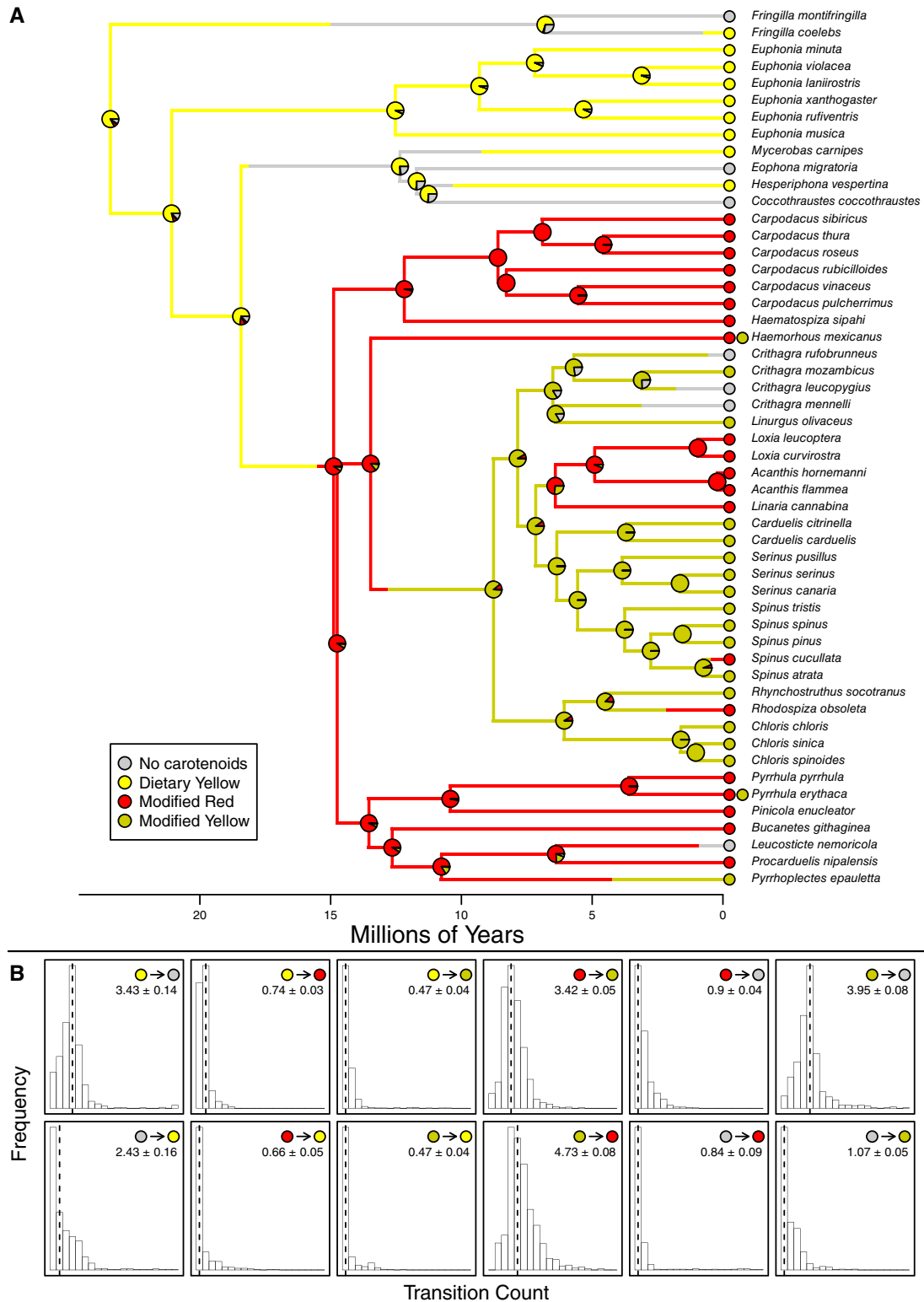


Figure 2. (A) Ancestral state reconstruction of carotenoid plumage states in Fringillidae. The number of simulations included for each model is proportional to their AICw. Colored circles at the tips of each branch represent the carotenoid plumage state of each extant species. Pie charts on each node 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).

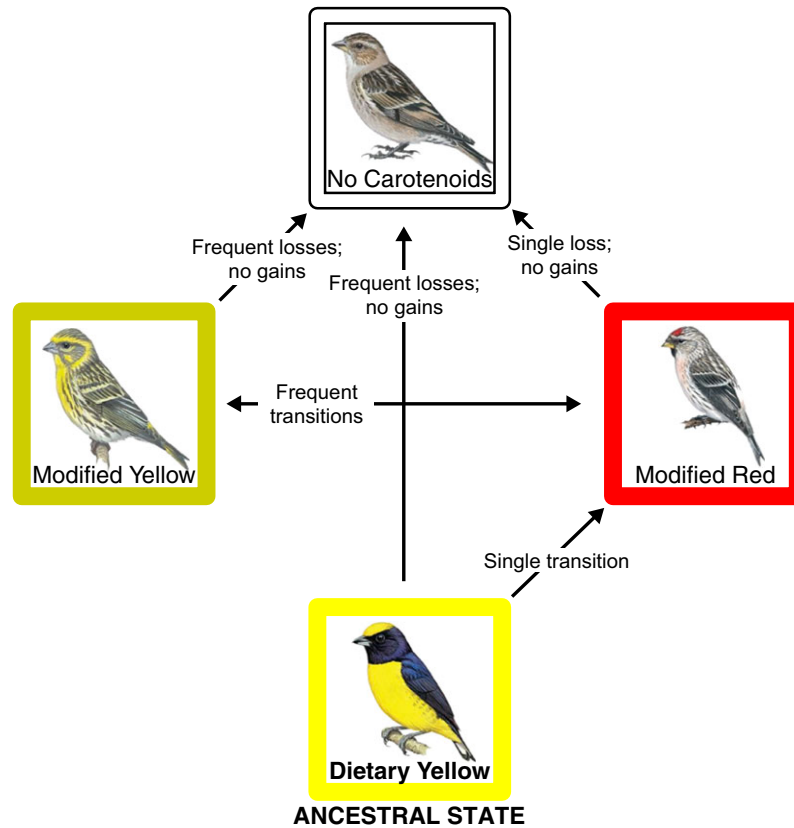


Figure 3. Summary of carotenoid character state changes throughout the evolutionary history of Fringillidae.

plumage. The lack of observed reversals in carotenoid metabolism 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 have 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.

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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.

SUPPLEMENTAL MATERIAL

Evolutionary innovation and diversification of carotenoid-based pigmentation in finches

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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.

Carotenoid Class	Species	Confirmed carotenoid type?	Location*
Dietary	<i>Dendroica petechia</i>	yes	AU
	<i>Dendroica discolor</i>	no	AU
	<i>Dendroica palmarum</i>	yes	AU
	<i>Dendroica coronata</i>	yes	AU
	<i>Dendroica pinus</i>	no	AU
	<i>Dendroica magnolia</i>	no	AU
	<i>Vermivora pinus</i>	no	AU
	<i>Icteria virens</i>	yes	AU
	<i>Geothlypis trichas</i>	yes	AU
	<i>Protonotaria citrea</i>	no	AU
	<i>Paradisea apoda</i>	yes	ASU
Modified	<i>Bombycilla cedrorum</i>	yes	AU
	<i>Carduelis tristis</i>	yes	AU
	<i>Carduelis pinus</i>	yes	AU
	<i>Carduelis psaltria</i>	no	ASU
	<i>Carduelis lawrencei</i>	no	ASU
	<i>Piranga ludoviciana</i>	yes	AU
	<i>Piranga olivacea</i>	yes	AU
	<i>Serinus canaria</i>	yes	AU

*Location where bird specimens were measured using a spectrometer. AU = Auburn University, ASU = Arizona State University.

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Table S2. Performance metrics of twelve different carotenoid evolution models. The model names correspond to those in Supplementary Figure S1.

Model Name	Log likelihood	Number of Parameters	AIC	Δ AIC	AICw
Equal Rates	-56.34	1	114.69	0.00	0.39
Symmetrical	-54.72	3	115.43	0.74	0.27
All Rates Different	-53.00	6	118.00	3.31	0.07
Stepwise Transition 1	-55.81	4	119.62	4.93	0.03
Stepwise Transition 2	-53.89	4	115.78	1.09	0.23

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

Catalog Number	Scientific Name
CUMV Bird 20827	<i>Euphonia xanthogaster</i>
CUMV Bird 20833	<i>Euphonia lanirostris crassirostris</i>
CUMV Bird 20851	<i>Euphonia rufiventris</i>
CUMV Bird 20861	<i>Euphonia musica intermedia</i>
CUMV Bird 20863	<i>Euphonia minuta minuta</i>
CUMV Bird 49242	<i>Euphonia violacea</i>

Table S4. Subjective, human-based description of putative carotenoid-based colors in finches. Classifications were made by evaluating color plates (del Hoyo et al. 2010). *Yellow* color classification corresponds to shorter wavelength colors, *red* color classification corresponds to all longer wavelength colors (oranges, pinks, reds).

Taxon	Description	Color class
<i>Bucanetes githagineus</i>	pinkish	red
<i>Bucanetes mongolicus</i>	pinkish	red
<i>Callacanthus burtoni</i>	red	red
<i>Carduelis ambigua</i>	yellow	yellow
<i>Carduelis atrata</i>	yellow	yellow
<i>Carduelis barbata</i>	yellow	yellow
<i>Carduelis cannabina</i>	red	red
<i>Carduelis carduelis</i>	red	red
<i>Carduelis chloris</i>	yellow	yellow
<i>Carduelis citrinella</i>	yellow	yellow
<i>Carduelis cucullata</i>	orange	orange
<i>Carduelis flammea</i>	red	red
<i>Carduelis flavirostris</i>	pinkish	red
<i>Carduelis hornemanni</i>	red	red
<i>Carduelis magellanica</i>	yellow	yellow
<i>Carduelis monguilloti</i>	yellow	yellow
<i>Carduelis pinus</i>	yellow	yellow
<i>Carduelis psaltria</i>	yellow	yellow
<i>Carduelis sinica</i>	yellow	yellow
<i>Carduelis spinoides</i>	yellow	yellow
<i>Carduelis spinus</i>	yellow	yellow
<i>Carduelis tristis</i>	yellow	yellow
<i>Carpodacus erythrinus</i>	red	red
<i>Carpodacus mexicanus</i>	red	red
<i>Carpodacus nipalensis</i>	purpleish	red
<i>Carpodacus pulcherrimus</i>	pinkish	red
<i>Carpodacus puniceus</i>	pinkish	red
<i>Carpodacus purpureus</i>	purpleish	red
<i>Carpodacus rhodochlamys</i>	pinkish	red
<i>Carpodacus rodochroa</i>	pinkish	red
<i>Carpodacus rodopeplus</i>	purpleish	red
<i>Carpodacus roseus</i>	pinkish	red
<i>Carpodacus rubescens</i>	red	red
<i>Carpodacus rubicilla</i>	pinkish	red
<i>Carpodacus rubicilloides</i>	pinkish	red
<i>Carpodacus synoicus</i>	pinkish	red
<i>Carpodacus thura</i>	pinkish	red
<i>Carpodacus vinaceus</i>	purpleish	red

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<i>Chlorophonia cyanea</i>	yellow	yellow
<i>Coccothraustes coccothraustes</i>	tawny	none
<i>Eophona migratoria</i>	tawny	none
<i>Euphonia cayennensis</i>	yellow	yellow
<i>Euphonia chlorotica</i>	yellow	yellow
<i>Euphonia finschi</i>	golden yellow	yellow
<i>Euphonia laniirostris</i>	yellow	yellow
<i>Euphonia minuta</i>	yellow	yellow
<i>Euphonia musica</i>	golden yellow	yellow
<i>Euphonia rufiventris</i>	yellow	yellow
<i>Euphonia violacea</i>	yellow	yellow
<i>Euphonia xanthogaster</i>	yellow	yellow
<i>Fringilla coelebs</i>	greenish yellow	yellow
<i>Fringilla montifringilla</i>	rufus	none
<i>Haematospiza sipahi</i>	red	red
<i>Hemignathus virens</i>	yellow	yellow
<i>Hesperiphona vespertina</i>	yellow	yellow
<i>Kozlowia roborowskii</i>	purpleish	red
<i>Leucosticte arctoa</i>	purpleish	red
<i>Leucosticte brandti</i>	purpleish	red
<i>Leucosticte nemoricola</i>	brown/gray	none
<i>Leucosticte tephrocotis</i>	pinkish	red
<i>Linurgus olivaceus</i>	yellow	yellow
<i>Loxia curvirostra</i>	red	red
<i>Loxia leucoptera</i>	red	red
<i>Loxia pytyopsittacus</i>	red	red
<i>Loxioides bailleui</i>	yellow	yellow
<i>Mycerobas carnipes</i>	yellow	yellow
<i>Paroreomyza montana</i>	yellow	yellow
<i>Pinicola enucleator</i>	pinkish	red
<i>Pinicola subhimachala</i>	pinkish	red
<i>Pyrrhoptes epauletta</i>	orange	orange
<i>Pyrrhula erythaca</i>	orangish pinkish	red
<i>Pyrrhula nipalensis</i>	pinkish	red
<i>Pyrrhula pyrrhula</i>	pinkish	red
<i>Rhodopechys sanguineus</i>	pinkish	red
<i>Rhodospiza obsoletus</i>	pinkish	red
<i>Rhynchostruthus socotranus</i>	yellow	yellow
<i>Serinus burtoni</i>	greenish yellow	yellow
<i>Serinus canaria</i>	yellow	yellow
<i>Serinus canicollis</i>	yellow	yellow
<i>Serinus citrinelloides</i>	yellow	yellow

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<i>Serinus estherae</i>	yellow	yellow
<i>Serinus albogularis</i>	yellow	yellow
<i>Serinus atrogularis</i>	yellow	yellow
<i>Serinus buehneri</i>	yellow	yellow
<i>Serinus citrinipectus</i>	yellow	yellow
<i>Serinus donaldsoni</i>	yellow	yellow
<i>Serinus dorsostriatus</i>	yellow	yellow
<i>Serinus flavigula</i>	yellow	yellow
<i>Serinus flaviventris</i>	yellow	yellow
<i>Serinus flavivertex</i>	yellow	yellow
<i>Serinus gularis</i>	gray/white	none
<i>Serinus nigriceps</i>	yellow	yellow
<i>Serinus capistratus</i>	yellow	yellow
<i>Serinus koliensis</i>	yellow	yellow
<i>Serinus leucopterus</i>	white/brown	none
<i>Serinus melanocephalus</i>	white/brown	none
<i>Serinus menachensis</i>	white/brown	none
<i>Serinus reichardi</i>	white/brown	none
<i>Serinus reichenowi</i>	yellow	yellow
<i>Serinus rothschildi</i>	yellow	yellow
<i>Serinus scotops</i>	yellow	yellow
<i>Serinus tristriatus</i>	gray/white	none
<i>Serinus xantholaemus</i>	yellow	yellow
<i>Serinus xanthopygius</i>	yellow	yellow
<i>Fringilla teydea</i>	blue	none
<i>Serinus leucopygius</i>	white/brown	none
<i>Serinus mennelli</i>	white/brown	none
<i>Serinus mozambicus</i>	yellow	yellow
<i>Serinus pusillus</i>	red	red
<i>Serinus rufobrunneus</i>	brown/gray	none
<i>Serinus serinus</i>	yellow	yellow
<i>Serinus striolatus</i>	yellow	yellow
<i>Serinus sulphuratus</i>	yellow	yellow
<i>Serinus syriacus</i>	yellow	yellow
<i>Serinus thibetanus</i>	yellow	yellow
<i>Uragus sibiricus</i>	pinkish	red
<i>Ammodramus humeralis</i>	yellow	yellow
<i>Anthus trivialis</i>	tawny	none
<i>Montifringilla ruficollis</i>	white/brown	none
<i>Motacilla alba</i>	white/black	none
<i>Parula pitayumi</i>	yellow	yellow
<i>Passer luteus</i>	yellow	yellow

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<i>Passer montanus</i>	tawny	none
<i>Petronia petronia</i>	yellow	yellow
<i>Plectrophenax nivalis</i>	white/brown	none
<i>Sturnella superciliaris</i>	red	red

50

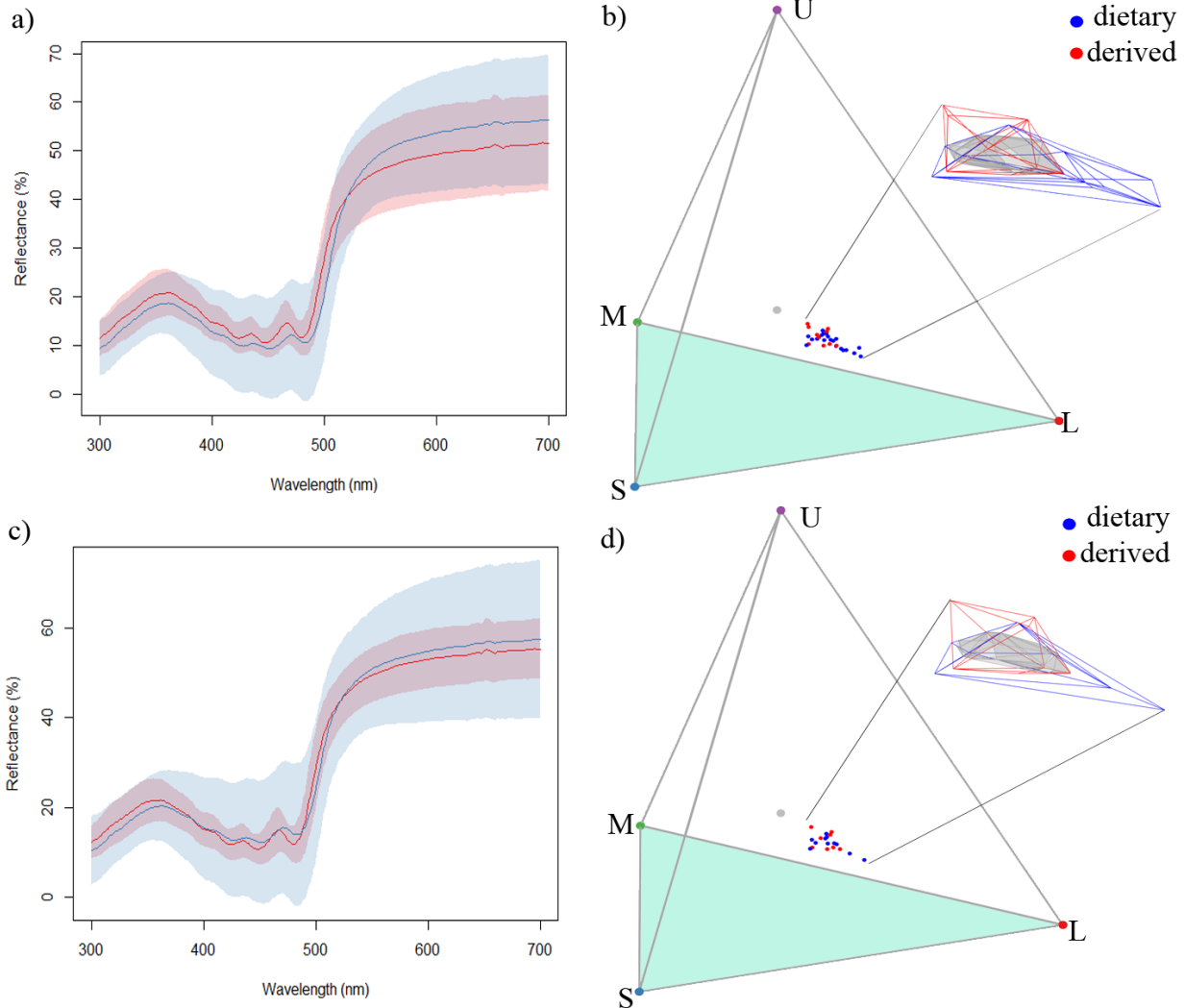


Figure S1. Yellow colors created with metabolically modified carotenoids (red) overlap substantially with yellow colors created with unmodified, dietary carotenoids (blue). Avian plumage colors were measured with a spectrometer (a,c), then plotted in avian colorspace (b,d) where vertices correspond to the ultraviolet (U), short-wavelength (S), medium-wavelength (M), and long-wavelength sensitive cones to quantify degree of perceptual overlap. A larger color dataset was used to create plots (a) & (b), though this dataset includes birds for which carotenoid mechanism was inferred based on phylogenetic relationships, rather than biochemically determined (Supplemental Table 3). A smaller dataset was also analyzed (c, d), containing only species with yellow plumage which has been biochemically analyzed. The degree of overlap between dietary pigments and metabolically modified pigments is substantial for both datasets, in particular the volume of colorspace that overlaps between dietary and metabolically modified pigments is 55% using the complete dataset (b), and 50% using the smaller conservative dataset (d). Panels for this figure were created using the R package *Pavo* (Maia et al. 2013).

70

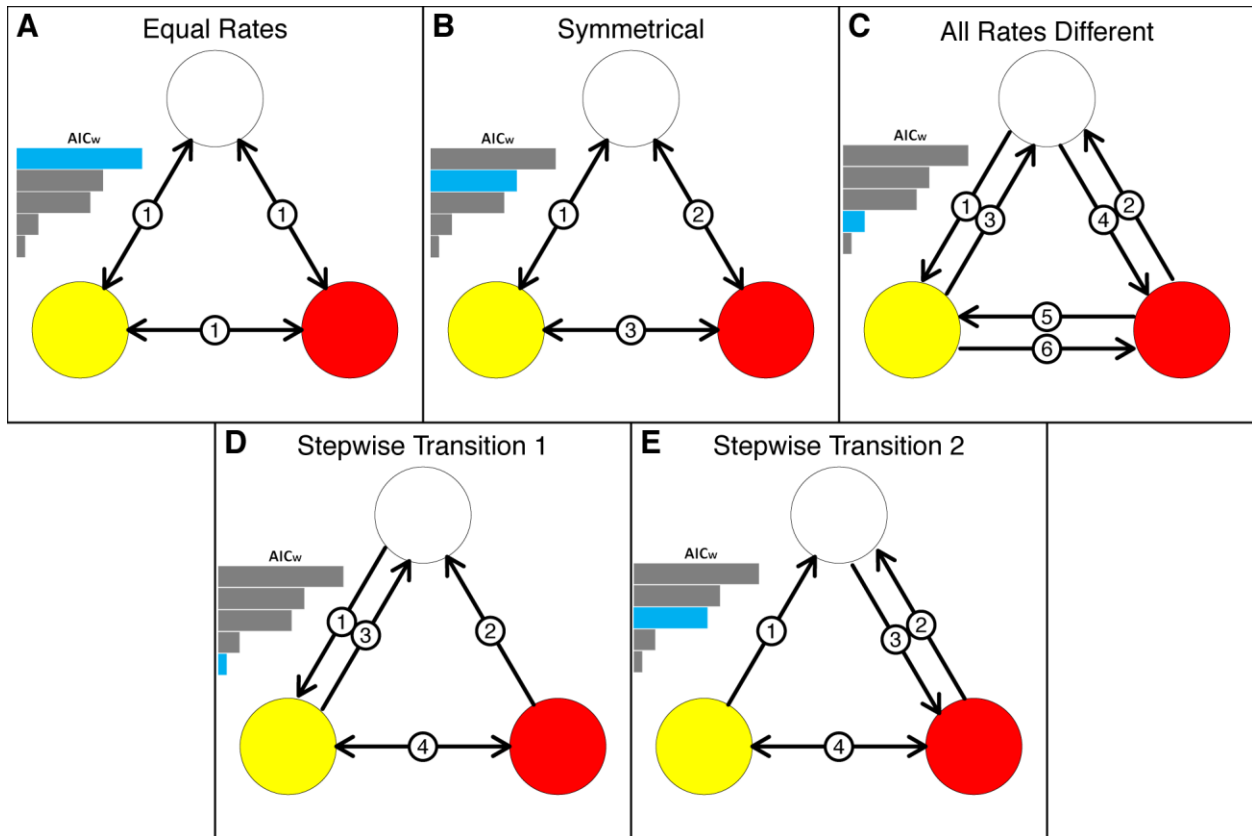


Figure S2: Five different plumage color evolution models tested via stochastic character mapping. Each number on a figure panel corresponds to a different rate shift parameter. Arrows with heads on both ends indicate symmetrical transition rates. Model support (AICw weight) values are indicated in the bottom left of each panel.

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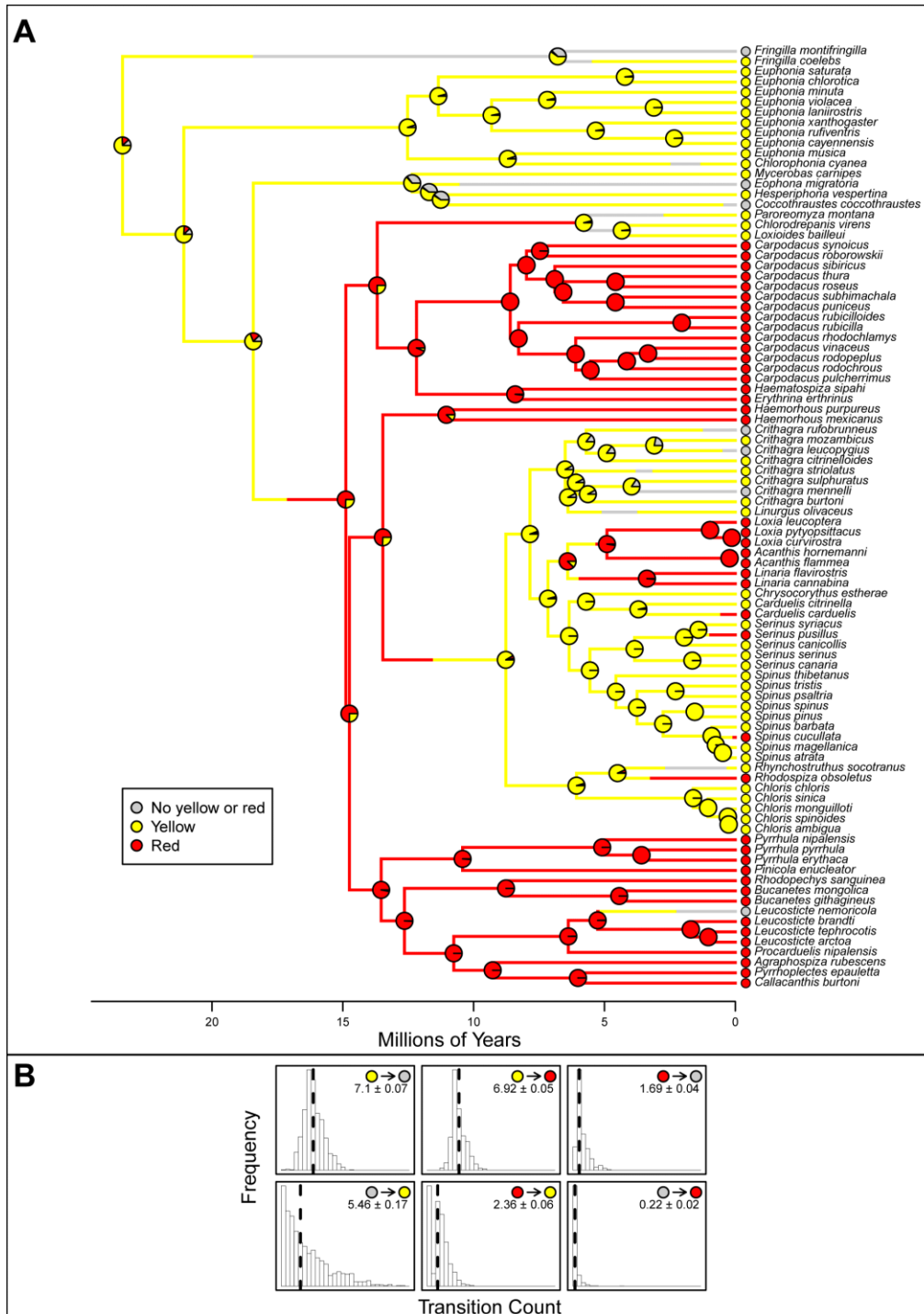


Figure S3: (A) Ancestral state reconstruction of plumage colors in Fringillidae. The number of simulations included for each model is proportional to their AICw. Colored circles at the tips of each branch represent the plumage color of each extant species. Pie charts on each node indicate the proportion 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 plumage color 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 color character states identified in (A).

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